

The known unknowns of antigen processing and presentation

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Abstract | The principal components of both MHC class I and class II antigen processing and presentation pathways are well known. In dendritic cells, these pathways are tightly regulated by Toll-like-receptor signalling and include features, such as cross-presentation, that are not seen in other cell types. However, the exact mechanisms involved in the subcellular trafficking of antigens remain poorly understood and in some cases are controversial. Recent data suggest that diverse cellular machineries, including autophagy, participate in antigen processing and presentation, although their relative contributions remain to be fully elucidated. Here, we highlight some emerging themes of antigen processing and presentation that we think merit further attention.

Endolysosomal compartments

Endosomes that have fused with lysosomes. This acidic environment allows degradation of antigens.

Since the discovery that T-cell immunity relies on 'denatured, unfolded, sequential determinants'¹ of proteins, whereas B-cell (that is, antibody) recognition of the same protein antigen is determined by its tertiary structure, immunologists have been fascinated with antigen processing and presentation. Decades of work have elucidated the pathways that generate peptide–MHC complexes. As a result, we can now explain most of the fundamental differences between B- and T-cell antigen recognition^{2,3} and such knowledge is useful for vaccine design and other immune-based interventions.

Recognition of antigens in the peptide-binding groove of surface-expressed MHC class I and class II molecules by specific T-cell receptors is central to T-cell activation. To fulfil their physiological function, MHC proteins must first acquire peptide antigens, a function that is executed differently by the two main classes of MHC molecules. For MHC class I molecules, the goal is to report on intracellular events (such as viral infection, the presence of intracellular bacteria or cellular transformation) to CD8⁺ T cells⁴. MHC class I molecules are composed of heavy chains and an invariant light chain, known as β_2 -microglobulin. The events of the biosynthesis of MHC class I molecules can be summarized in six steps: one, acquisition of antigenic peptides; two, tagging of the antigenic peptide for destruction by ubiquitylation; three, proteolysis; four, delivery of peptides to the endoplasmic reticulum (ER); five, binding of peptides to MHC class I molecules; and six, display of peptide–MHC class I complexes on the cell surface (FIG. 1). For MHC class II molecules, the goal is to sample the extracellular milieu and present

antigens to CD4⁺ T cells⁴. Similar to MHC class I molecules, the α - and β -chain of MHC class II molecules are synthesized in the ER and associate with the invariant chain (Ii; also known as CD74) for proper folding, trafficking and protection of the antigen-binding groove⁵. Newly assembled MHC class II molecules are then delivered by vesicular transport to endolysosomal compartments that supply peptide antigens. Following peptide loading, peptide–MHC class II complexes are delivered to the cell surface. Despite the involvement of different molecules and cellular compartments, the generation of peptide–MHC class II complexes can be stratified into the same six steps as those for peptide–MHC class I complexes.

The molecular expression of MHC class II molecules is mostly restricted to professional antigen-presenting cells (APCs), including macrophages and dendritic cells (DCs). DCs possess many unique features of antigen processing and presentation not seen in other cell types. Immature DCs reside in the tissue (for example, in the skin, lungs and gastrointestinal tract) and undergo remarkable transformation following exposure to pathogens. Pathogen-associated molecular patterns and their vertebrate receptors, including Toll-like receptors (TLRs)^{6,7}, influence the dynamics of antigen acquisition, cytoskeletal rearrangements and regulation of MHC biosynthesis, all of which affect both MHC class I and class II antigen processing and presentation^{8,9}. Likewise, the machinery of protein translation and degradation, which is required for generating antigenic peptides for presentation, is carefully regulated following DC activation¹⁰. DC activation by TLR ligands is also required for the formation

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Pathogen-associated molecular patterns

Molecular patterns that are found in pathogens but not in mammalian cells. Examples include terminally mannosylated and polymannosylated compounds, which bind the mannose receptor, and various microbial products, such as bacterial lipopolysaccharides, hypomethylated DNA, flagellin and double-stranded RNA, which bind Toll-like receptors.

Endolysosomal tubules

Highly dynamic subcellular structures that emanate from late endocytic–lysosomal and/or phagolysosomal compartments. They are known to contain MHC class II molecules, CD63, CD82 and lysosome-associated membrane protein 1 (LAMP1), and require microtubules for movement.

Endocytic pathway

A trafficking pathway used by all cells for the internalization of molecules from the plasma membrane to the endolysosomes.

Cross-presentation

The ability of certain antigen-presenting cells to load peptides that are derived from exogenous antigens onto MHC class I molecules. Cross-presentation is important for the initiation of immune responses to viruses that do not infect antigen-presenting cells.

Autophagy

An evolutionarily conserved process in which acidic double-membrane vacuoles, known as autophagosomes, sequester intracellular contents (such as damaged organelles and macromolecules) and target them for degradation, through fusion to lysosomes. This process does not involve direct transport through the endocytic or vacuolar protein sorting pathways.

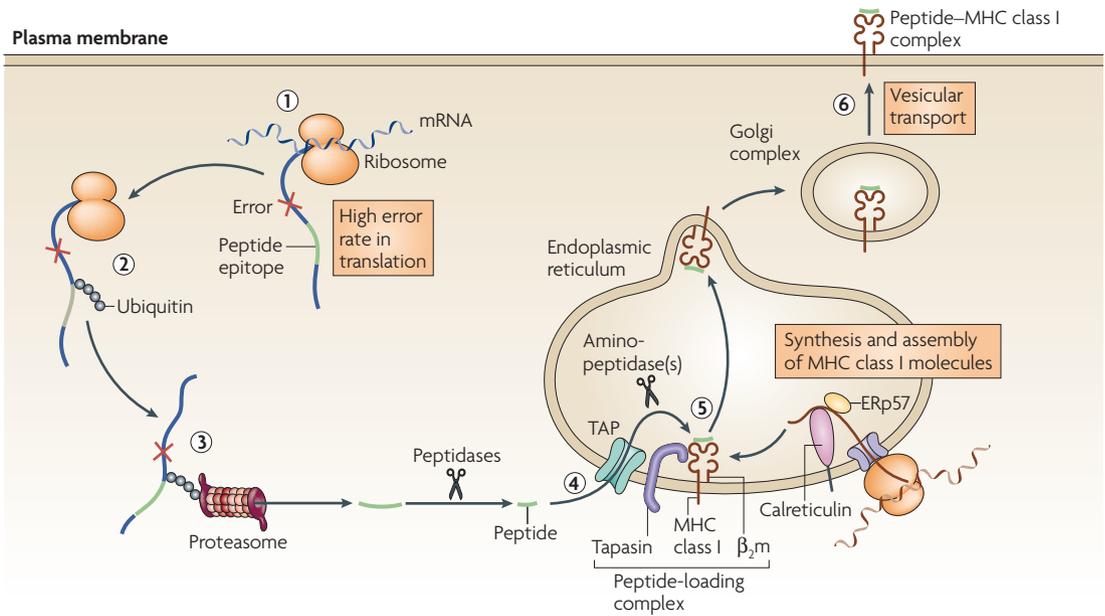


Figure 1 | Six steps for loading and trafficking of MHC class I molecules to the cell surface. Antigen processing and presentation by MHC class I molecules can be divided into six discrete steps. Step 1: acquisition of antigens from proteins with errors (for example, due to premature termination or misincorporation) or misfolded proteins that are tagged with ubiquitin for degradation. Step 2: misfolded proteins are degraded into peptides. Step 3: the proteasome degrades these ubiquitinated proteins into peptides. Step 4: the peptides are delivered to the endoplasmic reticulum (ER) by the transporter associated with antigen processing (TAP) complex. Step 5: peptide is loaded onto nascent MHC class I molecules; this process is facilitated by members of the peptide-loading complex, such as tapasin and two housekeeping ER proteins, known as calreticulin and ERp57. Step 6: peptide-loaded MHC class I molecules are transported via the Golgi complex to the cell surface. The steps for peptide–MHC class II loading conceptually follow this same path. β_2m , β_2 -microglobulin.

of endolysosomal tubules, which contain numerous proteins including MHC class II molecules, and deliver these proteins to the cell surface, where they are available to CD4⁺ T cells for potential activation^{11–13}.

