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Review

Cytosol to Lysosome Transport of Intracellular Antigens During Immune Surveillance

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The delivery of intracellular substrates such as misfolded proteins and damaged organelles from the cytosol to the lysosome for degradation is crucial for cell survival. Multiple transport pathways including bulk autophagy (microautophagy and macroautophagy) and chaperone-mediated autophagy (CMA) have been identified to efficiently facilitate this transit of macromolecules from the cytoplasm to acidic vacuolar organelles. While autophagy plays a role in the general housekeeping of cells, it also functions in more specialized processes such as development and differentiation, responses to physiological stress and immunity. The presentation of both exogenous and endogenous antigens (Ag) by major histocompatibility complex (MHC) class II molecules to $CD4^+$ T lymphocytes is critical for the induction of tolerance to self Ag as well as the development of immunity against intracellular pathogens and tumors. Here, we discuss the class II-mediated presentation of several endogenous Ag, dependent on either macroautophagy or CMA for their transport from the cytosol to endosomal/lysosomal compartments. Thus, the various pathways of autophagy as routes of cytoplasmic Ag delivery to lysosomes have significant implications for the MHC class II-mediated immune response to intracellular pathogens and cancer.

Key words: cancer, chaperone-mediated autophagy, immunity and host defense, lysosomal degradation, macroautophagy, MHC class II Ag presentation

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The continuous turnover of intracellular proteins is critical for the maintenance of cellular homeostasis. Cells have multiple mechanisms to achieve this, including the ubiquitin/proteasome system (1) as well as lysosomal degradation pathways including autophagy (2). Autophagy is a constitutively active process but is upregulated as a mechanism to salvage amino acids during periods of cellular stress such as nutrient deprivation. These pathways of lysosomal degradation have physiological relevance with respect to several cellular processes including immune recognition and responsiveness. This article reviews the pathways for cytosol to lysosome transport and their role in immunity and host defense.

MHC class I and class II molecules on antigen-presenting cells (APC) selectively acquire peptides from self and foreign Ag for presentation to $CD8^+$ and $CD4^+$ T lymphocytes, respectively. The spectrum of peptides displayed by class I and class II proteins is essential for self-tolerance as well as the development of immunity against invasive pathogens and tumors. Traditionally, immunologists believed that class I and class II molecules obtained their ligands from distinct subcellular pools of Ag. In this model, endogenous Ag processed in the cytosol gave rise to peptides for MHC class I presentation to $CD8^+$ T cells while engulfed exogenous Ag degraded in endosomes and lysosomes were destined for MHC class II presentation to $CD4^+$ T lymphocytes. While this paradigm remains true, alternative pathways for delivering exogenous Ag to MHC class I molecules have recently been characterized (3). Similarly, biochemical and functional studies indicate that MHC class II molecules also display peptides from cytoplasmic and nuclear proteins for $CD4^+$ T cell recognition with implications for viral, tumor and autoimmunity (4). Here, we discuss several pathways of cytosol to lysosome transport of potential importance to the development of host immune responses and specifically MHC class II-mediated Ag presentation.

The MHC Class II Ag Presentation Pathway

MHC class II molecules present antigenic peptides derived from exogenous as well as cytoplasmic proteins to $CD4^+$ T cells (4,5). MHC class II proteins are constitutively expressed on the surface of a number of professional APC such as dendritic cells (DC), B cells and macrophages as well as both cortical and medullary thymic epithelial cells. Treatment with an inflammatory signal such as interferon-gamma (IFN-γ) can induce MHC class II expression on the surface of non-professional APC such as endothelial cells, fibroblasts, epithelial cells and many tumors. MHC class II complexes consist of α and β subunits, which are first assembled in the endoplasmic reticulum (ER) with the chaperone molecule invariant chain (Ii) (6) (Figure 1). The precise role of Ii early in the transport pathway has been controversial with evidence, suggesting that Ii protects the

