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Autophagy and its role in MHC-mediated antigen presentation

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Abstract

Intracellular degradation by autophagy plays a role in the maintenance of cellular homeostasis under normal conditions and during periods of cellular stress. Autophagy has also been implicated in several other cellular processes including immune recognition and responsiveness. More specifically, autophagy has been identified as a route by which cytoplasmic and nuclear Ag are delivered to MHC class II molecules for presentation to CD4⁺ T cells. Autophagy has also recently been implicated in MHC class I cross-presentation of tumor Ag and the activation of CD8⁺ T cells. This review discusses the role of autophagy in modulating MHC class I and class II Ag presentation as well as its implication in regulating autoimmunity and tolerance, tumor immunity, and host defense against intracellular pathogens.

Pathways of autophagy

Autophagy is one mechanism that cells utilize to degrade cytoplasmic proteins and organelles during the maintenance of cellular homeostasis (1). There are multiple pathways of autophagy. In microautophagy, small portions of the cytosol are internalized via lysosomal membrane invaginations, and proteins are continuously degraded in the lumen of this organelle even under resting conditions (2). Microautophagy is upregulated under conditions of cellular stress such as nutrient deprivation, but studies of this pathway have been limited to yeast and cell-free systems (3,4). In mammalian cells, constitutively active autophagy pathways can also be upregulated as a mechanism to salvage amino acids during periods of cellular stress. Several types of selective autophagy have been identified in mammalian cells including macroautophagy and chaperone-mediated autophagy (CMA) (Fig. 1) as well as pexophagy and mitophagy (5). A related constitutive biosynthetic pathway, cytoplasm-to-vacuole targeting (Cvt), is involved in hydrolase sorting in yeast (6). Another pathway, xenophagy, involves the utilization of the autophagic machinery for the degradation of intracellular pathogens (7). Macroautophagy and CMA have been implicated in the presentation of Ag by MHC molecules, and thus, are the major focus of this review.

Macroautophagy is also a constitutively active cellular process modulating the degradation of long-lived proteins and organelles (1). During short periods of nutrient deprivation, this

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pathway is rapidly induced as a mechanism to salvage amino acids. The absence of growth factors or amino acids prevents signaling through phosphatidylinositol-3-kinase (PI3K) from activating the autophagy regulatory molecule, target of rapamycin (mTor); and thus, macroautophagy is induced (8). In macroautophagy, portions of the cytoplasm are sequestered into double-membrane structures known as autophagosomes. The formation of the autophagosome requires a number of autophagy-related gene (Atg) products which have been well-characterized in yeast (6) and are conserved in mammals; the specific details of autophagosome formation have been reviewed extensively elsewhere (2,8). Briefly, the Atg12-Atg5-Atg16 complex in conjunction with Atg9 mediates the induction of autophagosome formation. During this process, LC3 (Atg8) is conjugated to phosphatidylethanolamine (PE) with the assistance of the Atg4, Atg7, and Atg3 molecules and incorporated into the autophagosomal membrane. This membrane next expands and engulfs portions of the cytosol, including entire organelles. Upon formation of the autophagosome, the Atg12-Atg5-Atg16 complex dissociates from this structure while LC3 (Atg8)-PE remains in the autophagic lumen as an autophagosomal marker. The outer membrane of the autophagosome fuses with the lysosomal membrane forming an autolysosome within which the single membrane structure, or autophagic body, is degraded by lysosomal esterases, lipases, and proteases.

During periods of prolonged cellular stress, bulk autophagy declines and the process of CMA is upregulated. 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 (9)). 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 chaperone-mediated substrate unfolding likely enables proteins to bind and translocate across the lysosomal membrane. Lysosomal hsc70 (ly-hsc70) assists with the transport of substrate proteins into the organelle lumen where these molecules are degraded by mature acidic proteases.