DCs have a central role in the activation of naive T cells and therefore direct the adaptive immune response against invading microorganisms. But how do DCs participate in the immune response to intracellular microorganisms that do not directly infect APCs? First, whole microorganisms can transiently exist in the extracellular space and be taken up by DCs into the endocytic pathway, where relevant antigens are loaded onto MHC class II molecules in endolysosomes. In addition, DCs possess the capacity to take up these antigens and transfer them to the MHC class I pathway through a process referred to as cross-presentation, but the details of this process remain controversial.

But, how do antigens from the extracellular environment gain access to the MHC class I pathway, which is normally restricted to the presentation of intracellular antigens? Here, we review the evidence for the hypotheses that include the involvement of the ER dislocation machinery and channel-independent pathways in this process.

The generation of peptides for both MHC class I and class II pathways have previously been viewed as the exclusive domain of the proteasome and lysosomal-associated proteases, respectively. Recent data indicate that additional pathways can participate in this process. For example, a role for autophagy, which is a ubiquitous process by which cells remove damaged

organelles, in the generation of peptides for MHC molecules has been proposed. The pathways of antigen processing and presentation have recently been extensively reviewed^{4,14} and, therefore, in this Review we focus on aspects of antigen processing and presentation that are less well understood or that remain controversial.

MHC class II processing and presentation

Focused on the extracellular environment, the MHC class II antigen presentation pathway intersects with the endocytic pathway to sample antigens. Extracellular antigen is taken up by APCs and placed into a membrane-delimited compartment, known as the phagosome. This phagosomal compartment undergoes a series of modifications that are in part dictated by its content and finally fuses with lysosomes to form the phagolysosome, in which the contents of the phagosome can interact with MHC class II molecules (FIG. 2). Peptide-loaded MHC class II molecules are then transported to the cell surface where they engage antigen-specific CD4⁺ T cells. Despite the apparent simplicity of this pathway, important questions remain, including the nature of the modifications made to the phagosome, the modes of delivery of MHC class II molecules to the cell surface and the contribution of autophagy to the MHC class II pathway.

Phagosome maturation. Phagocytosis serves as an important mechanism for antigen acquisition and is restricted to professional APCs, which are responsible for the uptake of various particles, including microorganisms

Phagosome

A membrane-delimited compartment that confines ingested material such as microorganisms following phagocytosis. Unless counteracted by a pathogen survival strategy, the phagosome matures into a hostile environment (the phagolysosome) that is designed to kill and digest microorganisms by fusing with lysosomes.

Phagolysosome

An intracellular compartment that results from the fusion of phagosomes, which enclose ingested extracellular material, and lysosomes, which contain lytic enzymes.

and apoptotic bodies¹⁵. These particles are shuttled into phagosomes, which are then subjected to numerous modifications that serve to guide the recruitment of other proteins and the subsequent fusion events with other vesicles¹⁶ (FIG. 2). The formation of phagosomes has been extensively reviewed^{16,17}. Cellular-membrane protein recruitment to the phagosome — and therefore the fate of the phagosome — appears to be regulated in part by the capacity to engage TLRs early in the course of phagosome formation. Indeed, antigen that is complexed with the **TLR4** ligand lipopolysaccharide (LPS) and presented by DCs can activate T cells more potently than antigen alone¹⁸. Both MHC class II molecules and the tetraspanin member CD63 are specifically recruited to pathogen-containing phagosomes but not to phagosomes that contain polystyrene beads¹⁹ (note that latex beads and polystyrene beads are composed of distinct compounds and therefore the terms should not be used interchangeably). In addition, TLR4 signalling by LPS triggers the efficient peptide loading of

MHC class II molecules, whereas phagosomes that are devoid of LPS fail to contribute to the activation of antigen-specific T cells¹⁸. The connection between TLR signalling and the efficiency of MHC class II antigen presentation shows that the use of pathogens to induce phagocytic uptake, rather than the use of polystyrene beads, will inform us on the pathways relevant for antigen processing and presentation.

Given the importance of phagosome maturation in antigen processing, pathogens have devised strategies to modify their fate within the phagosome²⁰. *Mycobacterium tuberculosis*, for example, blocks phagosomal maturation, thereby enabling its own survival within macrophage phagosomes, from which it can then escape into the cytosol²¹. *Legionella pneumophila*, the causative agent of legionnaires' pneumonia, escapes the degradative lysosomal pathway by intercepting vesicular traffic from the ER to form an ER-like compartment that avoids fusion with lysosomes²². *Toxoplasma gondii* can modify the compartment in which it resides to allow long-term growth and prevent its own death by forming a parasitophorous vacuole that avoids fusion with vesicles of the endocytic pathway^{23,24}.

Fusion with lysosomes to form phagolysosomes is the final event in the life of a phagosome. It seems that the intravesicular conditions of lysosomes found in DCs might be different from those in other cell types. For example, DCs can be infected with HIV-1 through the cell-surface receptor DC-specific intracellular adhesion molecule 3-grabbing non-integrin (DC-SIGN), and HIV-1 is internalized by endocytosis in a form that retains the viability of the virus and allows its transfer to T cells, possibly through the immunological synapse^{25,26}. The fact that the otherwise fragile HIV-1 virions remain infectious after endocytosis highlights the innocuous environment of the DC lysosomes.

The degradation of antigen following the lysosomal acidification of endosomal compartments is tightly controlled in DCs in an activation-dependent manner¹⁰. Acidification of this compartment allows optimal activity of lysosomal acid hydrolases and cathepsins. **RAB27**, a RAB protein involved in vesicular docking and secretion, is rapidly recruited to phagosomes in DCs and controls acidification of this compartment by recruiting NADPH oxidase and by limiting the production of reactive oxygen species²⁷. Phosphorylation of extracellular-signal-regulated kinase 1 (ERK1) and ERK2 in DCs is also controlled by the action of the lysosomal cysteine protease cathepsin K, which has recently been shown to regulate **TLR9**-induced signalling, but not that of other TLRs²⁸. The inhibition or absence of cathepsin K in DCs greatly reduced TLR9-induced secretion of pro-inflammatory cytokines, linking cathepsin K activity specifically to the TLR9 signalling cascade and possibly to phagosome maturation. So, some TLR activation appears to be controlled by the enzymatic activity of its proteases in DCs.

The contributions of autophagy to the MHC class II pathway. Much of the emphasis on antigen degradation has been on phagosomal maturation through the endocytic pathway and fusion with lysosomal vesicles.

