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Involvement of autophagy in MHC class I antigen presentation

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Abstract

MHC class I molecules on the cellular surface display peptides that either derive from endogenous proteins (self or viral), or from endocytosis of molecules, dying cells or pathogens. The conventional antigen-processing pathway for MHC class I presentation depends on proteasome-mediated degradation of the protein followed by transporter associated with antigen-processing (TAP)-mediated transport of the generated peptides into the endoplasmic reticulum (ER). Here, peptides are loaded onto MHC I molecules before transportation to the cell surface. However, several alternative mechanisms have emerged. These include TAP-independent mechanisms, the vacuolar pathway and involvement of autophagy. Autophagy is a cell intrinsic recycling system. It also functions as a defence mechanism that removes pathogens and damaged endocytic compartments from the cytosol. Therefore, it appears likely that autophagy would intersect with the MHC class I presentation pathway to alarm $CD8^+$ T cells of an ongoing intracellular infection. However, the importance of autophagy as a source of antigen for presentation on MHC I molecules remains to be defined. Here, original research papers which suggest involvement of autophagy in MHC I antigen presentation are reviewed. The antigens are from herpesvirus, cytomegalovirus and chlamydia. The studies point towards autophagy as important in MHC class I presentation of endogenous proteins during conditions of immune evasion. Because autophagy is a regulated process which is induced upon activation of, for example, pattern recognition receptors (PRRs), it will be crucial to use relevant stimulatory conditions together with primary cells when aiming to confirm the importance of autophagy in MHC class I antigen presentation in future studies.

1 | INTRODUCTION

Work in the 1970s laid the foundation for discovery of pathways underlying MHC class I antigen presentation.[1-4] The pathways and involved molecules have been studied extensively since, yet, novel molecular mechanisms are still discovered.[5-7] Macroautophagy (hereafter autophagy) is an evolutionary conserved pathway which via receptors, specifically, can remove harmful material from the cytoplasm, for example, damaged organelles, viruses and bacteria into double-membrane vesicles termed autophagosomes.[8-12] Autophagy as a source of antigen has been studied mostly for MHC class II antigen presentation in antigen-presenting cells (APCs), and to some extent in thymic epithelial cells, but less for MHC class I antigen presentation.[13-15] Autophagy may provide cytoplasmic and nuclear antigens for MHC class II

antigen presentation by fusion of the autophagosome with the MIIC compartment (MHC class II containing compartment) in which antigens, also those derived from endocytosis, are loaded onto MHC class II molecules.[15-19] In this paper, original research articles which suggest involvement of autophagy in MHC class I antigen presentation will be reviewed. Some studies report that autophagy is not of importance [17,19,20]; others that autophagy promotes an antigen-specific $CD8^+$ T-cell response [21-25]; yet, others have shown that autophagy can reduce the antigen-specific $CD8^+$ T-cell response.[26] These differences might be explained by the use of different model systems, but might also reflect differences between cell types as well as differences for various pathogenic species. Autophagy is an important defence mechanism and culminates with autophagosome fusion to lysosomes, alternatively via endocytic compartments before degradation of the cargo in lysosomes.[27-30] However, many bacteria and viruses have developed strategies to evade the end station of the process, that is, complete degradation. Thus, it appears likely that autophagy intersects with the MHC class I presentation pathway to alert $CD8^+$ T cells of an ongoing intracellular infection. Nevertheless, further studies are needed to define the mechanisms and the importance of autophagy in MHC class I antigen presentation.