Autophagy and MHC class I Ag presentation

In the conventional paradigm, MHC class I molecules are restricted to surveying the cytosol for endogenous Ag from viruses, tumors, or self proteins for presentation to CD8⁺ T cells (reviewed in (10)). These endogenous Ag are degraded into peptide fragments by cytosolic proteases such as the proteasome and transported via a heterodimeric complex composed of Transporter associated with Antigen Processing 1 (TAP1) and TAP2 into the endoplasmic reticulum (ER). Once in the ER, peptides of the appropriate length (8 to 10 amino acids) bind nascent MHC class I heavy chains and β_2 -microglobulin and then transit through the secretory pathway to the cell surface for recognition by CD8⁺ T cells. A direct role for autophagy in the conventional pathway for MHC class I presentation has not been firmly established, yet recent studies discussed below demonstrate potential roles for autophagy in regulating class I-mediated cross-presentation of exogenous Ag.

During the process of cross-presentation, bone marrow-derived antigen presenting cells (APC) such as dendritic cells (DC) internalize and degrade Ag from the extracellular environment and display the resulting peptides in association with MHC class I molecules on their cell surface (11). The stimulation of naïve CD8⁺ T cells by these peptide-MHC class I complexes is known as cross-priming. The class I-restricted cross-presentation pathway plays an important role in the immune surveillance of infected cells as well as tumors and the subsequent development of an appropriate cytotoxic T cell response to these pathogens or malignancies. Precisely how peptides from exogenous Ag access the MHC class I presentation pathway is still under investigation although several routes have recently been proposed. These pathways

have been described extensively elsewhere (11–13); thus, only a brief summary of the current models of class I-mediated cross-presentation is offered here.

Studies have suggested that MHC class I molecules may acquire peptides derived from exogenous Ag internalized into phagosomes in a TAP-dependent or TAP-independent manner. In the TAP-dependent phagosome-to-cytosol-to-phagosome pathway, Ag is transported to the cytosol by a yet unknown mechanism, possibly Sec61, and degraded by the proteasome. Peptides are then re-imported into the phagosome which has acquired TAP as well as other ER proteins such as MHC class I. The phagosome-to-cytosol pathway calls for peptides generated in the cytosol by the proteasome to be directly transported to MHC class I molecules in the ER via TAP. Another possible mechanism of class I-restricted cross-presentation may involve the ER associated degradation pathway (ERAD). Here, exogenous Ag internalized in endosomes is transported into the ER, translocated for degradation in the cytosol by the proteasome, and finally transported back into ER by TAP. It has also been reported that DC may acquire exogenous peptides from other cells via GAP-junctions in order to cross-prime naïve CD8⁺ T cells. Lastly, in the vacuolar pathway, MHC class I molecules may acquire peptides that are generated in phagosomes by cysteine proteases such as cathepsin S in a TAP-independent manner. The mechanisms which potentiate MHC class I peptide loading in phagosomes via this latter pathway have not yet been determined.

Autophagy has recently been proposed as another possible mechanism for the MHC class I-mediated cross-presentation of exogenous Ag with results pertinent to tumor Ag (14). In this study, the authors induced macroautophagy in HEK 293T cells expressing a model Ag ovalbumin (OVA) by starvation or treatment with the compound rapamycin, which inhibits the mTor molecule (15). Following these treatments *in vitro*, enhanced cross-presentation of OVA to epitope-specific CD8⁺ T cells was observed. Conversely, the treatment of the OVA-expressing HEK 293T cells with 3-methyladenine (3-MA), an inhibitor of PI3K and macroautophagy (16), significantly reduced OVA cross-presentation *in vitro*. These authors also used rapamycin, 3-MA, and another PI3K inhibitor wortmannin (17) to modulate macroautophagy in melanoma cells and then measured the MHC class I-restricted cross-presentation of the tumor Ag gp100 *in vivo*. Similar to the results with the OVA-expressing HEK 293T cells, induction of macroautophagy enhanced the cross-presentation of gp100 while inhibition of macroautophagy reduced the cross-presentation of this Ag to epitope-specific CD8⁺ T cells. Macroautophagy was also specifically inhibited through the use of siRNA against the autophagy gene Atg6/beclin-1 in melanoma cells, and a significant reduction in the *in vivo* MHC class I-mediated cross-presentation of gp100 was again observed.

