The known unknowns of antigen processing and presentation

Jatin M. Vyas[*‡§], Annemarthe G. Van der Veen*[*] and Hidde L. Ploegh*[*||]*

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 cells[4]. 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 cells[4]. 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 groove[5]. 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 activation[10]. DC activation by TLR ligands is also required for the formation

*Whitehead Institute for Biomedical Research, Cambridge, Massachusetts 02142, USA.
‡Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115, USA.
§Division of Infectious Diseases, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts 02114, USA.
||Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.
Correspondence to H.L.P.
e-mail: ploegh@wi.mit.edu
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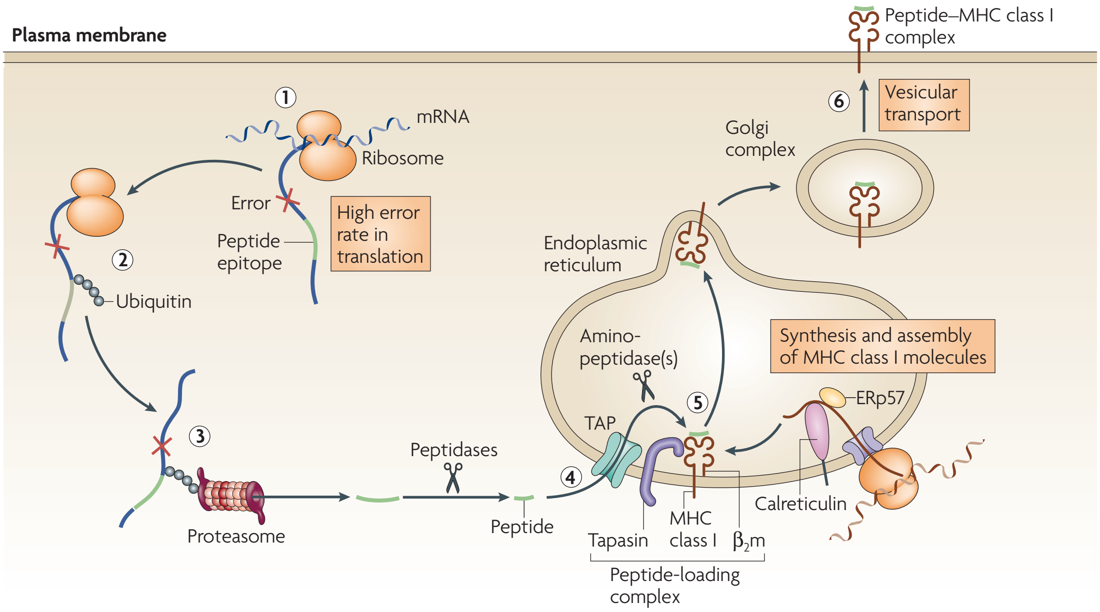

Figure 1 | **Six steps for loading and trafficking of MHC class I molecules to the cell surface.** Antigen processing and presentation by MHC class I molecules can be divided into six discrete steps. Step 1: acquisition of antigens from proteins with errors (for example, due to premature termination or misincorporation). Step 2: misfolded proteins are tagged with ubiquitin for degradation. Step 3: the proteasome degrades these ubiquitylated 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

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 eminate 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.

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 bodies[15]. 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 vesicles[16] (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 alone[18]. Both MHC class II molecules and the tetraspanin member CD63 are specifically recruited to pathogen-containing phagosomes but not to phagosomes that contain polystyrene beads[19] (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 cells[18]. 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.