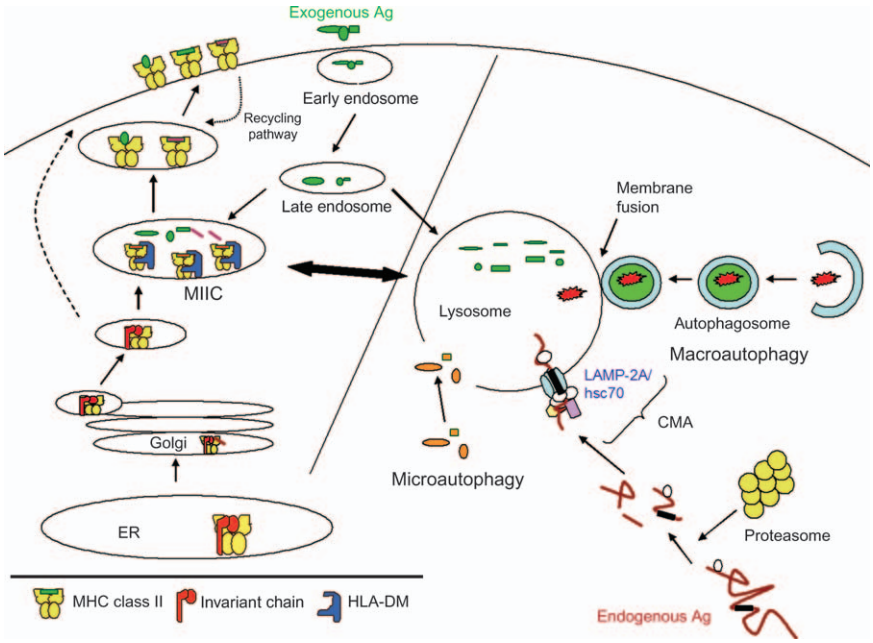

Figure 1: Pathways for MHC class II presentation of exogenous and endogenous Ag. Left: exogenous proteins are endocytosed and degraded by acidic proteases resident within endosomes and lysosomes. MHC class II molecules are assembled in the ER with the chaperone protein Ii, which targets these complexes to the endosomal pathway. Nascent as well as recycling MHC class II molecules acquire peptide ligands within endosomal compartments and then egress to the cell surface for presentation to $CD4^+$ T cells. Right: endogenous proteins may be degraded into antigenic peptides by the proteasome and transported into lysosomes through multiple pathways such as microautophagy, macroautophagy and CMA. In microautophagy, portions of the cytosol are continuously internalized through lysosomal invaginations. In macroautophagy, the cytoplasm is sequestered into double-membraned structures known as autophagosomes, which fuse with lysosomes. In CMA, specific cytosolic proteins are transported into lysosomes through a molecular chaperone/receptor complex composed of hsc70 and LAMP-2A. These antigenic fragments intersect with MHC class II molecules in a mature endosomal compartment known as the MIIC prior to presentation to $CD4^+$ T cells.

MHC class II molecule from associating with unfolded proteins in the ER (7) as well as from improper peptide loading as the complex traffics from the ER through the *trans* Golgi network (TGN) (8). The cytoplasmic tail of Ii contains a leucine-based motif that facilitates the sorting of MHC class II–Ii complexes through clathrin adaptor proteins found on clathrin-coated vesicles in the TGN (9). From the TGN, MHC class II–Ii complexes enter the endocytic pathway in order to interact with Ag (10). A portion of these complexes traffics directly from the TGN to endosomes and then to late endosomes and prelysosomes. Alternatively, a pool of newly synthesized MHC class II–Ii complexes egresses to the cell surface through the constitutive secretory pathway and then is rapidly internalized at the plasma membrane, thus gaining access to the endocytic pathway. Ii release from MHC class II is required for class II function and binding of antigenic peptides of 12–25 amino acids in length (6). Exogenous Ag delivered into the endocytic network through receptor-mediated or fluid-phase endocytosis are exposed to acidic proteases and denaturing reactions, yielding peptide ligands for class II molecules within the endosomal network (11). The binding of these peptides to class II molecules is facilitated by an editor protein, HLA-DM (12–14), which resides in a highly specialized organelle resembling a late endosome known as the MHC class II compartment (MIIC) (15). Recent studies have begun to reveal how peptides from cytoplasmic proteins might also access class II molecules within the endocytic pathway (16,17). Resultant peptide–MHC class II complexes are ultimately trafficked to the cell surface for immune surveillance by $CD4^+$ T cells.

