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REVIEW ARTICLE

Cross-presentation: dendritic cells and macrophages bite off more than they can chew!

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SUMMARY

As immunologists, our knowledge of the molecular mechanisms which underlie the presentation of antigens derived from extracellular or 'exogenous' sources to CD8 cytotoxic lymphocytes (CTL) has been limited. This process, termed 'cross-presentation', has been linked to the elicitation of protective CTL responses against tumours and may be extremely important in generating immune responses against clinically relevant pathogens that do not infect tissues of haemopoietic origin. It is now known that cross-presentation of exogenous antigens on major histocompatibility complex (MHC) class I occurs through several distinct cellular pathways. In this review we outline and discuss some recent advances in our understanding of these pathways.

Keywords antigen processing; cross-presentation; dendritic cell; exogenous antigen; MHC class I; phagosome

INTRODUCTION

The major histocompatibility complex (MHC) class-I restricted cytotoxic T-cell (CTL) response is the principal arm of the immune response that destroys intracellular pathogens such as viruses and some bacteria. CTL responses have also been implicated in the elimination of cells that have undergone malignant transformation. To initiate a protective CTL response, the antigens derived from pathogens and transformed cells must be processed and presented on professional antigen-presenting cells (APC) in the context of MHC class I molecules. Only professional APC such as dendritic cells (DC) and possibly some macrophages express a combination of co-receptors and MHC-class I molecules at levels high enough to stimulate naive CD8 T cells.[1]

Most peptides found on MHC class I molecules are derived from endogenous polypeptides synthesized on the cells' own ribosomes. Such endogenous antigens comprise peptides of host cell origin along with those from viruses and intracellular bacteria. The majority of antigenic peptides are generated by the proteolytic cleavage of malfolded proteins. This protein fraction has been collectively termed defective ribosomal products (DRiPs) and accounts for almost 30% of all synthesized proteins in cells.[2]

Upon DC activation, DRiPs accumulate as large cytosolic aggregates, called dendritic cell aggresome-like inducible structures (DALIS).[3] Ubiquitinating enzymes present within these complexes generate substrates for the cytosolic multi-catalytic proteasome complex which cleaves large ubiquitinated polypeptides into smaller peptides.[4] Peptides derived from proteasomal degradation are transported into the lumen of the endoplasmic reticulum (ER) by the transporter associated with antigen presentation (TAP).

In the lumen of the ER, newly imported peptides require further trimming by the ER-aminopeptidase I (ERAP).[5] The resulting peptides, 8–9 amino acids long, are loaded onto nascent MHC class I chains by the MHC class I loading complex. This involves the ER chaperones calnexin, calreticulin and tapasin. This is part of a process that forms the fully folded, mature MHC class I molecules. MHC class I–peptide complexes then dissociate from the MHC class I loading complex and are transported through the secretory pathway to the plasma membrane for display to the immune system.

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This presentation system evolved to exploit the dependence of replicating viruses on utilizing the host's protein synthesis machinery. However, it raises two important questions. How can we elicit CTL responses against tumour cells of a non-haematopoietic origin? How can we resolve viral infections where the virus does not infect professional APC? This paradox was solved when Bevan and colleagues characterized MHC class I-restricted CTL responses against peptides derived from 'exogenous' or extracellular protein sources.[6,7] This postulated pathway for the transfer of exogenous antigen into the MHC class I antigen processing machinery was subsequently termed 'cross-presentation'. With very few exceptions[8,9] cross-presentation is restricted to DC and some macrophages but is not normally seen in other nucleated cells.[10–14] In addition to 'cross-priming' naïve CD8 T cells, cross-presentation appears to be central to the maintenance of peripheral tolerance to self-antigens, that is 'cross-tolerance'.[15]

Cross-presentation has been shown for viruses, allografts, tumours, particulate antigens and purified proteins, both, *in vitro* and *in vivo*.[13,16,17]

THE ROLE OF DC IN CROSS-PRESENTATION

DC are the most potent professional APC capable of priming naïve T cells *in vivo*.[1] While resident in peripheral tissues DC exhibit high levels of receptor-mediated endocytosis, macropinocytosis and low levels of phagocytic activity and thus are efficient cells for capturing invading pathogens.[18–20] After capturing invading pathogens in the periphery, DC migrate to the local draining lymph nodes where they present antigens derived from the pathogens to circulating lymphocytes. Effective stimulation of naïve T cells occurs in the draining lymph nodes and depends on two main criteria: the efficient uptake and processing of antigens and a regulated maturation process in response to contact with inflammatory mediators. The exposure of DC to inflammatory stimuli induces their differentiation into professional APC. These unique aspects of DC function ensure that they are the ideal cell type for mediating cross-presentation *in vitro* and *in vivo*.[12,13,21–30] Deletion of DC abrogates cross-presentation of self-antigen.[31] Both TAP-dependent and independent routes for the presentation of exogenous antigen have been identified in DC.