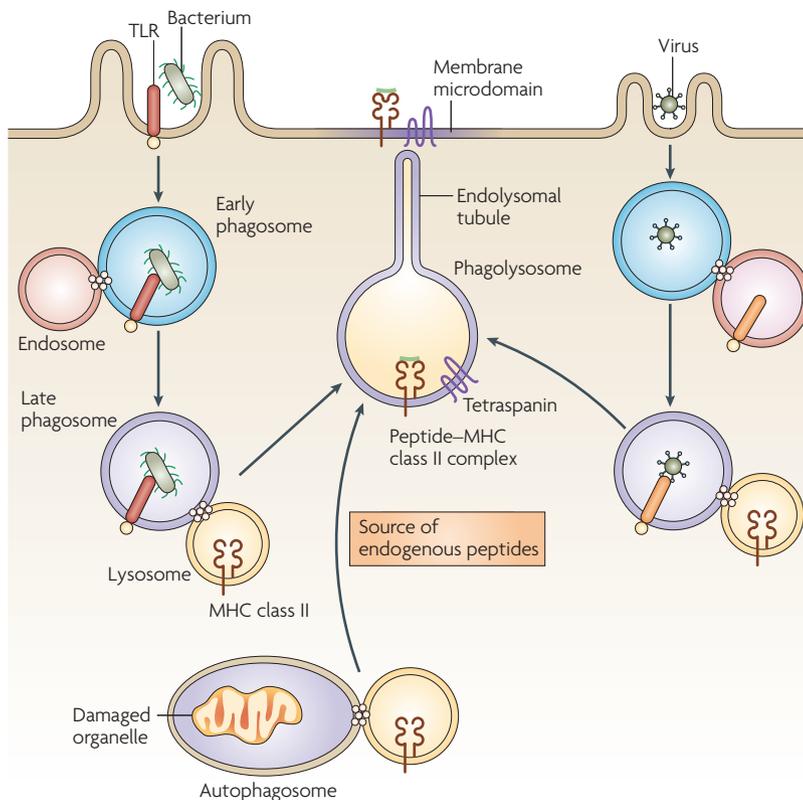


Figure 2 | Contribution of pathogens and self peptides to loading of MHC class II molecules in dendritic cells. Following phagocytosis of bacteria, surface-expressed Toll-like receptors (TLRs) become activated and influence the nature of phagosome maturation. Similarly, viruses engage TLRs found in endocytic vesicles that recognize nucleic acids. Following the maturation of phagosomes, these structures fuse with lysosomes to form phagolysosomes. MHC class II molecules that are contained in the lysosomes are loaded with peptide fragments of the bacteria or viruses that are formed by lysosomal proteases. Autophagosomes also fuse with lysosomes and serve as an additional source of peptides, including endogenous peptides, for MHC class II presentation. MHC class II molecules, as well as many other lysosomal proteins including tetraspanins, are transported in endolysosomal tubules to the cell surface. Surface MHC class II molecules can be found in membrane microdomains with other co-stimulatory proteins.

Tetraspanin

A family of transmembrane proteins that have four transmembrane domains and two extracellular domains of different sizes, which are defined by several conserved amino acids in the transmembrane domains. Their function is not clearly known, but they seem to interact with many other transmembrane proteins and to form large multimeric protein networks.

Immunological synapse

A large junctional structure that is formed at the cell surface between a T cell and an antigen-presenting cell. Important molecules that are involved in T-cell activation — including the T-cell receptor, numerous signal-transduction molecules and molecular adaptors — accumulate in an orderly manner at this site. Immunological synapses are now known to also form between other types of immune cells: for example, between dendritic cells and natural killer cells.

Cathepsins

Proteases that are mostly located in lysosomes and lysosome-like organelles and can be divided into cysteine, aspartate and serine cathepsin subgroups according to their active-site amino acid.

However, additional mechanisms have recently been implicated in antigen processing and presentation. Autophagy has an important role in maintaining cell homeostasis; it provides nutrients during periods of starvation and removes damaged organelles from the cytoplasm. There are at least three different types of autophagy: chaperone-mediated autophagy, microautophagy, and macroautophagy, of which macroautophagy is the best characterized. At least 30 autophagy-related genes (ATGs) contribute to autophagy in yeast, many of which have orthologues in mammalian cells (Supplementary information S1 (table)). Autophagosomal membrane formation and expansion is facilitated by two systems, the ATG8 (known as LC3 in mammals) system and ATG12 system, the details of which have been reviewed elsewhere²⁹. Autophagy is now considered an important process for the delivery of antigens to MHC class II molecules.

Autophagy can target pathogens that reside in the cytosol or within phagosomes for lysosomal degradation and can therefore participate in the effective elimination of viruses, bacteria and parasites^{30–32} (BOX 1). Autophagy might also contribute to several independent stages of antigen presentation, including the uptake of antigens from the cytosol or from phagosomes. The delivery of antigen to the endolysosomal degradation pathway, the loading of MHC class II molecules with endogenous peptides and the generation of functional, self-tolerant effector T cells.

Autophagy and TLRs. The signals that induce autophagy during the immune response have only recently begun to be elucidated. Although professional APCs engage in constitutive autophagy³³, cytokines can modulate this process¹⁴¹. In addition to cytokines, TLR ligands, including LPS and the TLR7 ligands imiquimod and single-stranded RNA, can induce autophagy in macrophages and enhance mycobacterial co-localization with autophagosomes, therefore resulting in the elimination of this pathogen^{34,35}. Several other TLR ligands induce the recruitment of the autophagosomal marker *beclin-1* (also known as BECN1) to the phagosome, followed by the ATG5- and ATG7-dependent recruitment of LC3 (REF. 36). A failure to do so was shown to result in

increased survival of engulfed *Saccharomyces cerevisiae*, further implicating TLR signalling in the induction of autophagy.

Conversely, autophagy also stimulates TLR signalling by delivering viral replication intermediates to TLR7, which is present in the endosomes of plasmacytoid DCs³⁷. As TLR signalling is a well-known maturation stimulus for professional APCs, autophagy can enhance antigen presentation through the delivery of TLR ligands to the endosome for functional maturation of DCs. The interplay between TLR signalling, autophagy and antigen presentation merits further investigation.

Autophagy and the phagosome. Autophagosomal proteins participate in the maturation of phagosomes. In one study, the autophagosomal markers LC3 and beclin-1 were found to translocate to the phagosomal membrane during the early stages of phagocytosis in the presence of a TLR ligand³⁶. However, the translocation of LC3 and beclin-1 to the phagosome was not associated with the formation of double-membrane structures³⁶ and therefore this phenomenon might be distinct from conventional autophagy. A proteomic study of polystyrene-bead-containing phagosomes derived from cultured *Drosophila melanogaster* cells identified ATG9, another autophagosomal marker, as one of 617 phagosomal proteins³⁸. However, other proteomic studies of phagosomes have not identified the presence of autophagosomal proteins on phagosomal membranes^{39–41}, perhaps because their association with the phagosomal membrane is transient³⁶ or because TLRs might not have been suitably engaged.

Autophagosomes also converge with endosomes^{42,43} and deliver exogenous peptides to endolysosomal compartments for loading onto MHC class II molecules³³. However, there is no direct evidence that autophagosomal degradation of pathogens in the endolysosomal compartment results in enhanced MHC class II presentation of the corresponding pathogen-derived peptides and therefore these two events have yet to be directly linked. The exact function of the recruitment of autophagy proteins to phagosomes remains to be determined, but it might affect antigen processing and presentation, and T-cell selection in the thymus (BOX 2).