2 | SUMMARY OF CONVENTIONAL MHC CLASS I ANTIGEN PRESENTATION

All nucleated cells express MHC class I molecules and can present peptides generated from proteins that are synthesized by the cell itself. This is called *direct* antigen presentation. The proteins derive from endogenous genes and, upon infection, from viral genes. In *classical/conventional* MHC class I antigen presentation, proteins are degraded into peptides by the proteasome, a multi-subunit enzyme complex present in the cytosol and the nucleus.[31] The proteasome exists in isoforms or subtypes designated constitutive, intermediate, thymoproteasome and immunoproteasome. Immune cells and cells exposed to pro-inflammatory cytokines such as interferon gamma (IFNγ) express high levels of the immunoproteasome.[31-33] This subtype shows a higher propensity than the others to generate peptides with C-terminal hydrophobic residues that fit in the cleft of the MHC class I molecule.[34-36] The peptides are further trimmed by peptidases, such as tripeptidyl peptidase II, in the cytosol.[37] Peptides that are not destructed may be translocated into the endoplasmic reticulum (ER) by the transporter associated with antigen-processing (TAP), which is integrated in the ER membrane. In the ER lumen, the peptide loading complex consisting of TAP, the MHC I heterodimer, two chaperones (calreticulin and tapasin), and a disulphide isomerase, is essential for loading of peptides onto MHC class I molecules. The chaperone tapasin edits peptide binding, and peptides which are too long for binding to MHC I, can be trimmed in the ER to a minimal length of 8 amino acids by an ER aminopeptidase (ERAAP).[38,39] Once a peptide with sufficient affinity is captured, the MHC I-peptide complex is transported from the ER via vesicles to the cell surface for presentation to $CD8^+$ T cells. In normal, healthy cells, the MHC class I molecules present self-peptides, and the $CD8^+$ T cells are normally tolerant. In contrast, in infected cells and in cancer cells, foreign and mutant sequences are expressed, respectively, and presentation of such peptides by MHC I class molecules will stimulate $CD8^+$ T cells.

Professional APCs are active phagocytic cells, and in particular, dendritic cells (DCs) have been extensively investigated for their ability to endocytose and process material from the extracellular milieu and present peptides on MHC class I molecules. The pathway is denoted cross-presentation and plays a key role in cancer immunosurveillance, as well as in immune responses against infections and transplants.[2,6,40-42] Two main pathways for antigen-processing and presentation on MHC class I molecules have been suggested for cross-presentation, that is the phagosomal-to-cytosol pathway and the vacuolar pathway.[6,7,43] In the phagosome-to-cytosol pathway, peptides generated in endocytic compartments are transported into the cytosol and further trimmed by the proteasome.[44,45] The next steps include translocation of peptides into the ER where peptides are loaded onto MHC class I molecules as in the pathway for direct antigen presentation. Alternatively, peptides generated by the proteasome are transported back into MHC I-containing endocytic compartments, further trimmed and loaded onto MHC class I molecules.[6,7,43] In contrast, the vacuolar pathway does not include translocation of antigen into the cytosol. Instead, processing of the internalized material and loading of peptides onto MHC class I molecules occurs inside an endocytic compartment, the phagosome.[43,46,47] Proteases such as cathepsin S and, as reported more recently, the proteasome may cleave the ingested material into peptides inside the phagosome.[46,48] Of note, APCs can present peptides on MHC I molecules both via direct antigen presentation and cross-presentation.

3 | AUTOPHAGY AND ATG PROTEIN-MEDIATED PROCESSES, A BRIEF INTRODUCTION

3.1 | Autophagy

The term autophagy (Greek for 'self-eating') was introduced in 1963 to describe the electron microscopy-based

observations of single- or double-membrane vesicles that contained parts of the cytoplasm including organelles in various states of disintegration.[49-52] Autophagy is defined as delivery of cytoplasmic cargo to the lysosome for degradation and can be divided in at least three distinct forms: macroautophagy, chaperone-mediated autophagy and microautophagy.[11] Macroautophagy depends on autophagy-related (ATG) proteins and encloses cytoplasmic cargo into a double-membrane vesicle termed autophagosome.[11,53,54] This process might be non-selective, that is, bulk cytoplasm is sequestered into autophagosomes; or selective, that is, cytoplasmic constituents are selectively sorted into autophagosomes by autophagy receptors.[11,12,55] Cytoplasmic constituents which are removed by selective autophagy include misfolded and aggregated proteins, ribosomes, organelles, and microorganisms, and the process is named after the cargo being eliminated.[11] For example, aggrephagy removes protein aggregates from the cytosol in a process which depends on an autophagy receptor, such as sequestosome-1 (SQSTM1/p62).[56-59] Xenophagy ('xeno' meaning 'other' or 'foreign') is autophagy-mediated, specific sequestering of pathogens from infected host cells.[60,61] Bacterial xenophagy removes bacteria by elimination of bacteria-containing, damaged phagosomes and bacteria that have escaped the phagosomal compartment.[60] Viral xenophagy can target fully formed cytoplasmic virions and viral components from the cytosol.[62,63] Xenophagy represents a first line of defence against infections. But to protect themselves from degradation, many pathogens have developed strategies to evade or block the xenophagic response by, for example, inhibiting the transition from autophagosome to autolysosome.[60,64]