These results support a potential role for macroautophagy in the MHC class I-restricted cross-presentation of tumor Ag, yet further mechanistic studies to define the role of autophagy pathways in class I-mediated Ag presentation are needed. The induction of macroautophagy by rapamycin in the OVA-expressing HEK 293T cells as well as the melanoma cells leads to the conversion of LC3-I to its LC3-II form, suggesting that the MHC class I-restricted cross-presentation of OVA and gp100 may require the initiation and formation of autophagosomes. Yet, treatment of cells with NH₄Cl, which blocks the acidification of lysosomes and prevents their fusion with autophagosomes, enhances class I-mediated cross-presentation. These results suggest that the degradation of the contents of autolysosomes during the late phases of autophagy hinders cross-presentation perhaps by destroying the Ag that serve as the sources of peptide epitopes for MHC class I molecules. In support of this, Li et al. show that purified autophagosomes from OVA-expressing HEK 293T cells or melanoma cells serve as a source of Ag for DC to promote cross-presentation of OVA or gp100 to the appropriate epitope-specific CD8⁺ T cells. While the authors demonstrate that autophagosomes may function as efficient carriers of Ag from donor cells, how and where these structures and their antigenic contents intersect with MHC class I molecules has yet to be determined (Fig. 2). Further insights

into the specific mechanisms by which autophagy plays a role in the presentation of tumor Ag by MHC class I molecules may lead to the development of new therapeutic strategies for the treatment of cancer.

In addition to its role in tumor immunity, autophagy may also contribute to the CD8⁺ T cell response to intracellular pathogens such as bacteria, parasites, and viruses. For example, autophagy is required for the clearance of several pathogens (reviewed in (18–20), including the parasite *Toxoplasma gondii* which is localized in autophagosomes in infected macrophages (21,22). Since CD8⁺ T cells are essential for protective immunity from *T. gondii* (23), it is possible that peptides derived from *T. gondii* access MHC class I molecules through autophagy. Several pathogens have been localized in autophagosomes that contain ER proteins (24), and perhaps components of the MHC class I presentation pathway such as class I molecules or TAP may also be found in autophagosomes. Furthermore, during periods of cellular stress, aggresome-like structures (ALIS) containing poly-ubiquitylated proteins are formed and serve as substrates for autophagy (25). ALIS can form in DC (DALIS) experiencing cellular stress, perhaps as a result of an infection by a pathogen, and the clearance of these DALIS by autophagy has been proposed as a source of peptides for MHC class I molecules (26).

Autophagy and MHC class II Ag presentation

Traditionally, MHC class II molecules present antigenic peptides derived from exogenous proteins to CD4⁺ T cells (27). MHC class II proteins are constitutively expressed on the surface of a number of professional APC such as DC, B cells, and macrophages as well as both cortical and medullary thymic epithelial cells (cTEC and mTEC, respectively). 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 ER with the chaperone molecule invariant chain (Ii) (28,29). The cytoplasmic tail of Ii contains a motif that targets the Ii-MHC class II complexes to endosomal/lysosomal compartments where acidic proteases degrade Ii to a small fragment known as the class II-associated invariant chain peptide (CLIP) which remains associated with the MHC class II peptide binding groove (30,31). Ag delivered into the endosomal/lysosomal network via receptor-mediated or fluid phase endocytosis are also exposed to proteases and denaturing reactions, yielding peptide ligands for class II molecules (32). CLIP removal and the capture of antigenic peptides by MHC class II proteins is catalyzed by the MHC-encoded molecule HLA-DM (33–35) and occurs in a highly specialized organelle resembling a late endosome/lysosome known as the MIIC (36). Resultant peptide-MHC class II complexes are ultimately trafficked to the cell surface for immune surveillance by CD4⁺ T cells.

Similar to the MHC class I-mediated cross-presentation of exogenous Ag to CD8⁺ T cells, recent studies have begun to reveal how cytoplasmic and nuclear Ag gain access to MHC class II molecules in lysosomal compartments for the presentation of peptides to CD4⁺ T cells (37, 38). Multiple pathways have been shown to contribute to the MHC class II-mediated presentation of cytoplasmic and nuclear viral, tumor, and self Ag in both professional and non-professional APC. These pathways include CMA, macroautophagy, a TAP-dependent pathway, and intercellular Ag transfer. The findings of these studies have been reviewed extensively elsewhere (39–42). Thus, here only a brief summary of these pathways for MHC class II-mediated endogenous Ag presentation is offered (Fig. 3).