Autophagy and endogenous antigens. Autophagy can also deliver endogenous antigens to the MHC class II pathway³⁰, as shown by its role in MHC class II presentation of the viral antigen Epstein–Barr virus nuclear antigen 1 (EBNA1) expressed at physiological levels⁴⁴. As judged by its co-localization with the autophagosomal marker monodansylcadaverine, EBNA1 accumulates in autophagosomal structures when lysosomal acidification, and therefore autophagosome maturation, is blocked. In addition, EBNA1 was shown to be presented to MHC class II-restricted EBNA1-specific CD4⁺ T-cell clones. This presentation was abrogated by the inclusion of 3-methyladenine and knockdown of ATG12, both of which inhibit autophagy. These studies demonstrate that cytosolic antigens degraded by autophagy can be delivered as peptides to the MHC class II pathway.

Box 1 | Pathogen interactions with the autophagy machinery

Autophagy can target pathogens that reside in the cytosol or within phagosomes for lysosomal degradation. Therefore, autophagy contributes to the effective elimination of viruses, bacteria and parasites^{30–32}. Autophagy eliminates *Mycobacterium tuberculosis*-containing phagosomes¹¹⁸. Similarly, clearance of pathogens such as *Streptococcus pyogenes*¹²⁹, *Salmonella enterica* serovar *Typhimurium*¹³⁰ and *Toxoplasma gondii*^{125,127} can also occur in an autophagy-dependent manner. Many pathogens strive to escape the autophagy machinery^{131,132}. The herpes simplex virus type 1 neurovirulence protein ICP34.5 antagonizes autophagy by interacting with the autophagy protein beclin-1 (REF. 133). Similarly, IcsB, a type III secretion effector protein from *Shigella flexneri*, allows the bacteria to escape autophagy by competing with the autophagy protein ATG5 for binding to the *S. flexneri* protein VirG¹³⁴. The ability of pathogens to escape autophagy might allow survival within host cells and, therefore, represent a new mechanism for immune evasion. Understanding the interactions between pathogens and the autophagy machinery will clarify the role of autophagy in the immune response.

Box 2 | Autophagy and T-cell selection and survival

RAB

A cytosolic protein that has GTPase activity, which, in the GTP-bound form, associates with membranes. Different RAB proteins associate with different intracellular compartments — for example, RAB5 associates with early endosomes, RAB7 with late endosomes and RAB11 with recycling endosomes.

Chaperone-mediated autophagy

The import and degradation of soluble cytosolic proteins by chaperone-dependent, direct translocation across the lysosomal membrane.

Microautophagy

The uptake and degradation of cytoplasm by invagination of the lysosomal membrane.

Macroautophagy

(Also known as autophagy). The largely non-specific autophagic sequestration of cytoplasm into a double-membrane-delimited compartment (an autophagosome) of non-lysosomal origin. Note that certain proteins, organelles and pathogens might be selectively degraded via macroautophagy.

Lysosomal degradation

The digestion of macromolecules in lysosomal organelles, which are the terminal organelles of degradative pathways, such as phagosomal, endosomal and autophagy pathways.

Endosomes

Membrane-delimited compartments that contain material ingested by endocytosis. Some material will be recycled to the cell surface whereas some cargo will transit to late endosomes and eventually fuse with lysosomes to form endolysosomes. Endosomes may also fuse with phagosomes to allow maturation of the phagosomal compartment.

Central tolerance

Deletion of self-reactive T cells in the thymus.

Peripheral tolerance

Control of self-reactive T cells in the periphery.

Professional antigen-presenting cells (APCs) and interferon- γ (IFN γ)-stimulated epithelial cells show a high level of constitutive autophagy, continuous fusion between autophagosomes and MHC class II-expressing compartments, and efficient delivery of endogenous antigens to these compartments by autophagosomes³³. Of note, the thymic compartment in transgenic mice in which LC3, an autophagosome marker, was tagged with green fluorescent protein shows a high level of constitutive autophagy in thymic epithelial cells, even under nutrient-rich conditions¹³⁵. Medullary thymic epithelial cells, which mirror virtually all tissues in the body by promiscuous expression of tissue-restricted self antigens, have an important role in the induction of central tolerance¹³⁶. These poorly phagocytic cells might use autophagy for MHC class II-restricted antigen presentation for positive and negative selection of T cells. Similarly, the recently described autoimmune regulator (AIRE)⁺ fibroblast reticular-like cells in peripheral lymph nodes might use a similar mechanism to present self antigens to peripheral T cells¹³⁷.

Although not formally shown, in the absence of autophagy, endogenous peptides might not be presented by MHC class II molecules, and result in a failure to tolerize potentially self-reactive CD4⁺ T cells. Defects in autophagy could therefore result in impaired clonal selection and defects in central or peripheral tolerance. Indeed, mutations in the autophagy-related gene *ATG16L1* and the potentially autophagy-inducing *IRGM* (immunity related GTPase family, M) gene (a member of the p47 GTPases), have been associated with the development of autoimmunity in patients with Crohn's disease^{138–140}. How *ATG16L1* or *IRGM* mutations influence autoimmunity is not known, but the possibility of defective presentation of the necessary self peptides to allow maturation of CD4⁺ T cells in the absence of autophagy is a testable hypothesis.

Autophagy influences many steps in the MHC class II pathway and has profound effects on T-cell development, although further work is needed to understand the molecular interactions between the autophagy machinery and known components of the MHC class II pathway.

In addition to viral antigens, MHC class II-restricted antigen presentation of certain self⁴⁵, tumour⁴⁶ and model⁴⁷ antigens depends on autophagy. A substantial number of peptides recovered in a complex with MHC class II molecules originate from proteins that are usually found in the cytosol or the nucleus^{48–51} and the size of the fraction of these peptides was increased following starvation-induced autophagy⁵¹. Therefore, by means of autophagy, the peptide repertoire presented by MHC class II molecules is extended from exogenous antigens to include some endogenous antigens.

Although the above examples all rely on macroautophagy, chaperone-mediated autophagy also contributes to MHC class II-restricted presentation of endogenous antigens⁵². In this case, lysosome-associated membrane protein 2 isoform A (*LAMP2a*), together with heat shock cognate protein 70 (*HSC70*; also known as *HSPA*), can transport a cytoplasmic antigen to MHC class II molecules and contribute to antigen presentation⁵².

How the phagocytic uptake of antigens influences the balance between MHC class II-restricted presentation of endogenous versus exogenous antigens is not fully understood. One study briefly addressed this issue and showed that exogenous antigen does not compete with endogenous antigen for MHC class II-restricted presentation in mature DCs⁵³. The maturation status of APCs might also affect their ability to present endogenous antigens. Autophagy is a constitutive process in both immature and mature human monocyte-derived DCs³³. However, TLR4 and TLR7 stimulation in a macrophage cell line increases autophagy^{34,35}, which suggests that maturation of APCs could enhance MHC class II-restricted antigen presentation of endogenous proteins. Although autophagy is clearly involved in the MHC class II pathway, further work is needed to define the extent by which autophagy influences the peptide–MHC class II repertoire during an immune response to pathogens.

Delivery of MHC class II molecules to the cell surface.