ROUTES OF ENTRY FOR EXOGENOUS ANTIGENS

There are four major cellular processes used by DC and macrophages to internalize exogenous antigens (Fig. 1). These are endocytosis, pinocytosis, phagocytosis and marcopinocytosis. The form of the antigen, its solubility, and whether it is part of an immune complex or still associated with a pathogen all determine the route of entry.

Endocytosis is the term used to describe the formation of vesicles of between ~150–200 nm that form at sites of membrane invaginations, termed coated pits. Antigens induce the formation of coated pits by clustering specific cell surface receptors.[32] In pinocytosis, soluble antigen is taken up at the same time as extracellular fluid present in the vicinity of the budding endosome. Uptake of these vesicles can be both clathrin dependent and independent.

Phagocytosis is the clathrin-independent process by which cells internalize large particulate material such as apoptotic cells, cellular debris, or bacteria, which are usually destined to be degraded by lysosomal enzymes. The phagosome is a membrane-bound organelle formed when a phagocytic cell engulfs particulate material.[33] The phagocytic process can be divided into three stages: attachment of the particle to the cell surface, mediated by surface receptors; engulfment, characterized by the flow redistribution of the plasma membrane to surround the particle; and formation of the phagosome (phagolysosome). Macropinocytosis is a process whereby large vacuoles, termed macropinosomes, form at the plasma membrane; these nonspecifically trap large volumes of the extracellular media. Macropinosomes are usually 200–500 nm in diameter and are thought to form at sites of membrane ruffling.

RECEPTORS INVOLVED IN THE RECOGNITION AND UPTAKE OF EXOGENOUS ANTIGENS

Phagocytosis and receptor-mediated endocytosis are mediated by an increasing number of characterized cellular receptors; many of which have been implicated in cross-presentation. Targeting antigens to Fc receptors for immunoglobulin G (FcγR)[1] or administered in the form of immune complexes,[34] opsonized liposomes,[35] or dead cells[36] strongly augments cross-presentation by DC and macrophages. Mice lacking these receptors fail to cross-present efficiently.[34]

The uptake of cell-associated antigens from apoptotic and necrotic bodies is largely accomplished by complement receptors such as the calreticulin/CD91 receptor complex, αv integrins,[24,37] class A-scavenger receptors and CD36.[24,29,38] Bacterial antigens are recognized by a large panel of surface receptors, including the mannose receptor,[39,40] dectin-1[41] and scavenger receptors.[42]

Bacterial and cellular antigens are also efficiently cross-presented when transferred as peptides associated to heat-shock proteins (hsp). Cross-priming activities have been reported for cytosolic hsp (hsp 70, hsc 70, hsp 90) and ER chaperones grp94/gp96,[43–47] as well as their bacterial homologues.[44,48,49] The uptake of hsp–peptide complexes appears to be mediated by specific cellular receptors such as the α2-macroglobulin receptor CD91,[50,51] and the scavenger receptors LOX-1[52] and SR-A.[53]

THE CELL BIOLOGY OF CROSS-PRESENTATION

Nearly all cells can bind and represent peptides by β_2-microglobulin-stabilized MHC class I dimers.[54,55] However, this form of antigen presentation is restricted to a very limited number of antigens that are derived either by extracellular processing in the surrounding plasma, or by regurgitation of endosomally processed polypeptides.