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 glutamic acid decarboxylase (GAD) or a mutant form of human Ig κ light chain, designated

SMA (37). Expression of anti-sense cDNA for LAMP-2 reduced the MHC class II-restricted presentation of GAD while over-expression of the LAMP-2A isoform resulted in an increased presentation of epitopes from both GAD and SMA. In B cells, SMA, one of the causative agents of light chain amyloidosis, fails to fold properly after synthesis and is immediately translocated out of the ER to the cytosol (43), suggesting that CMA may contribute to the immune recognition of mis-folded ER proteins. Stress induced autophagy pathways also appear to play a role in the presentation of multiple cytoplasmic and nuclear Ag. Human B cells in which autophagy is induced by serum starvation display an increase in those MHC class II-associated peptides derived from intracellular proteins as measured by mass spectrometry (44). Studies using PI3K inhibitors 3-MA and wortmannin or siRNA targeting the Atg12 gene to block macroautophagy demonstrated that multiple epitopes from cytoplasmic and nuclear proteins are dependent on this pathway for their presentation by MHC class II molecules. These epitopes include the cytosolic and nuclear versions of the bacterial protein neomycin (45,46), mucin-1 (47), and the EBV Nuclear Ag (EBNA) 1 (48). A recent report by Munz and colleagues demonstrates that in B cells and DCs, autophagosomes are constitutively formed and continuously fuse with MIIC (38). Furthermore, when the influenza matrix protein 1 (MP1) was targeted to autophagosomes by fusion with LC3 (Atg8), an increase in the MHC class II-restricted presentation of this epitope to CD4⁺ T cells was observed.

Two additional pathways have been shown to contribute to the MHC class II-mediated presentation of endogenous viral Ag in B cells and DC including a TAP-dependent pathway and intercellular Ag transfer. Both the proteasome and TAP were shown to be required for the generation of two MHC class II-restricted epitopes encoded within two distinct transmembrane glycoproteins from influenza virus using murine B cells and DC (49). These results differ from several reports suggesting that TAP plays no role in the presentation of cytoplasmic Ag to MHC class II molecules (37,46,50–53), thus further studies regarding this transport pathway in specialized immune cells such as DC are clearly needed. In the second pathway, two Epstein Barr virus (EBV) nuclear Ag, EBNA 2 and EBNA 3C, were shown to gain access to the MHC class II pathway via intercellular Ag transfer (54). Treatment of EBV-transformed human B cells with 3-MA to block macroautophagy failed to inhibit the MHC class II-restricted presentation of these endogenous viral Ag. Whether intact viral Ag or antigenic fragments complexed with other proteins or within exosomes are transferred to recipient APC for MHC class II-mediated presentation is unknown. However, Ag processing by lysosomal proteases within the recipient cell is required for the class II-restricted presentation of EBNA 2 and EBNA 3C viral Ag, strongly supporting intercellular or cross-presentation.

Autophagy and the regulation of immune responses

Autophagy pathways may be induced or altered during infection and immunity as cellular stress responses are initiated. *In vitro* studies have demonstrated recognition of viral, self, and tumor Ag dependent upon autophagy in the context of MHC class II Ag presentation. A role for autophagy in the cross-presentation of tumor Ag via MHC class I molecules has also been reported both *in vitro* and *in vivo* (14). Less is known about whether autophagy influences either CD4⁺ or CD8⁺ T cell responses to bacterial pathogens. A recent report on autophagy in the thymus provides a clue as to the importance of this process in the presentation of self Ag and its impact on the induction and maintenance of CD4⁺ T cell tolerance (55). The potential role of autophagy in regulating immune responses to pathogens, tumors, and self Ag has been reviewed extensively elsewhere (18,41,56,57) with highlights of recent studies discussed here.