The molecular mechanisms of macroautophagy, hereafter referred to as autophagy, have been described in many excellent reviews, for example,[11,53,54,65] and only a brief introduction aiming to give a background for understanding of this review will be included here. Autophagosome formation is a tightly regulated process and includes multiple protein complexes and various post-translational modifications.[30,65,66] A master regulator of initiation is the so-called UNC51-like kinase (ULK) complex, which is translocated to a membrane source at the ER.[65] Phosphorylation by the ULK complex leads to recruitment of class III phosphatidylinositol complex I (PIK3C3-C1) encompassing vacuolar protein sorting 34 (VPS34), p150, BECN1, NRFB2, AMBRA and ATG14L. This together with the production of phosphatidylinositol 3-phosphate supports the formation of the initial membrane structure of the autophagosome, designated phagophore or isolation membrane. Several cellular sites have been implicated as a membrane source for the autophagosome, and transported lipids might be transferred via ATG2 to the expanding phagophore. After activation of ATG8/LC3 (microtubule-associated protein light chain 3) by ATG7, a complex consisting of ATG5, ATG12 and ATG16L1 facilitates the transfer of LC3 to phosphatidylethanolamine (PE) (LC3-II), which inserts LC3 into the autophagosomal membrane. LC3 is a key molecule for selective autophagy as autophagy receptors such as SQSTM1, NBR1, NDP52, TAX1BP1 and OPTN typically interact with LC3 via a motif designated LC3 interacting region (LIR).[12,59,67] In addition, the receptors contain ubiquitin-binding domains and can thereby bind ubiquitinated cytoplasmic material and sort such cargo into the autophagosome by simultaneous interaction with LC3.[12,59,68] LC3 is also important for membrane expansion, whereas members of the same protein family (ATG8) likely facilitate closure of the autophagosome and in the end, fusion of the autophagosome with lysosome.[59,65,69,70] Finally, cargo as well as the inner membrane is degraded, before end products are released into the cytosol for reuse. The rate of the complete process of autophagy which encompasses the inclusion of cargo into the forming autophagosomes, and in the end fusion with lysosomes for degradation of the content, is referred to as autophagic flux.[71,72] Most schematic drawings depict autophagosomes fusing with lysosomes only. However, studies using mammalian cells suggest that the autophagic pathway intersects with the endocytic pathway at an earlier stage, and that there are multiple entry sites for endocytic vesicles during autophagosome maturation.[27,29,30] For example, by use of electron microscopy and endocytic tracers, it has been shown that autophagosomes fuse with early endosomes and late endosomes to form a vacuole known as amphisome.[27,73-75] The intersection with endosomes enables delivery of MHC I molecules to the autophagosome.[76] Upon fusion with lysosomes, the amphisomes mature into autolysosomes containing a single membrane layer and lysosomal enzymes (Figure 1).

Although not being the main topic of this review, several studies have shown that autophagy within antigen-containing, dying cells can promote $CD8^+$ T-cell responses in a process depending on cross-presentation.[77-80] The studies point towards autophagosomes as possible carriers of antigen. Uhl et al showed that autophagy within influenza-infected Bax/Bak$^{-/-}$ mouse embryonic fibroblasts facilitated cross-priming of virus-specific $CD8^+$ T cells and proposed 'macroautophagy as the dominant means of packaging antigen for cross-presentation'.[77] Similar findings were reported in 2008 by Li et al, who observed enhanced $CD8^+$ T-cell stimulation upon loading of DCs with enriched, cell-derived, antigen-containing $LC3^+$ autophagosomes.[78] Subsequently, it was shown that antigen in autophagosome enriched samples were more efficiently cross-presented than antigen from tumour cell lysates, and that injection of APCs loaded with enriched autophagosomes partially protected against tumour in mouse models of cancer.[79,80]