The final step in antigen processing and presentation is the transport of vesicles that contain MHC class II molecules and proteins that are usually found in the immunological synapse from the endolysosomal compartment to the cell surface (FIG. 2). The mechanism of transport to the cell surface, as determined by direct visualization of MHC class II molecules in primary DCs, is thought to involve the transformation of MHC class II-containing compartments into tubular structures that are directed towards the site of T-cell interaction at the plasma membrane^{54,55}. Tubulation of MHC class II-expressing endosomal compartments requires loading of DCs with antigen, maturation of DCs in response to a TLR ligand and T cells specific for the antigen presented by MHC class II molecules. The direction of the MHC class II-containing tubules towards the interacting T cell is proposed to promote the clustering of MHC class II molecules at the site of T-cell contact, and might assist in controlling the formation of the immunological synapse⁵⁴. MHC class II-containing endolysosomal tubules use microtubule-based movement with *RAB7A* and RAB-interacting lysosomal protein (*RILP*), which are involved in the engagement of the necessary motor proteins⁵⁶. The composition of the immunological synapse on the DC side has not been fully identified. Spinophilin, a scaffolding protein of neuronal dendritic spines that regulates synaptic transmission, has been found in DCs and is dynamically directed to contact sites in an antigen-dependent manner⁵⁷. Other proteins involved in the polarized movement of these compartments to the cell surface remain to be identified.

At the plasma membrane, MHC class II molecules cluster in lipid rafts or tetraspanin-rich microdomains⁵⁸. Although incorporation of MHC class II molecules into these microdomains is thought to be functionally important, the exact mechanism by which microdomains participate in effective MHC class II-mediated antigen

presentation remains to be defined. In addition, what controls the incorporation of MHC class II molecules into microdomains is still unclear. The cytoplasmic tail of the MHC class II β -chain is ubiquitylated in mouse immature DCs, which is essential for recycling MHC class II molecules from the cell surface in these cells⁵⁹. The number of ubiquitylated MHC class II molecules in DCs decreases following maturation, resulting in the accumulation of MHC class II molecules at the cell surface. However, no motif for microdomain sorting in the MHC class II molecule has been described to date and the functional importance of this ubiquitylation reaction remains to be established. Analysis of mice in which the endogenous MHC class II β -chain locus is replaced with a mutated version that cannot be ubiquitylated should prove informative.

The translocation of MHC class II molecules from tubular compartments to specialized membrane subdomains (that is, lipid rafts, tetraspanin-rich microdomains and the immunological synapse) following DC maturation suggests a highly controlled and polarized transport mechanism. Given its role in polarized exocytosis, the exocyst complex is an attractive candidate to mediate MHC class II-mediated transport to the plasma membrane. The 743 kD exocyst complex is composed of Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 (REF. 60), and is involved in the docking of secretory vesicles to specific sites at the plasma membrane before soluble-*N*-ethylmaleimide-sensitive-factor accessory-protein receptor (SNARE)-mediated fusion takes place⁶¹. In addition, proteins of the exocyst complex have been found on phagosomes from the *D. melanogaster* S2 cell line³⁸. This expression of the exocyst complex in DCs might also allow for the regulated fusion of this compartment with the plasma membrane following the completion of the peptide-MHC class II-loading reactions, although direct evidence for this role remains to be established.

MHC class I processing and presentation

Antigenic peptides derived from cytosolic proteins intersect the MHC class I biosynthetic pathway in the ER, where the MHC class I heavy chains and β_2 -microglobulin are synthesized. Proteins destined for degradation undergo ubiquitylation and are then processed by the proteasome. In some viral infections, interferon- γ (IFN γ) production leads to the recruitment of distinct proteins to the proteasome to allow increased production of octamer-to-decamer peptides that are suitable for binding to MHC class I molecules. These peptides are transported from the cytoplasm into the ER via the transporter associated with antigen processing (TAP) complex where they associate with nascent MHC class I molecules and β_2 -microglobulin. The trimeric complex of MHC class I heavy chains, β_2 -microglobulin and peptide allow for optimal folding, glycosylation and delivery to the cell surface.

Cross-presentation. Important intracellular changes in non-immune cells, which can be induced by events such as viral infection or malignant transformation, must be reported to the immune system to ensure the induction of a CD8⁺ T-cell response. DCs possess

the unique capacity to stimulate naive T cells and can take up and degrade infected non-immune cells or cell-derived fragments and subsequently deliver the peptide fragments to the MHC class I pathway for display on the cell surface to CD8⁺ T cells⁶². This property is atypical, because most cells exclusively present peptides derived from endogenous proteins on MHC class I molecules. This process of presenting exogenous peptides on MHC class I molecules is known as cross-presentation or cross-priming and was first described by Bevan in 1976 (REFS 63,64). Cross-presentation requires the requisite peptide precursors to gain access to the cytosol for processing by the proteasome, followed by their active transport into the ER where newly assembled MHC class I molecules are found. DCs appear to be uniquely equipped for cross-presentation⁶⁵. Nonetheless, the routes by which exogenous antigens access newly formed MHC class I molecules remain unclear.

A tool that has been used to examine the delivery of exogenous antigens to the cytosol is infected cell protein 47 (ICP47), which is produced by human herpes simplex virus type 1 and is a potent inhibitor of the TAP complex^{66,67}. ICP47 can be reduced to a 35-residue peptide without loss of its inhibitory potency⁶⁷. This ICP47 peptide can freely access the cytosol of a DC after phagocytic uptake, where it can interfere with the cytoplasmic face of the human TAP complex and therefore inhibit peptide translocation into the ER. Exposure of the human DC-like cell line KG-1 to the ICP47 peptide fragment blocked TAP-dependent maturation of the mouse MHC class I molecule H-2K^b, which had previously been introduced into these cells, indicating that extracellular proteins can access the cytosol in DCs, although the subcellular route remains unclear⁶⁸.

The lipid bilayer is not a passive barrier that separates one compartment from another; it actively participates in the translocation of substrates. The transport of peptide antigens across the lipid bilayers can principally occur either in a protein-dependent or protein-independent manner. During protein-dependent transport of peptides across lipid bilayers, there is a precedent for the translocation of proteins from the ER into the cytoplasm for the purpose of degradation. The ER dislocation machinery serves as a quality control complex that is responsible for removing misfolded proteins for degradation. Alternatively, peptides might pass across membranes without the need for dedicated protein channels (see later). In the following sections, we explore the possible role of protein-dependent and protein-independent transport of exogenous proteins across lipid bilayers to gain access to MHC class I molecules for cross-presentation (FIG. 3).

Is the ER dislocon complex involved in cross-presentation?

How do extracellular peptides escape from the phagosome to the cytosol? The delivery of proteins through a membrane pore to the cytosol might use the same protein complex responsible for transporting misfolded proteins out of the ER. The ER dislocon is a complex of an incompletely identified collection of ER-resident proteins, the principal function of which appears to be the ejection of misfolded proteins from

Lipid rafts

Microdomains of the cell membrane that are enriched in sphingolipids. Several membrane-associated signalling molecules are concentrated in these rafts.

Exocytosis

The release of material contained within vesicles by fusion of the vesicles with the plasma membrane.

SNARE

(Soluble-*N*-ethylmaleimide-sensitive-factor accessory-protein receptor). A class of proteins that is required for membrane fusion events that occur in the course of vesicle trafficking and secretion.