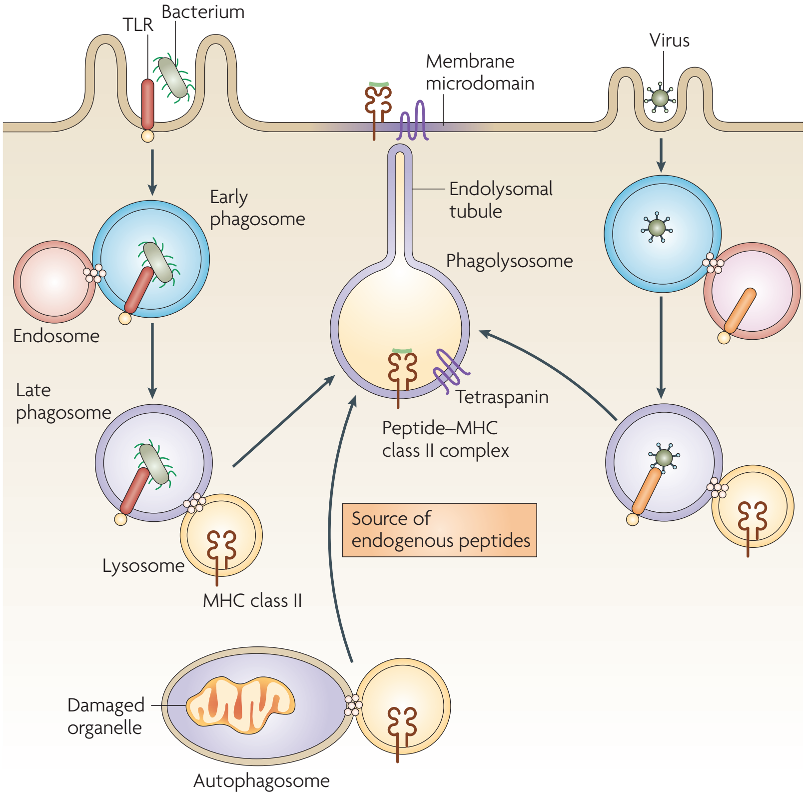

Figure 2 | **Contribution of pathogens and self peptides to loading of MHC class II molecules in dendritic cells.** Following phagocytosis of bacteria, surface-expressed Toll-like receptors (TLRs) become activated and influence the nature of phagosome maturation. Similarly, viruses engage TLRs found 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 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.

Given the importance of phagosome maturation in antigen processing, pathogens have devised strategies to modify their fate within the phagosome[20]. *Mycobacterium tuberculosis*, for example, blocks phagosomal maturation, thereby enabling its own survival within macrophage phagosomes, from which it can then escape into the cytosol[21]. *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 lysosomes[22]. *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 manner[10]. 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 species[27]. 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 TLRs[28]. 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.

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 elsewhere[29]. 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 autophagy[33], cytokines can modulate this process[141]. 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.

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 phagosomes[118]. 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 VirG[134].
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.

Conversely, autophagy also stimulates TLR signalling by delivering viral replication intermediates to TLR7, which is present in the endosomes of plasmacytoid DCs[37]. 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 ligand[36]. However, the translocation of LC3 and beclin-1 to the phagosome was not associated with the formation of double-membrane structures[36] 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 proteins[38]. 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 transient[36] 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 molecules[33]. 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 pathway[30], as shown by its role in MHC class II presentation of the viral antigen Epstein–Barr virus nuclear antigen 1 (EBNA1) expressed at physiological levels[44]. 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 2 | Autophagy and T-cell selection and survival

Professional antigen-presenting cells (APCs) and interferon-γ (IFNγ)-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 autophagosomes[33]. 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 conditions[135]. 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 tolerance[136]. 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 cells[137].

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.

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.

In addition to viral antigens, MHC class II-restricted antigen presentation of certain self[45], tumour[46] and model[47] 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 autophagy[51]. 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 antigens[52]. 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 presentation[52].

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 DCs[53]. 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 DCs[33]. 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 synapse[54]. 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 proteins[56]. 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 manner[57]. 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 microdomains[58]. 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 cells[59]. 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 place[61]. In addition, proteins of the exocyst complex have been found on phagosomes from the *D. melanogaster* S2 cell line[38]. 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.

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.

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 (IFNγ) 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 cells[62]. 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-presentation[65]. 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 potency[67]. 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 unclear[68].

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