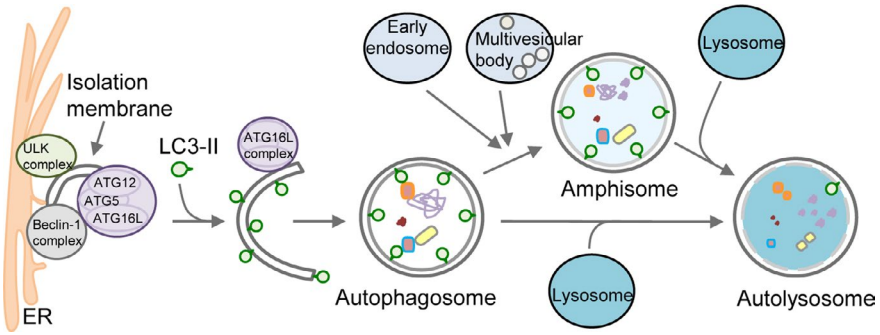

FIGURE 1 Schematic drawing of autophagosome formation and intersection with the endocytic pathway. Autophagy is initiated by recruitment of the UNC51-like kinase (ULK) complex to a membrane source at the ER. Next, the Beclin-1 (BECN1)-containing complex is important for formation of the initiating membrane/isolation membrane (phagophore) of the autophagosome. A complex of ATG5, ATG12 and ATG16L1 promotes ligation of ATG8 proteins such as LC3 to phosphatidylethanolamine (PE) (LC3-II) so that it becomes integrated in the autophagic membrane. Lipids are transported to the expanding membrane of the forming autophagosome, and several cellular sites have been implicated as a membrane source. Endosomal compartments are essential for autophagosome maturation and autophagic flux. Autophagosomes may fuse with lysosomes and form autolysosomes, or fuse with early endosomes and multivesicular bodies, and form amphisomes. Autophagy culminates with fusion of autophagosomes or amphisomes to lysosomes, degradation of the inner membrane as well as the cargo, and release of the end products into the cytosol for reuse

3.2 | ATG protein-mediated processes

Most molecular components of the autophagy machinery can also mediate autophagy-independent functions, such as protein release, Golgi apparatus to ER transport, and endocytosis including LC3-associated phagocytosis (LAP).[81,82] Such processes, in particular ATG-mediated protein release and LAP, are important to rule out when aiming to identify the involvement of autophagy in direct MHC class I antigen presentation. LAP is important for clearance of dead cells and pathogens and shares several ATG components with autophagy. After formation of the single membrane phagosome upon receptor activation, LC3 may become conjugated to the membrane.[81-84] VPS34, UVRAG, Beclin-1 and the conjugation systems ATG7-ATG3 and ATG12-ATG5-ATG16L are shared between autophagy and LAP, but the two pathways can be separated by the requirement of VPS15, ATG14, and AMBRA-1 (autophagy), and Rubicon (LAP).[85,86] Autophagy receptors such as SQSTM1 and NDP52 appear not to be involved in LAP. LAP has been studied mostly in macrophages and to some extent in DCs.

Protein release mediated via ATG proteins, denoted secretory autophagy or ATG gene-dependent secretion, includes exocytosis of lysosomes and secretory granules, and secretion of proteins lacking an N-terminal signal peptide (unconventional protein secretion).[81,82] The mechanisms are only beginning to be understood, and different molecular pathways for ATG gene-dependent secretion appear to exist. A double-membrane autophagosome might be formed, but it is still unclear how secreted molecules are transported to the cell surface and whether other vesicles such as the multivesicular body are involved. LC3, ATG5, ATG7 and ATG12 have been reported to be important.[11,82,87] Most studies on secretory autophagy in immune cells have focused on secretion of IL-1β and to a lesser extent on other cytokines.[88] Different pathways, ATG gene-dependent as well as independent pathways, have been reported for IL-1β secretion, and further studies are needed to identify the molecular mechanisms.[81,82,87-89]

4 | EVIDENCE FOR INVOLVEMENT OF AUTOPHAGY IN MHC CLASS I ANTIGEN PRESENTATION

Below is a review of studies which have investigated whether autophagy is involved in pathways of MHC class I antigen presentation. The studies have been performed on APCs infected with virus or bacteria, and APCs exposed to soluble or cell-associated antigen. Thus, the studies will describe pathways both for direct antigen presentation and cross-presentation. Relatively few such studies have been performed, in particular when it comes to direct MHC class I antigen presentation.