TAP complex

A heterodimeric complex composed of transporter associated with antigen processing 1 (TAP1) and TAP2 in the membrane of the endoplasmic reticulum. The complex transports peptides from the cytoplasm to the endoplasmic reticulum, where peptides can be loaded onto MHC class I molecules. Without these peptides, MHC class I molecules are unstable and are much less likely to transit to the cell surface or to remain there.

the ER to the cytosol for degradation. Dislocated proteins are deglycosylated, ubiquitylated and eventually degraded by the proteasome in a pathway known as the ER-associated degradation (ERAD) pathway⁶⁹. Some proteins involved in dislocation are now known (see later), and include several ubiquitin ligases, members of the derlin family, which are the human homologues of the *S. cerevisiae* protein Der1 (degradation in the ER 1), and ATPase p97.

Some data suggest that ER-derived proteins might reside on the phagosomal membrane. Proteomic analysis of the phagosome revealed the presence of several ER-derived proteins, such as the chaperones calnexin, calreticulin, GRP78 (glucose-regulated protein, 78 kD; also known as HSPA5 or BIP) and *SEC61* (REFS 39,70). In addition, Gagnon *et al.* claimed that the ER (or a subcomponent thereof) fused with nascent phagosomes (referred to as the ER–phagosome fusion model)⁷¹. Based on this model, it was proposed that the presence of ER-derived components in phagosomes would allow

the ER dislocon to participate in cross-presentation at the phagosomal membrane by allowing the escape of proteins from the phagosomes into the cytosol^{72–74}. Although not definitive, these data suggest that the ERAD machinery may be involved in translocating peptides from the phagosome to the cytosol.

However, the ER–phagosome fusion model^{70,71} is controversial because potential ER contamination remains a confounding problem, despite rigorous efforts to purify phagosomes⁷⁵. In addition, biochemical experiments that fail to control for post-lysis associations with ER proteins have limited use in understanding the nature of protein–protein interactions. Nonetheless, we cannot consider the question of whether the ER fuses with phagosomes settled without an exact replication of the original report⁷¹. Cell-type differences are also a factor worthy of consideration.

The ERAD protein *SEC61* (also referred to as the translocon) is a protein channel that is responsible for the transport of newly synthesized proteins into

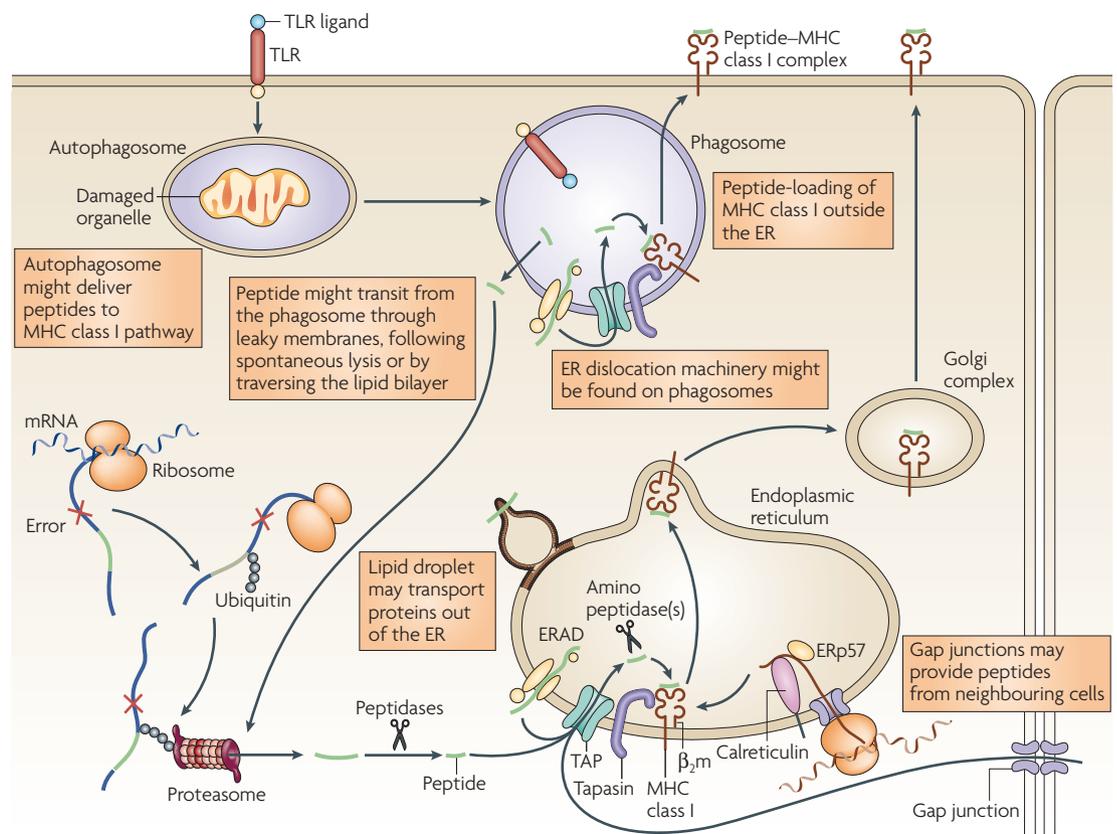


Figure 3 | Additional pathways that might be relevant for antigen processing and presentation by MHC class I molecules in TLR-stimulated dendritic cells. How peptides traffic to MHC class I molecules during cross-presentation in dendritic cells (DCs) is still unknown. Here, we propose several possibilities that could allow peptides to access MHC class I molecules. Autophagosomes containing both endogenous peptides and pathogen-derived proteins could potentially serve as a source of peptides for the MHC class I pathway. Peptides might transit from the phagosome through leaky membranes, following spontaneous lysis of the phagosomal compartment or by traversing the lipid bilayer. Components of the peptide-loading complex and the endoplasmic reticulum (ER) dislocation machinery, including the ER-associated degradation (ERAD) protein complex, might be shuttled from the ER to the phagosome, perhaps involving lipid droplets for transport. Transfer of these components to the phagosome might permit loading of MHC class I molecules with peptides at this site rather than in the ER. Gap junctions may serve as conduits that allow neighbouring cells to donate peptide epitopes for loading of MHC class I molecules. β_2m , β_2 -microglobulin; TAP, transporter associated with antigen presentation.

the ER and also has been proposed to dislocate proteins from the ER to the cytoplasm with the help of other ERAD proteins⁷⁶. In addition, SEC61 has been shown to localize to polystyrene-bead-containing phagosomes in macrophages⁷¹ and, therefore, SEC61 might be involved in the dislocation of proteins from the phagosome into the cytosol that in turn access the cross-presentation pathway. It has been proposed that the *Pseudomonas aeruginosa* exotoxin A inhibits the function of the SEC61 channel and has been used to block SEC61-dependent protein transport across the ER and phagosome membranes^{68,77}. Incubation of cells with ovalbumin in the presence of exotoxin A failed to induce interleukin-2 (IL-2) production by a peptide-specific CD8⁺ T-cell hybridoma, which suggests a role for SEC61 in cross-presentation⁶⁸. However, evidence that exotoxin A binds to SEC61 is indirect and rests on an immunoprecipitation–immunoblotting protocol that does not exclude exotoxin A targets other than SEC61 (REF. 77), including the potent inhibitory effect of exotoxin A on protein synthesis. In fact, there is no evidence for a direct role for SEC61 in the escape of any bacterial toxins from the ER. More recent work suggests that cholera toxin uses ER luminal proteins that participate in quality control, as well as a derlin-1-dependent step for escape from the ER⁷⁸.