Pathways of Lysosomal Proteolysis

Of critical importance in Ag presentation by MHC class II molecules is the degradation of proteins to antigenic peptides. To achieve this, APC exploit the intracellular proteolytic machinery common to most cells. Short-lived cytoplasmic Ag may be degraded by cytosolic proteases such as the proteasome and calpain. Proteolysis of cytoplasmic Ag, like exogenous Ag, may also occur in lysosomes, which are morphologically heterogenous with a low pH environment conducive to protein unfolding and the activation of acid-dependent hydrolases (18). Protein transit from the cytosol to lysosomes, also known as autophagy, typically leads to substrate degradation (2). Autophagy occurs through three distinct mechanisms: microautophagy, macroautophagy and CMA (Figure 1). In microautophagy, small portions of the cytosol are

internalized through lysosomal invaginations, and proteins are continuously degraded in the lumen of this organelle even under resting conditions (19). Microautophagy is upregulated under conditions of cellular stress such as starvation, but studies of this pathway have been limited to yeast and cell-free systems so little is known about its role in mammalian cells (20,21). Macroautophagy is also a constitutively active process in cells for the degradation of long-lived proteins and organelles (2), and during short periods of nutrient deprivation, this pathway is induced as a mechanism to salvage amino acids. In macroautophagy, the cytoplasm is sequestered into double-membraned structures known as autophagosomes, which fuse with lysosomes forming an autolysosome. Following fusion, the vacuolar material is degraded and the products are recycled back into the cytosol. Because these pathways show no specificity or selectivity in terms of the material sequestered, they have been termed bulk autophagy.

During periods of prolonged cellular stress, bulk autophagy declines and the process of CMA is instead activated. In CMA, specific cytosolic proteins displaying homologous pentapeptide motifs bind to a molecular chaperone complex composed of multiple heat-shock proteins including the heat-shock 70-kDa protein (hsc70) (reviewed in 22). This molecular chaperone complex transports the substrate protein to the lysosomal membrane, where it associates with an isoform of the lysosome-associated membrane protein-2, LAMP-2A, which functions as part of the lysosomal receptor for CMA. The cytoplasmic domain of LAMP-2A serves as a potential docking site for the molecular chaperone–substrate complex, and these chaperones likely unfold substrate proteins, enabling them to bind and translocate across the lysosomal membrane. Lysosomal hsc70 assists with the transport of the proteins into the organelle lumen, where these molecules are degraded by mature acidic proteases.

Cytosol to Lysosome Transport of Ag in APC

While bulk autophagy and CMA are essential for general protein turnover in most cells under normal conditions and periods of cellular stress, respectively, these pathways may also play a more specialized role in APC such as DC, B cells and macrophages. For example, the multiple forms of autophagy could function as routes for antigenic proteins located in the cytosol to reach the lysosome for processing into peptides prior to binding to MHC class II molecules in the MIIC compartment (Figure 1). Blocking these pathways of cytoplasmic to lysosomal transport may result in a reduction in the presentation of endogenous Ag by MHC class II molecules to $CD4^+$ T lymphocytes. Here, we summarize the recent evidence linking macroautophagy and CMA to the processing and class II-restricted presentation of cytosolic and nuclear Ag in APC.