Human herpesviruses (family Herpesviridae) have been studied for their ability to modulate autophagy.[63,90] Herpesviruses are enveloped, double-stranded DNA viruses, and have in common that they can establish a persistent, lifelong latent phase in the infected host. Herpes simplex virus type I (HSV-1) infection of cells can induce autophagy by activation of the kinase EIF2AK2, which phosphorylates eukaryotic translation initiation factor 2 subunit 1 (eIF2α).[91-93] However, HSV-1 also encodes several proteins which show subverse effects on autophagy, and the first anti-autophagic viral protein to be discovered

was the HSV-1-encoded neurovirulence factor named infected cell protein (ICP) 34.5.[93] ICP34.5 recruits a phosphatase which dephosphorylates eIF2α, and in addition, it may inhibit autophagy by binding to Beclin-1.[91,92,94] Accordingly, infection with ΔICP34.5 HSV-1 induces typical autophagosomes consisting of LC3-positive, double-membrane structures.[95] Of note, the inhibitory effect of ICP34.5 on autophagy is reported to be cell type dependent, and wild-type HSV-1 has also been shown to induce autophagy in various cell types.[90] English et al observed that infection with HSV-1 resulted in LC3-positive, four-layered membrane structures as well as typical autophagosomes in the mouse macrophage cell line BMA3.1A7, and both compartments contained viral particles.[95] The four-layered membrane structures seemed to emerge from the inner and outer nuclear membrane and were positive for HSV-1 glycoprotein B, a protein which is conserved among herpesviruses and is required for viral entry.[96] By use of a $CD8^+$ T cell hybridoma, specific for a peptide of HSV-1 glycoprotein B, the authors could show that at early stage of infection, MHC class I antigen presentation was proteasome-dependent.[95] However, at 10-12 hours after infection, $CD8^+$ T-cell activation was reduced substantially when different treatments (bafilomycin, 3-methyladenine or Atg5siRNA) were used to inhibit autophagy. Remaining $CD8^+$ T-cell activation was abolished by inhibitors of the proteasome. Based on the study, the authors proposed a model for the autophagy-dependent antigen presentation where the virus first is processed in autophagosomes, next, protein fragments are further degraded by the proteasome before peptides are loaded onto MHC class I molecules in the ER.[95,97] The model resembles the phagosome-to-cytosol pathway in cross-presentation, but the initial compartments for antigen-processing differ.

Viruses and viral antigens might be secreted from the infected APCs by secretory autophagy or other mechanisms, but English et al ruled out that endocytosis and cross-presentation of potentially secreted antigen were involved 8 hours after HSV-1 infection.[95] Although it might have strengthen their model if involvement of cross-presentation had been dismissed also at later time points, the study clearly suggests that autophagy contributes to MHC class I HSV-1 antigen presentation at the late stage of infection. Interestingly, the importance of autophagy varied with the stimulatory conditions applied to the macrophages. In heat-shock treated or IL-1β stimulated macrophages, both autophagy and the proteasome contributed to MHC I antigen presentation. In contrast, IFNγ-stimulated macrophages depended on the proteasome for MHC I presentation of glycoprotein B. It should be mentioned that IFNγ-stimulated macrophages were much more efficient at stimulating the $CD8^+$ T cells than the heat-shock or IL-1β-stimulated macrophages. This was not so surprising because, as written by the authors, the stimulatory effect of IFNγ on MHC class I antigen presentation is well established. Nevertheless, the data support that there are different pathways for MHC class I direct antigen presentation. The authors argue that heat-shock treatment and IL-1β stimulation could mimic stress induced by fever or stimulation with pyrogenic cytokines, and propose that, during such conditions, there will be more efficient processing of viral antigens in vacuolar organelles.[95]

Recently, Budida et al[25] performed some similar experiments to those reported by English et al,[95] but bone marrow-derived DCs (BMDCs, resembling primary DCs) were used instead of a macrophage cell line. Proliferation of glycoprotein B-recognizing $CD8^+$ T cells was used as readout for MHC class I HSV-1 antigen presentation. Budida et al confirmed in BMDCs, the previously reported ability of HSV-1 ICP34.5 to counteract late autophagosomal maturation in a macrophage cell line[95] and a DC cell line (DC2.4).[98] Budida et al also showed that compared to HSV-1 infection, HSV-1Δ34.5 led to significantly enhanced MHC I antigen presentation via a process depending on autophagosomal maturation since blockade of autophagic flux impaired antigen presentation.[25] The authors concluded that the data were "consistent with a potential contribution of autophagolysosomal processing to classical MHC class I antigen presentation".