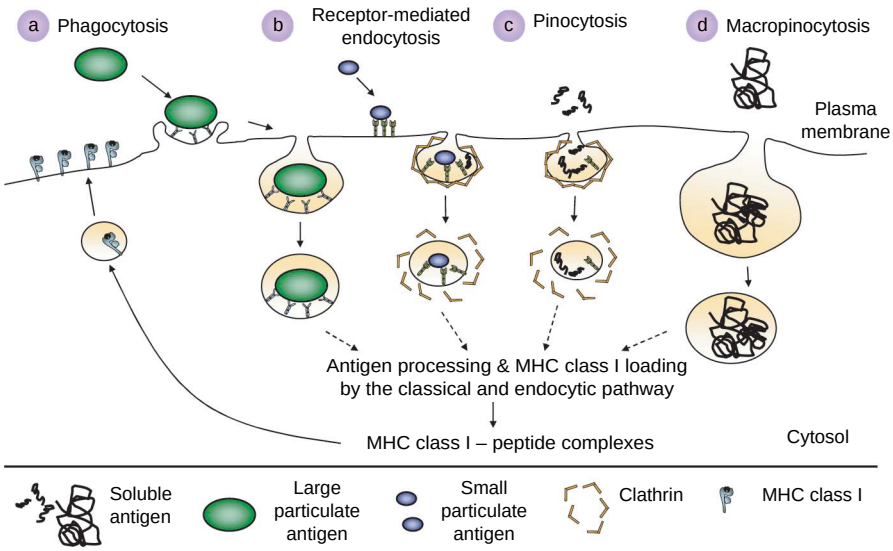

Figure 1. Common pathways for the internalization of exogenous antigens by professional antigen presenting cells (pAPC). DC and macrophages acquire exogenous antigens through four major pathways. The nature of the antigen determines which internalization route is used. (a) Large particulate antigens (such as opsonized/complement fixed bacteria, apoptotic cells and biologically inert particles) are internalized by phagocytosis. (b) Small particulate antigens enter the cell by receptor-mediated endocytosis. (c) Pinocytosis describes the uptake of soluble antigens as part of the extracellular fluid present in the vicinity of the budding endosome. Uptake of endocytic vesicles can be both clathrin dependent and independent. (d) Large fluid volumes are internalized by marcopinocytosis. Antigen from endocytic vesicles is either translocated into the cytosol for presentation via the classical pathway, or loaded on MHC class I molecules within the endocytic compartment.

Efficient loading of MHC class I molecules has been considered to be restricted to the ER because cells lacking TAP or tapasin are severely impaired in their ability to present MHC class I-associated antigens.[56,57] How can exogenous antigens access the MHC class I pathway? So far evidence has supported two principal pathways. Rodriguez and colleagues showed that exogenous antigen internalized by phagocytosis can escape lysosomal degradation by translocation into the cytosol in a murine DC cell line.[22] A similar observation was made with antigens from internalized cytomegalovirus-infected fibroblasts in human DC[58] and vaccinia virus-infected fibroblasts in murine macrophages and DC.[59]

Once in the cytosol, antigenic peptides derived from the translocated material by proteasomal degradation access the ER lumen via TAP. Three observations support this model: Cross-presentation is abrogated by the inhibition of proteasomal degradation (using lactacystin);[22] by inhibition of TAP or in TAP-deficient professional APC;[16] and by inhibition of the secretory pathway and the trans-Golgi network using brefeldin A.[60]

The second model proposes that MHC class I molecules encounter and bind exogenously derived peptides in post-Golgi or endolysosomal compartments, in the same way as MHC class II molecules, before being transported to the cell surface. Indeed, MHC class I–β_2-microglobulin dimers are present in the endosomes of immature DC and can traffic rapidly to the surface upon encounter with a maturation signal.[61–63] Specific sorting to endolysosomal compartments is mediated by a highly conserved tyrosine motif within the cytoplasmic tail region of MHC class I. Intriguingly, deletion or mutations of this motif abrogate acquisition and cross-presentation of exogenously derived peptides *in vitro* and attenuate T lymphocyte responses to immunodominant viral epitopes *in vivo*.[64] Cross-presentation within a endolysosomal compartment is independent of TAP.

PHAGOCYTOSIS AND CROSS-PRESENTATION

Recent revelations regarding the phagocytic process support an alternative molecular model for cross-presentation. This may occur autonomously through an involvement of the ER in the generation of phagosome compartments but in a TAP-dependent manner. It has been a paradigm for more than two decades that the plasma membrane, through invagination, provides all of the membrane required to form complete phagosomes.[65] However, active phagocytes are capable of engulfing large numbers of particles without any apparent loss from their plasma membrane. This observation is difficult to explain by membrane regeneration processes alone. Desjardins and colleagues characterized phagosomal membranes by mass spectrometry and two-dimensional gel electrophoresis.[66] Surprisingly, these studies revealed that several ER-resident proteins are present in phagosomes. Further analysis showed that phagosomes fuse with the ER during particle engulfment, and that ER membranes constitute a large part of phagosomal

membranes.[67] Other studies have shown that phagosomes from murine macrophages[68] and DC,[69] as well as human DC,[63] acquire the entire MHC class I loading complex and other elements involved in peptide processing and translocation. They also obtain the sec61 translocon complex, which is capable of transporting entire proteins through membranes. Phagosome compartments retain these ER elements until maturation to phagolysosomes at which point they display increasing numbers of lysosomal markers in parallel with a decrease in luminal pH.