Macroautophagy

Macroautophagy appears to play an essential role during development and oncogenesis as well as in response to physiological stress (23), but its role in immunological processes, in particular MHC class II-restricted Ag presentation, has only been recently studied. Dengjel et al. employed mass spectrometry to examine how the induction of autophagy alters the complex spectrum of peptides displayed by MHC class II molecules (24). Following serum starvation of human B-lymphoblastoid cell lines (B-LCL) to induce autophagy for different time periods, the authors observed an increase in those MHC class II-associated peptides derived from intracellular proteins. They also demonstrated that the induction of autophagy by starvation alters the balance of active proteases such as the cathepsins within lysosomes, suggesting that starvation-induced autophagy may alter the Ag processing machinery for MHC class II-mediated presentation.

Macroautophagy is a highly regulated process in both yeast and mammalian cells, and the nutrient-sensing target of rapamycin (Tor) molecule plays a key role in regulating macroautophagy (19). Excess nutrients or growth factors activate phosphatidylinositol 3-kinase (PI3K), resulting in the downstream activation of Tor and a decrease in macroautophagy. Macroautophagy is sensitive to the PI3K inhibitors 3-methyladenine (3-MA) (25) and wortmannin (26), and several investigators have used these compounds to examine how inhibiting autophagy affects MHC class II Ag presentation. Nimmerjahn et al. demonstrated that the treatment of human B cells with 3-MA or wortmannin completely abrogated the MHC class II-restricted presentation of the transfected cytosolic bacterial protein neomycin phosphotransferase II (NeoR) (27). Interestingly, the authors also showed that the generation of the NeoR epitope is dependent on lysosomal acidic proteases rather than cytosolic enzymes, suggesting that this Ag expressed in the cytoplasm must gain access to lysosomal compartments for proper epitope formation. A more recent report showed that the MHC class II-mediated presentation of mucin-1 (MUC-1) peptides in DC transfected with MUC-1 RNA was also sensitive to both 3-MA and wortmannin (28). However, the investigators found that the generation of the MUC-1 epitope was dependent on processing in both the cytosol and the lysosomal compartments, suggesting that the MUC-1 Ag is first partially degraded in the cytosol and then transported through macroautophagy to the lysosome for further processing.

Nuclear proteins, like cytosolic proteins, may also be sources of antigenic peptides for MHC class II-restricted $CD4^+$ T lymphocytes. Paludan et al. recently showed that the inhibition of lysosomal acidification by chloroquine in Epstein–Barr virus (EBV)-transformed human B-LCL resulted in the accumulation of the EBV nuclear antigen 1 (EBNA 1) in intracellular vesicles positive for the lysosome-associated membrane protein-1 (LAMP-1) and

monodansylcadaverine (29), a fluorescent marker specific for autophagosomes (30). Treatment of the B-LCL with 3-MA reduced the MHC class II-restricted presentation of EBNA 1, suggesting that macroautophagy may be the mechanism by which this nuclear Ag gains access to lysosomes for epitope processing. During macroautophagy, autophagosome initiation and formation require several autophagy-related gene (Atg) products, first identified and characterized in yeast (19,31). Paludan et al. further demonstrated that silencing the essential autophagy gene *Atg12* with small interfering RNA also diminished the activation of EBNA 1-specific $CD4^+$ T cells (29). Similar to the generation of the NeoR epitope, the processing of the EBNA 1 epitope for MHC class II presentation may depend on the activation of acid-dependent hydrolases resident in lysosomes rather than cytosolic proteases such as the proteasome. In the case of the long-lived protein EBNA 1, autophagy may serve as the main degradative pathway whereas the proteasome is more likely responsible for the degradation of short-lived proteins.

While it appears that macroautophagy regulates the lysosomal processing of several endogenous Ag, the question remains how the antigenic peptides generated in lysosomes gain access to the MIIC compartments in APC. Previous electron microscopy studies had shown that the endocytic pathway intersects the autophagy pathway (32), and it is possible that MHC class II-positive endosomes might converge with autophagosomes in APC. Recently, Schmid et al. reported that in APC such as B cells and DC, autophagosomes are constitutively formed and that these vacuolar-like structures continuously fuse with MIIC (17). In these studies, the authors showed that the autophagosome marker LC3 colocalizes with resident proteins of the MIIC such as MHC class II molecules, the chaperone HLA-DM and the lysosome membrane protein LAMP-2. Furthermore, when the cytosolic Ag influenza matrix protein-1 (MP-1) was directly targeted to autophagosomes by fusion with LC3, an increase in the MHC class II-mediated presentation of MP-1 to $CD4^+$ T cells was observed. These results suggest that macroautophagy is an effective route for delivery of endogenous Ag to MHC class II molecules for the activation of $CD4^+$ T lymphocytes.