Human cytomegalovirus (HCMV) belongs to the family of Herpesviridae. During the early stage of HCMV infection, independent on de novo viral protein synthesis, there is an increase in autophagy, whereas later on HCMV is associated with autophagy inhibition.[99,100] Tey and Khanna have reported that the epitope LPL from the HCMV latency-associated protein pUL138 is presented on MHC class I molecules via an autophagy-mediated pathway.[21] pUL138 itself is not incorporated into the HCMV particle, but is a type I integral membrane protein that localizes in the Golgi apparatus of infected cells, and the LPL peptide resides within the putative transmembrane domain.[101] Tey and Khanna suggested that this alternative, autophagy-mediated pathway for MHC I antigen presentation "may have a role in circumventing viral immune evasion strategies that primarily target the conventional pathway". In this study, they used human monocyte-derived DCs (MoDCs) as APCs as well as the human TAP1 and TAP2-deficient T2.B35 cell line, which is a hybrid of B and T lymphoblasts.[21] MHC class I antigen presentation was evaluated by measurement of IFNγ-secretion from a pUL138-specific $CD8^+$ T-cell line. Several highly interesting findings were presented in this study: (a) MHC I presentation of LPL by T2.B35 cells infected with replication-deficient adenovirus encoding pUL138 (AdUL138) was not affected by inhibitors of the proteasome (lactacystin or epoxomicin), but was significantly reduced by an inhibitor of endosomal acidification (chloroquine) and an inhibitor of cysteine and serine proteases (leupeptin),

suggesting that endosomal compartments were critical for presentation of the epitope, whereas the proteasome was not. The process was TAP-independent. (b) The involvement of autophagy was supported by the finding that eGFP fused to pUL138 co-localized with LC3 and lysosome membrane-associated glycoprotein 2 (LAMP2), and that MHC I presentation of LPL was reduced upon shRNA of ATG12. (c) Endogenous antigen synthesis was required for MHC class I presentation of LPL. (d) MHC I presentation of LPL following natural HCMV infection of moDCs mainly depended on the alternative pathway as MHC I presentation of LPL was significantly inhibited by 3-methyladenine and chloroquine, and there was only a trend for inhibition by lactacystin (proteasome inhibitor). (e) MHC I pUL138 presentation followed partly different pathways in AdUL138- and HCMV-infected fibroblasts: In AdUL138-infected fibroblasts, the proteasome was critical for MHC I antigen presentation, whereas in HCMV-infected fibroblasts, both the vacuolar/autophagy-dependent pathway and the conventional pathway were involved. Collectively, these data suggest that for MHC I presentation by APCs of the HCMV-derived peptide LPL, an alternative, TAP-independent pathway was critical. The pathway probably involved processing as well as loading of peptides onto MHC I molecules in vacuoles. Interestingly, the alternative pathway appeared to be more important in APCs than in fibroblasts. One interpretation of the data is that the used pathway for MHC I antigen presentation depends on the cell type and pathogen and/or ability to replicate, that is, AdUL138-infected fibroblasts used the conventional pathway; HCMV-infected APCs, the alternative pathway; and HCMV-infected fibroblasts made use of both the conventional and alternative pathway. Also MHC I presentation of another HCMV-derived epitope, pp65 by infected fibroblasts, depended on both the proteasome and the alternative pathway.[21] In another study, Khanna et al examined cross-presentation in Epstein-Barr virus-transformed B cells and found that autophagy and the proteasome cooperate synergistically.[102] The pathway was TAP-independent, and the authors suggested that $CD8^+$ T-cell epitopes are loaded onto MHC I molecules in the autophagolysosomal compartment rather than in the ER.[102]