Intracellular pathogens

Studies have begun to examine the role of autophagy in a host cell's defense against intracellular pathogens such as viruses and bacteria although the precise molecular steps involving autophagy or autophagy-linked gene products are still poorly understood (reviewed

in (41,58)). Autophagy limits the virus-induced encephalitis observed during Sindbis virus infection through the interaction of the Atg6 gene Beclin 1 with Bcl-2 (59). Additionally, during the process of xenophagy, autophagosomes engulf and destroy viruses (7). For example, the herpes simplex virus-1 (HSV-1) is degraded within autophagosomes in a dsRNA-activated protein kinase (PKR) dependent manner (60). Autophagy has recently been shown to play a role in the innate immune response to vesicular stomatitis virus (VSV) (61). In plasmacytoid DC (pDC), autophagy facilitates the transport of VSV replication intermediates from the cytosol to lysosomes potentially to promote Toll-like receptor 7 (TLR7) engagement. Activation of TLR7 in DC results in the induction of important mediators of anti-viral immunity such as IFN- α and IL-12. Plasmacytoid DC also recognize viruses through TLR9, localized in endosomes, and signaling through TLR9 recruits and activates the transcription factor interferon response factor 7 (IRF7) which is essential for the production of type I interferons (62). Using rapamycin and siRNA against mTOR, Cao et al recently demonstrated that virus-induced TLR9 dependent signaling through the mTOR pathways is also critical for the production of type I interferons in pDC as well as bone-marrow derived DC and macrophages (63). Whether signaling through mTOR following virus-induced activation of TLR9 affects the autophagy pathway during an innate anti-viral immune response has yet to be investigated. Lastly, as discussed above, the MHC class II-mediated presentation of the EBV Ag EBNA1 by autophagy suggests that this pathway may also play a role in the adaptive immune response to viruses (48). The demonstration that targeting influenza MP1 to autophagosomes enhanced MHC class II presentation of the MP1 epitope (38) may suggest that in virally infected cells, fusion of autophagosomes containing viral particles with lysosomes could promote viral Ag association with class II for presentation to CD4⁺ T cells.

Host cells also utilize autophagy pathways in their defense against bacteria and parasites. Autophagy limits bacterial replication by enveloping free bacteria such as group A *Streptococcus* in autophagosomes for delivery to lysosomes (64) or by targeting phagosomes containing bacteria such as *Mycobacterium tuberculosis* for fusion with lysosomes (65,66). The innate immune response initiated as a result of pathogenic infections also influences autophagy pathways. For example, signaling through TLR7 during infection of macrophages with *M. bovis* bacilli Calmette-Guerin (BCG) induces macroautophagy resulting in the elimination of the intracellular bacteria (67). Furthermore, cytokine expression may modulate macroautophagy in order to limit pathogen replication. Type II IFN enhances the degradation of *M. tuberculosis* and *Rickettsia conorii* in infected cells (65,66), and the TNF family member CD40L induces macroautophagy to facilitate the fusion of phagosomes containing *T. gondii* with lysosomes during parasite infection (21). Finally, although the role of autophagy in the adaptive immune response to bacteria and parasites has not yet been carefully examined, it is possible that in infected cells, fusion of autophagosomes containing the intracellular pathogens with lysosomes could be a mechanism by which bacterial or parasitic Ag associate with MHC class II molecules for presentation to CD4⁺ T cells.

Cancer

Similar to its role in infectious disease, autophagy is believed to contribute to both cancer development and tumor suppression (57). Autophagy may be induced during the later stages of oncogenesis as the rapid proliferation of the tumor depletes critical nutrients, thus suppressing further tumor growth. Additionally, following chemotherapy, tumor cells may initiate autophagy as a protective measure to recycle nutrients or remove damaged cellular components. For example, the induction of macroautophagy increases the survival of breast cancer cells undergoing treatment with 4-hydroxytamoxifen (68). Yet, how modulation of the autophagy pathways in tumor cells influences immune recognition and clearance has not yet been thoroughly investigated. As discussed above, one recent report suggests autophagy as a potential mechanism for the cross-presentation of tumor Ag onto MHC class I molecules. It is

also possible that during the induction of autophagy within a tumor cell, the formation of autophagosomes might provide a source of tumor Ag which could intersect with MHC class II molecules upon fusion of the autophagosomes with lysosomes.