The MHC class II-mediated presentation of several cytoplasmic Ag requires the induction of macroautophagy and subsequent lysosomal processing, yet the presentation of other cytosolic Ag by MHC class II molecules is insensitive to 3-MA treatment and less dependent on the activity of lysosomal proteases. For example, treatment of human B cells expressing the cytoplasmic autoantigen glutamate decarboxylase (GAD) with proteasome inhibitors or calpain selectively blocked the MHC class II-restricted presentation of this Ag (33). Conversely, cytoplasmic GAD presentation is only slightly reduced in B cells treated with chloroquine or lysosomal protease inhibitors, suggesting that processing of GAD in the cytosol before transport to the MIIC is key for the class II-mediated presentation of this Ag. Furthermore, the treatment of human B cells with 3-MA has no significant effect on the MHC class II-restricted presentation of cytoplasmic GAD or another cytosolic autoantigen, SMA, a mutant form of the human immunoglobulin kappa light chain (D. Zhou and J. S. B., unpublished data). For these cytoplasmic Ag, macroautophagy may not be the primary route of transport from the cytosol. Similarly, the MHC class II-restricted presentation of the I-Eα protein expressed in the cytosol was dependent on a functional proteasome (34) but insensitive to 3-MA treatment (35). In contrast, tumor cells transfected with hen egg lysozyme (HEL) in the cytoplasm do not require the proteasome or macroautophagy for the MHC class II-mediated presentation of the HEL epitope (36). Lastly, Taylor et al. recently showed no inhibition of the MHC class II-restricted presentation of two EBV nuclear Ag, EBNA 2 and EBNA 3C following 3-MA treatment of B-LCL (37). Instead, the authors demonstrated that these Ag gain access to the MHC class II pathway through intercellular Ag transfer. Whether intact Ag or antigenic fragments complexed with other proteins or within exosomes are transferred is unknown, but Ag processing by lysosomal proteases in the recipient cell is required for the MHC class II-restricted presentation of these EBV Ag. Overall, these findings indicate that macroautophagy may be an important mechanism for the processing of some cytoplasmic Ag and their delivery to MHC class II molecules. Conversely, for those cytoplasmic Ag dependent on the activity of cytosolic proteases for their processing, macroautophagy may play a less significant role in MHC class II-mediated presentation.

Chaperone-mediated autophagy

Other mechanisms of cytosol to lysosome transport must exist for those cytoplasmic Ag which do not utilize bulk autophagy as the pathway to reach the lysosome. Previous studies have shown that components of the MHC class I Ag presentation pathway such as the proteasome are important in the processing of cytoplasmic Ag (33,34). Thus, the TAP heterodimer, which delivers the majority of MHC class I peptides from the cytosol to the ER, might play an important role in the transport of cytoplasmic Ag fragments into endosomal or lysosomal compartments. Recently, the proteasome- and TAP-dependent generation of two MHC class II-restricted epitopes encoded within two transmembrane glycoproteins of infectious influenza virus was reported in both murine B cells and DC (38). These results differ from several reports from a number of laboratories, suggesting that TAP plays no role in the presentation of cytoplasmic Ag to MHC class II molecules (16,27,33,35,36,39). To explain the discrepancy in these results, the authors suggest that in cells with more permeable endosomal membranes such as DC, cytosolic Ag might have more access to this TAP-dependent pathway than in B cells and fibroblasts containing more rigid endosomal membranes. Further studies regarding this transport in specialized immune cells such as DC are clearly needed.