Exogenous antigens from within phagosomes are rapidly translocated to the cytosol within 1–2 hr post-internalisation.[68,69] This translocation was also observed with the cholera toxin subunit 1 (CTA1), a known substrate for Sec61,[70] suggesting a role for this complex in the translocation events observed. Following export from the phagosome to the cytosol, antigens would become available for degradation by proteasomes into shorter peptides which are suitable for TAP-mediated import into the ER, and thus access the classical pathway for MHC class I presentation. Desjardins and colleagues found that the proteasomal α- and β-subunits are transiently recruited to the cytoplasmic surface of phagosomal membranes. This recruitment peaks approximately 1 hr after phagocytosis and is time independent from the acquisition of ER-luminal proteins. This raises the alternative possibility that peptides derived from phagocytic antigens can be retro-translocated back into the lumen of the phagosome compartment for loading onto MHC class I molecules after proteasomal degradation. Data demonstrating that the TAP molecules and the MHC class I loading complex in phagosomes are fully functional support the idea that the phagosome could function as an autonomous cross-presentation organelle. Indeed, the import of peptides is dependent on TAP, as demonstrated by both antibodies against TAP and the inhibitory peptide ICP47, derived from herpes simplex virus.[63] When antigenic peptides were added to isolated phagosomes, or fed to macrophages in the form of whole proteins, MHC class I peptide complexes were detectable 1–2 hr after phagocytosis. Membranes extracted from these vesicles could stimulate interferon-γ production from T-cell receptor transgenic CD8 T cells and responses were inhibited by lactacystin.

Amigorena and colleagues have presented strong evidence that MHC class I loading occurs in the same phagosome compartment utilized for internalization of the antigen.[69] Thus, in the current model (Fig. 2), phagocytosed antigens for cross-presentation on MHC class I are internalized in a process identical to that used for degradation of apoptotic bodies and for presentation on MHC class II molecules. After ER-phagosome formation and internalization of antigens, maturation of ER-phagosomes down the endocytic pathway results in acquisition of hydrolases that aid the breakdown of internalized antigens and export into the cytosol.[71] In the cytosol, these polypeptides are polyubiquitinated and further degraded by recruited proteasomes. Peptides that are suitable for transport by TAP are then re-imported and loaded onto MHC class I.

MACROPINOCYTOSIS AND CROSS-PRESENTATION

The extension of the phagosome loading compartment model to DC remains controversial as the phagocytic activity of these cells is thought to be significantly less than that of macrophages or neutrophils. However, DC are highly efficient in macropinocytosis. Watts and colleagues demonstrated that exogenous antigenic material delivered to the interior of the DC via macropinocytosis could be processed and presented on MHC class I.[72] Whether the formation of macropinosomes involves ER membrane fusion remains to be formally demonstrated, but there are emerging data suggesting that soluble antigens can be cross-presented in the same way as phagocytosed antigens. DC form large numbers of membrane-bound macropinocytic vesicles without a detectable loss from the plasma membrane.

Peter Cresswell and colleagues first demonstrated that soluble proteins internalized by marcopinocytosis can access the MHC class I peptide-loading complex. Internalization of soluble ovalbumin and an anti-tapasin antibody appeared in similar peripheral vesicles, some of them positive for the ER marker calnexin.[63] Also, the group utilized a soluble form of the TAP inhibitor US6, a human cytomegalovirus membrane protein, to show the TAP dependency of this process. Cross-presentation of soluble ovalbumin in the presence of US6 was inhibited in a dose-dependent manner.

CONCLUDING REMARKS

Data linking aspects of ER membrane trafficking with the immunological phenomena of cross-presentation provides compelling evidence for the existence of a specific cross-presentation organelle. From a mechanistic point of view, this model is advantageous as internalized antigens could potentially avoid direct competition with DRiPs for access to TAP and the class I loading complex in the ER. Moreover, it has been demonstrated that phagosome loaded MHC class I peptide complexes are rapidly displayed to the immune system within just 1 hr 'post' internalization.[69]

However, a number of questions about this model remain. It has been suggested (but not formally demonstrated) that the export of polypeptides from phagosome compartments is mediated by the ER translocon Sec61. Localization of Sec61 is usually restricted to the rough ER where it associates with ribosomal subunits.[73] The presence of ribosomes on phagosomes has never been demonstrated.