Autoimmunity and tolerance

Autophagy appears to also play a role in the breakdown of tolerance and the development of autoimmunity (reviewed in (41,69)). During autophagy, contents of the cytosol and nucleus, including potential self Ag, are degraded in lysosomal compartments where MHC class II molecules reside. Thus, alterations in autophagy pathways may lead to a breakdown in tolerance or to insufficient tolerance induction and the development of autoimmunity. A recent report by Nedjic et al demonstrated that autophagy plays a key role in the generation and maintenance of tolerance in the thymus (55). In this study, autophagosomes were not readily detected in murine peripheral APC, yet cortical thymic epithelial cells (cTEC), which express MHC class II molecules, were found to display high levels of constitutive macroautophagy. The authors postulated that macroautophagy within cTEC may favor the presentation of a broader spectrum of self Ag via class II on these cells. Using thymi from *Atg5*^{-/-} mice, the authors evaluated the positive and negative selection of several MHC class II-restricted transgenic TCRs and found that the loss of *Atg5* altered the selection of some, but not all, TCRs. These results suggested that in the absence of autophagy, the composition of peptide-MHC class II complexes on the surface of cTECs was altered, thus skewing the T cell repertoire that was positively selected. Additionally, the lack of autophagy may also prevent MHC class II presentation of certain tissue specific Ag in medullary thymic epithelial cells (mTEC), thus impairing negative selection and allowing autoreactive T cells to exit into the periphery. In support of this, the authors observed severe colitis and inflammation of multiple organs in athymic mice which received thymi from *Atg5*^{-/-} mice. Studies of T cells deficient in *Atg5* revealed defects in cellular proliferation and survival (70), suggesting further analysis of the role of this gene product in regulating T cell responses as well as the thymic microenvironment are needed.

In humans, recent studies have identified ATG16L1 (autophagy-related 16-like 1), which is involved in autophagosome formation, and IRGM GTPase, which stimulates autophagy, as susceptibility loci for Crohn's disease (71–74). It has been proposed that mutations in these genes may lead to defective macroautophagy, thus impairing the innate immune response to bacterial infection and increasing the bacterial load in the gut, resulting in enhanced mucosal inflammation and the development of disease. A recent study using *Atg16L1*-deficient mice demonstrated an increase in the production of the inflammatory cytokines IL-1 β and IL-18 and in the susceptibility to colitis (75). Alternatively, as the data from the *Atg5*^{-/-} mice would suggest, inhibition of autophagy pathways might also lead to the failure to induce tolerance to mucosal self Ag. Lastly, the tissue damage associated with certain autoimmune diseases such as systemic lupus erythematosus (SLE) has been attributed to the defective clearance of apoptotic cells. Macroautophagy plays a role in the removal of these apoptotic cell corpses, and the impairment of this process may result in the build-up of necrotic material in tissues, and thus, inflammation.

Conclusions

Multiple mechanisms, including CMA and macroautophagy, have been implicated in the delivery of several endogenous Ag to lysosomes for their eventual presentation by MHC class II molecules. While substantial research has been conducted into the role of autophagy in the MHC class II presentation of endogenous Ag, far less is known about autophagy in MHC class I presentation and cross-presentation pathways. Further studies are also needed to more clearly define the relationship between the various autophagy pathways and MHC class I and class II

Ag presentation during the immune response to intracellular pathogens, tumors, and self Ag. Inhibition or enhancement of autophagy may alter the MHC class I and class II presentation of Ag to CD8⁺ or CD4⁺ T cells, respectively, thus providing novel therapeutic strategies for the treatment of infectious diseases, cancer, and autoimmunity.

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Abbreviations used in this paper

CMA, chaperone-mediated autophagy
 Cvt, cytoplasm-to-vacuole targeting
 mTor, target of rapamycin

Atg, autophagy-related gene
PE, phosphatidylethanolamine
hsc70, heat shock 70 kDa protein
LAMP, lysosome-associated membrane protein
ly-hsc70, lysosomal hsc70
ER, endoplasmic reticulum
DC, dendritic cells
ERAD, ER associated degradation pathway
3-MA, 3-methyladenine
ALIS, aggresome-like structures
DALIS, DC aggresome-like structures
cTEC, cortical thymic epithelial cells
mTEC, medullary thymic epithelial cells
Ii, invariant chain
GAD, glutamic acid decarboxylase
EBNA, EBV Nuclear Ag
MP1, influenza matrix protein 1
PKR, dsRNA-activated protein kinase
VSV, vesicular stomatitis virus
pDC, plasmacytoid DC
BCG, bacilli Calmette-Guerin
ATG16L1, autophagy-related 16-like 1
SLE, systemic lupus erthematosus.