Another possible mechanism for the transfer of cytosolic Ag to endosomal or lysosomal compartments for processing and binding to MHC class II molecules is CMA. Recently, our laboratory demonstrated a role for CMA in regulating cytoplasmic Ag presentation by MHC class II molecules by modulating the cellular levels of two components of the CMA pathway, LAMP-2A and hsc70, in human B cells expressing cytoplasmic Ag GAD and SMA (16). Expression of antisense complementary DNA (cDNA) for LAMP-2 reduced the MHC class II-restricted presentation of GAD, while overexpression of the LAMP-2A isoform resulted in an increased presentation of epitopes from both GAD and SMA. Alternative splicing of the LAMP-2 gene gives rise to three isoforms in humans, termed LAMP-2A, 2B and 2C (40) (D. Zhou and J. S. B., unpublished data). While LAMP-2A and 2B are ubiquitously expressed, LAMP-2C displays a tissue-specific distribution and is not found in human B cells (D. Zhou and J. S. B., unpublished data). Interestingly, overexpression of the LAMP-2B isoform had no effect on the presentation of cytoplasmic GAD or SMA yet reductions in total LAMP-2 isoforms perturbed both cytoplasmic and exogenous Ag presentation by MHC class II. LAMP-2A overexpression did not change the overall cellular morphology of APC (D. Zhou and J. S. B., unpublished data), alter the content of autophagosomes in APC or affect the maturation or distribution of the lysosomal protease, cathepsin D (16). These results suggested that while the overexpression of LAMP-2A in APC enhanced cytoplasmic Ag presentation, it did not alter the normal cellular processes attributed to lysosomes. In addition to LAMP-2, the chaperone hsc70 is essential for the transport of substrates from the cytosol to lysosomes during CMA. We demonstrated that the expression of antisense cDNA hsc70 reduced the MHC class II-restricted presentation of cytoplasmic GAD while hsc70 overexpression enhanced the presentation of this Ag (16). To more definitely show that LAMP-2A plays a role in the transport of cytosolic Ag to the lysosome, we electroporated a biotinylated version of the GAD peptide directly into the cytosol of human B cells overexpressing LAMP-2A and observed both an increase in the relative abundance of MHC class II:biotin–GAD peptide complexes within the cells and an enhancement in the GAD-specific T cell response (16). Thus, our results suggested that LAMP-2A, possibly in association with hsc70, facilitates the delivery of epitopes in human B cells from cytoplasmic Ag to endosomal or lysosomal compartments accessible to MHC class II molecules, ultimately resulting in the activation of specific $CD4^+$ T lymphocytes.

Autophagy and the Immune Response

Autophagy is essential for cell survival because this pathway is responsible for the removal of excessive or defective subcellular structures such as protein aggregates or damaged organelles and for a cell's ability to adapt during periods of physiological stress (23). Thus, it may not be surprising that autophagy pathways are induced or altered during infection and immunity, both of which can involve cell stress responses. Autophagy is often a component of the immune response to intracellular pathogens; thus, some microorganisms have evolved strategies to evade the autophagy pathway. Similarly, autophagy is believed to play a role not only in tumorigenesis but also in tumor suppression and eradication, again potentially linking this pathway to host defense and immunity.

Intracellular pathogens

The relationship between autophagy and intracellular microorganisms such as bacteria and viruses has been extensively reviewed previously (41); thus, we provide here a few examples to illustrate this relationship. Bacteria can enter a cell through phagocytosis, and once inside, they either escape from the phagosome into the cytosol prior to fusion with lysosomes or remain within this compartment and block its fusion with lysosomes (42). The host cell may initiate autophagy as a defense mechanism in order to clear bacteria located in the cytosol or in isolated vacuoles (43). For example, upon entering the cytosol from endosomes, *Streptococcus pyogenes* becomes enveloped by autophagosomes and the bacteria are destroyed upon fusion of the autophagosomes with lysosomes (44). During infection of macrophages with *Mycobacterium tuberculosis*, treatment with IFN-γ induces the small GTPase LRG-47 important in autophagosome formation, thus facilitating the fusion of pathogen-containing vacuoles with lysosomes (45). Not surprisingly, bacteria have evolved strategies to subvert the pathways of autophagy. For example, the cytosolic pathogen *Shigella flexneri* produces the virulence factor IcsB that inhibits autophagy (46). Some bacteria such as *Legionella pneumophila* and *Coxiella burnetii* induce the formation of double-membrane compartments, resembling autophagosomes, for sites of bacterial replication and then block the maturation of these compartments to autolysosomes to prevent degradation (47,48).