The complete molecular composition of the ER dislocon remains to be resolved but several components of the ER dislocation machinery, in addition to SEC61 and ATPase p97, might have a role in the cross-presentation of exogenous antigen. For example, mammalian cells have several ubiquitin E3 ligases that reside in the ER lumen, such as gp78, HRD1 (HMG-CoA reductase degradation 1; also known as *SYVN1*), MARCH VI (membrane-associated ring finger (C3HC4) 6), SCF (SKP1–cullin–F-box), CHIP (C terminus of HSC70-interacting protein), and Parkin (implicated in Parkinson's disease; also known as PARK2), all of which are involved in tagging proteins that are destined for degradation⁶⁹. Other components of the dislocation machinery include the homologue of the yeast protein Hrd3p, SEL1L⁷⁹, signal peptide peptidase (SPP)⁸⁰ and the newly identified UBC6E, AUP1 (U-rich element RNA-binding protein 1) and UBXD8 (REF. 81), but the role of these components in the context of antigen presentation remains to be investigated.

Proteomic studies of polystyrene-bead-containing phagosomes identified only a small number of dislocation components on the phagosomal membrane^{38–41}. However, the nature of the polystyrene beads and the presence or absence of TLR ligands might lead to differential recruitment of the dislocation machinery to the phagosome. The analysis of the composition and distribution of components of the dislocation machinery in professional APCs is in its infancy, and no systematic attempt has been made to link most of these components to cross-presentation.

Even in the absence of ER–phagosome fusion, ER components might participate in the dislocation of proteins from the phagosome. The ER and the

endocytic pathway might be connected by a specific transport pathway that shuttles cargo between them and in this way, components of the ER could redistribute to phagolysosomal compartments following TLR stimulation. In primary immature DCs, TLR3, TLR7, TLR9 and TLR13 move from the ER to endolysosomes⁸² under the control of UNC93B1 in response to stimulation with TLR agonists⁸³. This phenomenon, if extendable to other ER proteins, could allow for the activation-dependent trafficking of the components of the ER dislocon complex to an endosomal or phagosomal compartment. The evidence that the ER dislocon complex contributes to cross-presentation is limited and further experiments are needed to address whether the ER dislocon complex is truly involved and if so, how it arrives at the phagosome.

One possible mode of transport of proteins from the ER to the phagosomes might involve lipid droplets. We have proposed a model whereby lipid droplets might form on the surface of organelles to deliver lipids and other protein cargo to different destinations within the cell, consistent with the previously described idea that lipid bilayers are not passive participants in antigen processing and presentation⁸⁴. In this manner, the lipid droplets could serve as an escape hatch for both proteins and viruses, such as hepatitis C virus⁸⁵. The model does not exclude other protein-based pathways for antigen translocation and almost certainly requires proteins for its formation. Whether the model is relevant for antigen processing and presentation remains to be determined. The presence of ubiquitylated apolipoprotein B, a protein that is usually secreted from the cell, on lipid droplets targets the protein for proteasomal degradation and provides at least one documented example that would support such a role of substrate transport⁸⁶.

Alternatives to membrane channels for cross-presentation.

In addition to ER-derived protein channels, other hypotheses of how peptides and/or proteins travel from the phagosome to the cytoplasm for cross-presentation exist. The delivery of hydrophilic substrates across membranes typically requires protein channels, but this is not universally true. Here, we present three models by which peptides might traverse the lipid bilayer and gain access to another subcellular compartment without the need for a conventional energy-dependent protein channel.

The first model proposes that proteins might 'leak' from the phagosomes into the cytosol. The HIV-1 Tat protein is an example of a polypeptide that can spontaneously traverse a lipid bilayer⁸⁷. A decamer of the HIV-1 Tat protein (⁴⁷YGRKKRRQRR⁵⁷) can translocate itself — and cargo of various sizes appended to it — across the membrane in an energy-independent manner. But how can a highly charged peptide traverse a largely hydrophobic boundary? Models to explain this transport suggest that charged residues of the Tat decamer interact with the phosphate groups on either side of the membrane, and that the peptide might form small pores (~3 nm in diameter) to allow additional peptides to pass through the membrane⁸⁷. Other endogenous peptides might have similar features, negating the need for a

dedicated peptide transporter in the plasma membrane. The penetration of non-enveloped viruses⁸⁸ or the action of certain microbial peptides⁸⁹ and proteins⁹⁰ could also affect host membranes, such that the permeability barrier for proteins is compromised or lost completely. The effects of such pathogen-derived products could also be limited to certain intracellular compartments, such as the endosomes or the ER (as reported for listeriolysin⁹¹ and papova virus, respectively^{92,93}). Therefore, peptide antigens might simply leak out of phagosomes and thereby gain access to the cytoplasm.

A second possibility for peptide escape into the cytosol is the rupture of the phagolysosomal membrane. Peptides might thereby be released from the phagolysosome and directly interact with the cytoplasmic processing machinery. Support for this hypothesis comes from studies of *Cryptococcus neoformans*; phagosomes that contain *C. neoformans* lose membrane integrity, which relates in part to the *de novo* synthesis of its polysaccharide capsule following phagocytosis⁹⁴. *C. neoformans* slips out of these compartments and is extruded from the cell, leaving the APC intact and the fungal organism viable^{95,96}. Equally interesting is the proposal that *M. tuberculosis* might not remain confined to the endocytic structures in which it initially resides by escaping from the phagolysosome into the cytosol²¹. Both the *C. neoformans*-containing and the *M. tuberculosis*-containing compartments, if not perfectly sealed while the microorganisms are in transit, might release other phagosomal content into the cytoplasm.

In addition, peptides may travel out of the phagolysosome as a result of rupture of this compartment. The notion of the lysosome as a 'suicide bag' could lead to the assumption that the release of lysosomal contents into the intracellular milieu leads to cell death⁹⁷. Because only a few hundred protons are sufficient to maintain the topical lysosomal pH in these modestly sized organelles, the rupture of such a compartment might not be as disastrous as it would seem at first glance⁹⁸; the appropriate cytoplasmic pH can be rapidly restored. Indeed, osmotic lysis of pinosomes is an effective means of introducing endocytosed soluble materials into the cytoplasm for presentation by MHC class I molecules⁹⁹ and could mimic normal disruption as it might occur in the phagolysosomal compartment.

Lysosomal membrane stability can be regulated by host proteins, such as sphingosine, which affects lysosomal membrane permeability in a dose-dependent manner¹⁰⁰. Other host proteins such as apolipoprotein L-1 and serpin 6 (SRP6), which regulates proteolytic activity of lysosomal enzymes, can also perturb the lysosomal membrane¹⁰¹. In fact, hypotonic shock of *Caenorhabditis elegans* lacking SRP6 results in cell necrosis by release of lysosomal contents¹⁰¹. The extent to which these changes might occur in a cell-type specific manner (for example in DCs) is not known. Nonetheless, such perturbations might allow peptides to gain access to the cytosol and therefore to the peptide-loading machinery for MHC class I molecules.

A third possibility is that MHC class I molecules might be loaded with peptide outside the ER. The biosynthetic pathway of MHC class I molecules might

intersect with the phagosome, allowing its loading with peptide in the endolysosomal compartments themselves. Another pathway might allow internalized antigens to be exported to the cytosol and processed by the proteasome before translocation back into the phagosomes in a TAP-dependent manner^{73,74}. In addition, surface MHC class I molecules internalized during phagocytic uptake of cargo might end up in phagolysosomal compartments (FIG. 3).