Moreover, the relevance of this model for *in vivo* cross-presentation has not been addressed and the efficiency of MHC class-I loading in phagosomes remains unclear. How many molecules can be loaded within this compartment, are they all derived from the ER, or to what extend from recycling MHC class I. Using an approach designed for looking at intracellular protein trafficking, Cresswell and colleagues demonstrated that most loaded phagosomal MHC class I molecules are sensitive to endoglycosidase H (endoH) digestion, which results from a lack of

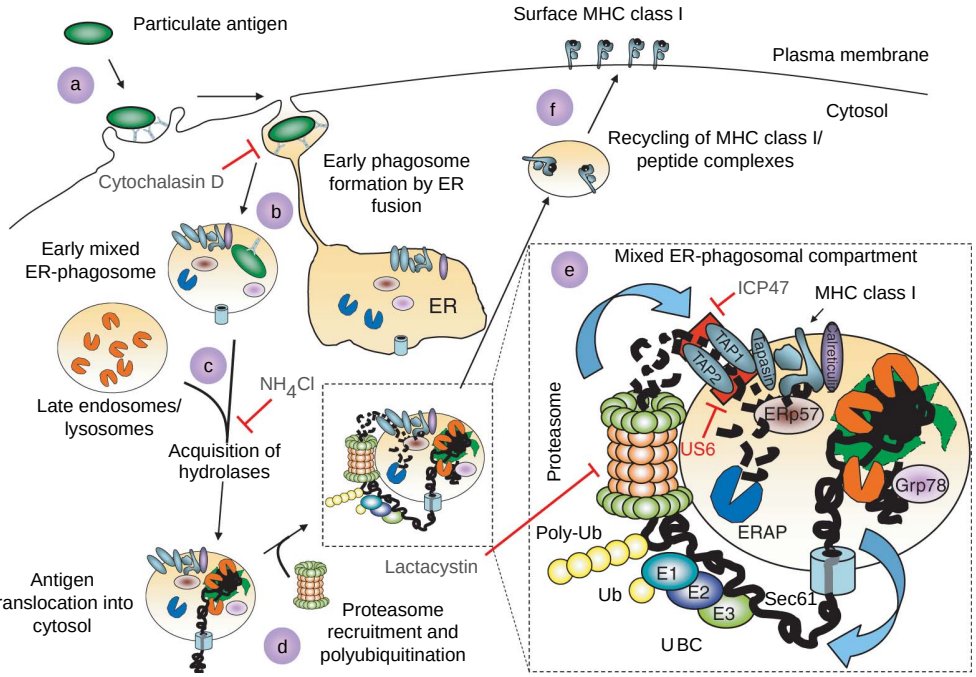

Figure 2. Schematic representation of ER-mediated phagocytosis and antigen cross-presentation in a mixed ER-phagosome compartment. (a) Phagocytosis is initiated by binding and cross-linking of cell surface receptors by particulate antigens. (b) ER membranes fuse with the plasma membrane to form the complete phagosome with a large proportion of ER-derived membranes and proteins including all the major elements of the MHC class I loading complex such as TAP, tapasin, calnexin, MHC class I heavy chain, ER chaperones and disulphide isomerase (Grp78, ERp57), ER-aminopeptidase associated with peptide trimming (ERAP) and the peptide translocation channel (Sec61). (c) Phagosomes gradually progress through the endocytic pathway and acquire hydrolases such as cathepsin D. (d) Partial proteolysis by cathepsins generates polypeptides which are suitable for export into the cytosol by Sec61. (e) Cytosolic peptides are poly-ubiquitinated by the ubiquitinating enzyme complex (UBC) and are substrates for proteasomal degradation. Processed peptides are re-imported by the TAP and trimmed by ERAP. MHC class I molecules are loaded by the MHC class I loading complex. (f) Phagosomes containing loaded MHC class I may recycle back to the plasma membrane by exocytosis. Specific inhibitors (depicted in red) that interfere at various points in the cross-presentation pathway have allowed the development of the current model.

post-translational modification within the medial-Golgi.[63] This suggests that MHC class I molecules in phagosomes must be derived primarily as newly synthesized protein from the ER rather than by recycling from the plasma membrane.[63] In addition, such nascent MHC class I/peptide complexes are also present on the plasma membrane. This is at least suggestive of direct transport back to the cell surface.

Hence, by utilizing similar approaches based on a greater understanding of the cell biology that underlies cross-presentation, immunologists will be able to provide definitive answers to the remaining questions.

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