Less is known about the role of autophagy in the clearance of viral infections in host cells. One candidate mechanism is the activation of the protein kinase PKR by double-stranded RNA viruses (41); activation of PKR and the subsequent downstream signaling events have been shown to induce autophagy during herpesvirus infection. Recently, Lee et al. showed that autophagy is required for single-stranded RNA viruses to be detected by a component of the innate immune system, the Toll-like receptor 7 (TLR7) (49). In plasmacytoid DC (pDC), autophagy facilitates the transport of viral replication intermediates from the cytosol to lysosomes where TLR7 is activated. Additionally, autophagy is required for the production of important mediators of antiviral immunity, the type 1 interferons, in pDC following infection with

either single-stranded RNA or double-stranded DNA viruses. Like bacteria, viruses have evolved mechanisms to utilize autophagy to their advantage. For example, positive-strand RNA viruses such as polioviruses and coronaviruses as well as large cytoplasmic DNA viruses such as vaccinia virus use components of the autophagy pathway to facilitate viral replication (41,50). These viruses initiate the formation of double-membrane structures, similar to autophagosomes, upon which to build their viral replication complexes. Overall, these examples illustrate how autophagy and membrane remodeling contribute to the host cell's defense against intracellular pathogens and how these microorganisms have developed strategies to evade these pathways.

Cancer

In addition to its role in infectious disease, autophagy is thought to contribute to both cancer development and tumor eradication. During the early stages of cancer, autophagy may be suppressed to promote tumor growth, while in the later stages of oncogenesis, autophagy may be induced as rapid proliferation of the tumor depletes critical nutrients (51). Additionally, following chemotherapy, tumor cells may initiate autophagy as a protective measure to recycle nutrients or remove damaged cellular components (51). In contrast, overexpression of autophagy-specific genes such as *beclin-1* inhibits tumor formation in mice while inactivation of these genes results in significant tumorigenesis (52). Furthermore, several tumor suppressor genes that facilitate the induction of autophagy are silenced in cancer cells yet genes such as PI3K that downregulate autophagy are often activated in tumors (52). Given the complex nature of the relationship between autophagy and cancer, more research is needed to further elucidate the contribution of this pathway to tumorigenesis. Additionally, whether changes in the autophagy pathways in tumor cells influence immune recognition and clearance has not yet been explored.

Conclusions

The transport of intracellular substrates from the cytosol to lysosomes is important not only for the maintenance of cellular homeostasis but also for more specialized functions in immune cells such as the MHC class II-mediated presentation of cytoplasmic and nuclear Ag. Multiple mechanisms of cytosol to lysosome transport, including but not limited to macroautophagy and CMA, have been implicated in the delivery of several endogenous Ag to lysosomes for their eventual presentation by MHC class II molecules. The discrepancies in the results of these studies suggest that the type of APC as well as the nature, half-life and subcellular distribution of endogenous Ag may influence which transport pathway is utilized. Thus, additional research is needed to clarify those factors that ultimately determine the route of Ag delivery. Further studies are also needed to more clearly define the relationship between the various autophagy pathways and the immune response to intracellular pathogens and tumors. Blocking or enhancing these pathways of cytosol to lysosome transport may alter the MHC class II-mediated presentation of endogenous Ag to $CD4^+$ T lymphocytes, thus providing novel therapeutic strategies for the treatment of infectious diseases and cancer.

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