The idea that MHC class I molecules might traffic to different compartments for peptide loading is not new¹⁰². MHC class I molecules might visit phagolysosomal compartments to acquire peptides prior to their surface display^{103–105}. The conserved tyrosine residue in the cytoplasmic tail of the heavy chains of MHC class I molecules is important for the internalization of this complex and might assist in trafficking MHC class I molecules into the phagolysosomal compartment¹⁰⁶. MHC class I molecules that lack this tyrosine residue were found to be inferior in their capacity to present antigens to CD8⁺ T cells during viral infections¹⁰⁶. However, presentation of endogenous antigens to CD8⁺ T cells was unaffected. In this model, it is proposed that internalization of MHC class I molecules is therefore a prerequisite for cross-presentation¹⁰⁶.

Alternative sources of cytosolic peptides. As described above, most peptides for MHC class I presentation are thought to be derived from proteasomal breakdown of cytosolic proteins. However, emerging data suggest that this proteasome-mediated process might not be the exclusive provider of such antigens; both intercellular protein channels and autophagy might have a role.

Gap junctions are intercellular channels that connect the cytoplasm of two adjacent cells by generating a functional channel, composed of six connexins on each side¹⁰⁷, and might facilitate intercellular transport of peptides. Although the role of these channels in the translocation of peptides into the cytosol for presentation by the MHC class I pathway is intriguing, to date the data that support this mechanism are limited¹⁰⁸. Gap junctions might transport peptides from infected cells into neighbouring cells in an effort by the immune system to inflict damage to adjacent cells and so limit the spread of a viral infection¹⁰⁹ (FIG. 3). This proposed mechanism for the prevention of viral dissemination is therefore crucially dependent on adequate levels of peptide–MHC complexes and antigen-specific CD8⁺ T cells. A single peptide–MHC complex will suffice to induce the cytotoxic activity of CD8⁺ T cells and so the criterion might easily be met¹¹⁰. The availability of gene-targeted mice with defects in gap junctions is likely to clarify their involvement in antigen presentation.

The role, if any, of autophagy in MHC class I-restricted antigen presentation remains to be determined. Autophagy clears ubiquitylated cytoplasmic protein aggregates¹¹¹. Indeed, in hepatocytes and neurons of mice deficient for ATG5 or ATG7, such cytoplasmic protein aggregates accumulate in the cytoplasm^{112–114}. DC aggresome-like structures (DALIS) contain poly-ubiquitylated proteins, and peptides derived from

Sphingosine

An amino alcohol that can be linked to a fatty acid via the amino group to form the basic structure of sphingolipids.

Necrosis

A form of cell death that results from a decline in cellular ATP or ADP levels below the concentration that is required to maintain cellular organization and integrity.

Aggresome

Proteinaceous inclusion body that forms when the cellular degradation machinery is impaired or overwhelmed and that leads to an accumulation of protein for disposal.

Unfolded-protein response

A response that increases the ability of the endoplasmic reticulum to fold and translocate proteins, decreases the synthesis of proteins, and causes the arrest of the cell cycle and apoptosis.

DALIS can be presented by MHC class I molecules¹¹⁵. Aggresome-like structures (ALIS), which are similar to DALIS, are observed in many immune and non-immune cells in response to stress, in which they serve as substrates for autophagy¹¹⁶. Therefore, one might speculate that autophagy-mediated clearance of DALIS could serve as a source for MHC class I-restricted peptides.

H2-M3^a (histocompatibility 2, M region locus 3), a member of the non-polymorphic MHC class Ib family, presents *N*-formylated peptides to CD8⁺ T cells¹¹⁷. These peptides are present only in mitochondria and in prokaryotes, such as *Listeria monocytogenes* and *M. tuberculosis*. Given that autophagy is implicated in the clearance of these organisms^{117,118} and in the clearance of mitochondria, the H2-M3^a molecule might acquire its peptides by an autophagy-dependent process.

Presentation of DALIS, ubiquitylated proteins or *N*-formylated peptides requires autophagosomal degradation products to access the peptide-MHC class I-loading complex. The autophagosomal membrane has been proposed to originate from the ER^{119,120}. In addition, during ER stress and the unfolded-protein response, autophagy is induced and consumes portions of the ER, serving as an alternative to ER-associated degradation of proteins^{119,121-123}. The peptide-MHC class I-loading complex and/or dislocation machinery might therefore be present on the autophagosome. Indeed, *Brucella abortus*, *L. pneumophila*, and *Porphyromonas gingivalis* can be found in autophagosomes containing ER proteins, including SEC61 (REF. 124). Alternatively, peptides might 'escape' from autophagosomes and be transported into the ER in a TAP-dependent manner.

Although a role for autophagy in MHC class I presentation seems plausible, treatment with wortmannin or 3-methyladenine, which inhibit autophagy by inhibiting phosphoinositide 3-kinase activity, and knockdown of ATG12 failed to affect MHC class I-restricted presentation of an endogenous antigen by Epstein-Barr-virus-transformed B cells^{44,47}. In addition, co-localization between MHC class I molecules and green-fluorescent-protein-labelled LC3 was not observed in epithelial cells³³. Although B cells and epithelial cells express MHC class I molecules, their behaviour and regulation might differ from DCs. In both immature and mature DCs, the MHC class I-restricted presentation

of a peptide derived from influenza matrix protein 1 (MP1) fused to LC3 (to direct the peptide-MHC class I complex to autophagosomes) remains unaltered when compared with unfused cytosolic MP1 (REF. 33). However, the cytosolic MP1 is presented very efficiently and almost saturates the MHC class I pathway. These features might mask any contribution of the autophagosomal MP1-LC3 complex to cross-presentation.

Several invasive bacteria and parasites that can be cleared through autophagy induce a CD8⁺ T-cell response. *T. gondii* is cleared after invasion of macrophages through autophagy in a process that requires either IFN γ -inducible p47 GTPases and disruption of the parasitophorous vacuole that encloses the parasite, or CD40 ligation¹²⁵⁻¹²⁷. Protective immunity from this organism requires the induction of a CD8⁺ T-cell response, and therefore cross-presentation is essential¹²⁸. But how do peptides derived from *T. gondii* gain access to the peptide-MHC class I-loading complex? Perhaps, the fusion of ER membrane with the autophagosome in *T. gondii*-infected cells brings the MHC class I-loading complex into close contact with peptides derived from the parasitophorous vacuoles. However, the net contribution of autophagy to the generation of most of the peptide-MHC class I complexes found on the surface of an infected cell remains to be determined.

Concluding remarks

The rules that govern antigen processing and presentation in DCs are complex. Numerous important cellular changes take place following exposure of DCs to TLR ligands. Although the pathways of cross-presentation, autophagy and ER dislocation have been established as important for antigen processing and presentation, many questions remain unanswered. Future work that elucidates the molecular details of these pathways in DCs will need to relate this information to what is known to occur following TLR stimulation. The confirmed importance of TLR signals for antigen processing and presentation requires us to focus on the interaction of whole microorganisms and their products with DCs to obtain insight on the pathways used by antigens to gain access to MHC molecules. It is likely that the observations summarized in this Review are just the tip of the iceberg.

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 ATG8 | ATG12 | beclin-1 | LAMP2a | RAB27 | RAB7A | RILP | SEC61 | TAP | TLR4 | TLR7 | TLR9

FURTHER INFORMATION

Hide L. Ploegh's website: <http://www.wi.mit.edu/research/faculty/ploegh.html>
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