Chlamydia is an obligate intracellular bacterium and is known to infect epithelial cells, fibroblasts, macrophages and DCs. Fiegl et al investigated MHC I presentation of antigen from *Chlamydia psittaci* in a mouse DC cell line (JAWS II) and observed that autophagy was important.[22] The authors proposed a model where cytosolic Chlamydia is engulfed by autophagosomes. Next, these compartments may fuse with MHC I-containing endosomes and form amphisomes, which contain cathepsin D. Thereafter, protein fragments are translocated into the cytosol for further processing by the proteasome, before peptides are reimported into the amphisome and loaded in a pH-dependent manner onto MHC I molecules, which primarily derive from endosomal recycling. The model was based on several observations.[22] For example, electron microscopy and immunofluorescent staining showed that Chlamydia localized in autophagosomal vacuoles and co-localized with cathepsin D. Upon Chlamydia infection, TAP translocated to cathepsin D-positive vacuoles but was also found in the ER; MHC class I antigen presentation (evaluated by $CD8^+$ T-cell stimulation) was dependent on cathepsin D and S, the proteasome, and TAP, but not tapasin nor ER to plasma membrane transport.

4.1 | Involvement of ATG genes in different MHC class I presentation pathways

There are also studies suggesting that autophagy is not involved in direct MHC class I antigen presentation. For example, fusion of influenza matrix protein 1 to LC3 and expression of the construct in a B lymphocyte cell line or moDCs did not affect MHC I presentation of the influenza antigen.[19] The same construct resulted in strongly enhanced MHC class II antigen presentation to $CD4^+$ T cells.[19] General control nonderepressible 2 (GCN2) is a kinase which phosphorylates eIF2α, and phosphorylated eIF2α is suggested to be important for autophagy induction.[93,103] Ravindran et al performed an experiment with the aim to verify that $GCN2^{-/-}$ BMDC retain MHC class I antigen presentation.[23] For this purpose, they infected BMDCs with the yellow fever vaccine (YF-17D) with or without a gene encoding ovalbumin (OVA) and evaluated MHC I antigen presentation by $CD8^+$ T cells specific for the OVA peptide SIINFEKL or the male antigen H-Y. The authors concluded that there was no difference in direct MHC I antigen presentation between wild-type and $GCN2^{-/-}$ BMDCs.[23] It is tempting though to speculate that there was a tendency for reduction of MHC I antigen presentation in the $GCN2^{-/-}$ BMDCs. Nevertheless, this study demonstrated that in BMDCs, the genes GCN2, ATG5 and ATG7 were critical for MHC I antigen cross-presentation of cell-associated OVA expressed in BHK cells infected with YF-17D-OVA.[23] Still, there are conflicting observations on the importance of ATG genes in MHC I antigen cross-presentation, as for example, Mintern et al found that ATG7 in primary mouse DCs is important for cross-presentation of soluble OVA, but not cell-associated OVA nor OVA targeted to the C-type lectin receptor DEC205.[24] Moreover, Lee et al reported that ATG5 gene deletion in DCs did not affect MHC class I antigen presentation of soluble OVA nor cell-associated OVA.[20] The discrepancies are likely due to different assay conditions including DC activation status, and as pointed out by Mintern et al, the use of different DC populations. Mintern et al proposed that a possible role of ATG genes observed in their

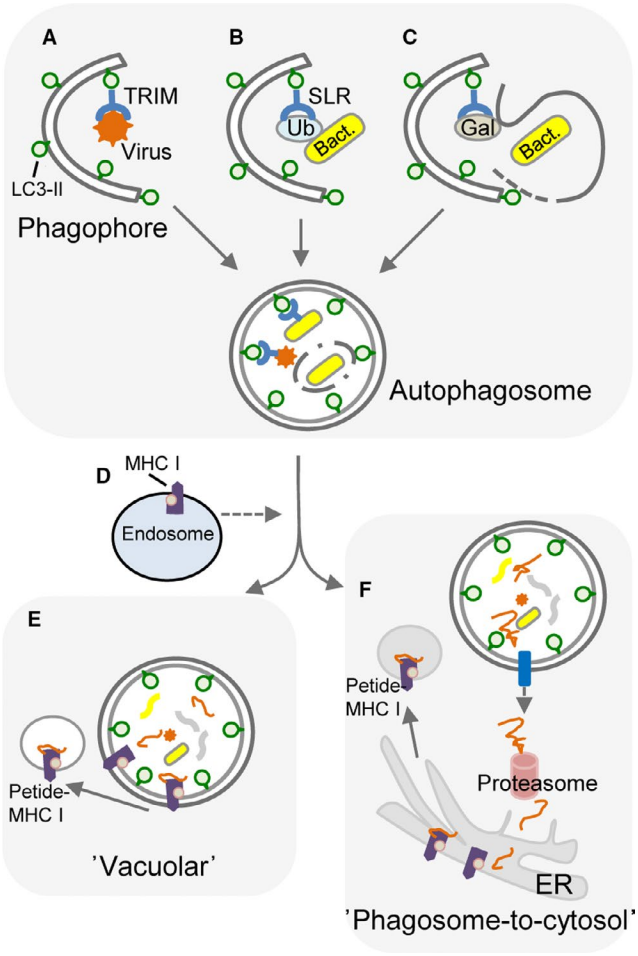

FIGURE 2 Elimination of microorganisms and suggested pathways for autophagy-mediated MHC class I antigen presentation. Pathogens like viruses, bacteria and fungi can be removed by selective autophagy, a process termed xenophagy. A, Viruses can be recognized by tripartite motif-containing (TRIM) proteins which act both as receptors and inducers of autophagy. B, Cytosolic bacteria (Bact.) or host cell components associated with the bacteria are tagged by ubiquitin (Ub). Autophagy receptors such as sequestosome 1-like receptors (SLRs) bind to both ubiquitin and LC3-II, and can thereby sequester bacteria into autophagosomes. C, Galectins, for example, galectin-8 can accumulate on bacteria-containing, damaged vacuoles. Recognition of galectin by autophagy receptors mediates targeting of the vacuole to autophagosomes. D, The pathogen-containing autophagosome may fuse with MHC I molecule-containing vesicles. E, The 'vacuolar' pathway is TAP-, and proteasome-independent. Instead, the antigen is degraded into peptides in the autophagic compartment/amphisome, and peptides are loaded onto MHC I molecules in the vacuole before transport in vesicles to the cell surface. F, In the 'phagosome-to-cytosol' pathway, degraded material is transported into the cytosol for further degradation by the proteasome. Next, the peptides are transported into the ER and loaded onto MHC I molecules before transportation in vesicles to the cell surface

study is through creation of a compartment where antigen is preserved rather than degraded and refer to LAP.[24] Finally, ATG5 and ATG7 have been reported to play a role in controlling MHC class I molecule levels on DCs and thereby shape $CD8^+$ T-cell responses.[26] $Atg5^{-/-}$ DCs showed elevated surface display of MHC class I molecules and increased $CD8^+$ T-cell responses.[26] The authors proposed that the underlying mechanism was via LC3 which is required for adaptor protein kinase-1-mediated internalization of MHC class I molecules. Such a function of ATG proteins may affect both direct presentation and cross-presentation of antigen on APCs.

5 | CONCLUDING REMARKS

Relatively few studies have investigated the involvement of autophagy in MHC class I antigen presentation, and the reported results may be interpreted as conflicting. However, the experimental conditions used varied substantially, for example, cell lines versus primary cells, different stimulatory conditions or lack of such, and pathogens versus soluble and cell-associated antigens. Some studies have focused on the involvement of autophagy in cross-presentation, others in direct antigen presentation. It is indeed important to exclude cross-presentation when examining involvement of autophagy in direct MHC class I antigen presentation, because antigen might be secreted by the APC, taken up via LC3-associated phagocytosis (LAP) and cross-presented. ATG gene-dependent secretion and LAP depend on several molecular components of the autophagy machinery including LC3 and its conjugation system. Therefore, genetic deletion of ATG5 and/or ATG7 is not sufficient to prove autophagy. Still, several of the reviewed studies suggest that autophagy can be important in direct MHC class I antigen presentation (Figure 2). Multiple studies have also shown that the conventional proteasome-TAP-dependent pathway represents the main mechanism for MHC class I antigen presentation. Therefore, autophagy may represent a compensatory mechanism during conditions of immune evasion such as down-regulation of TAP and dislocation of MHC I molecules from the ER. It appears likely that a cell with the machinery for autophagy would utilize this not only for intracellular destruction of pathogens, but also to warn the immune system by presenting pathogen-derived peptides on MHC I molecules. Further studies are needed to confirm such a concept.

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CONFLICT OF INTEREST

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