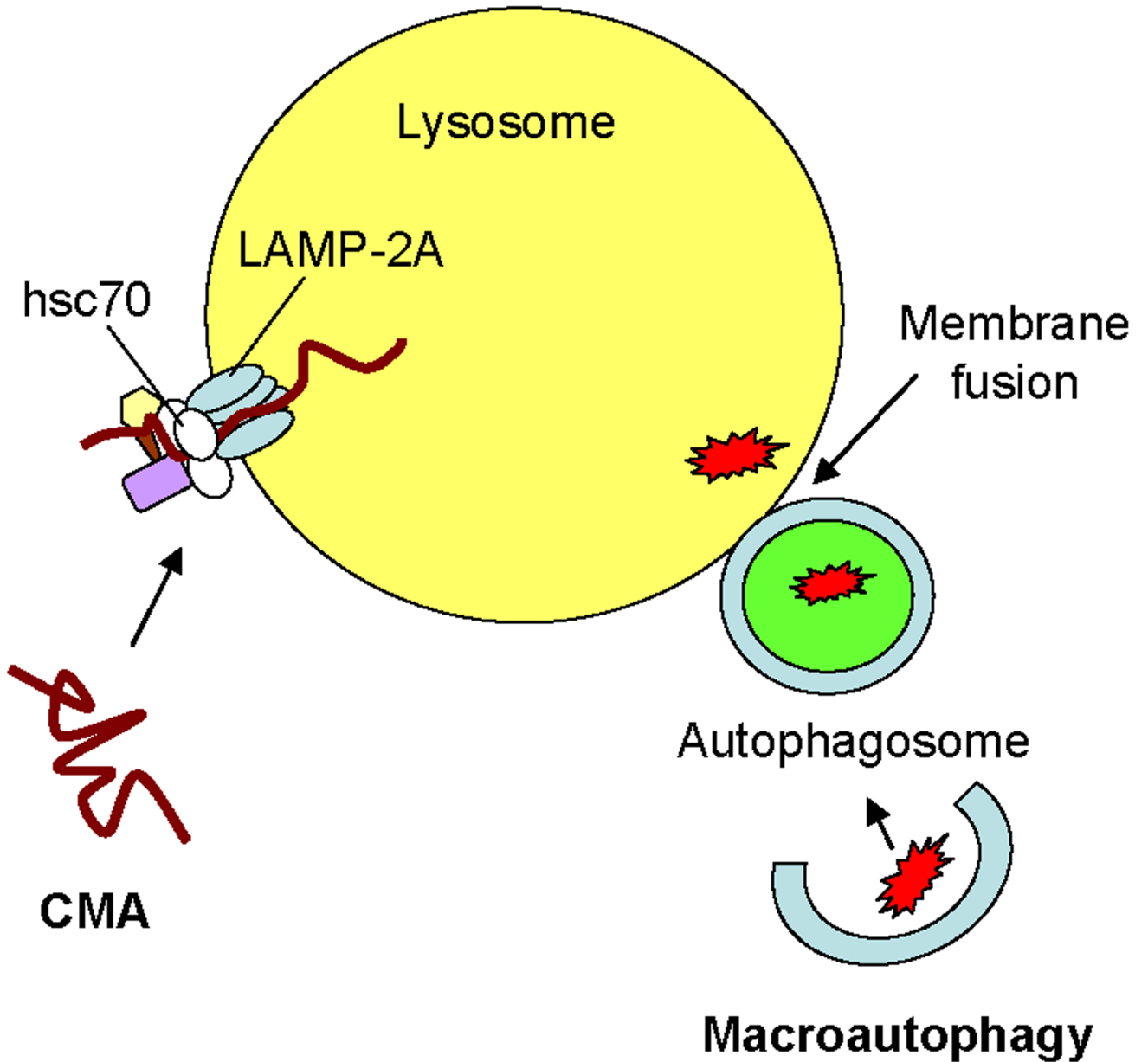


FIGURE 1. Pathways of autophagy in MHC class II-mediated Ag presentation

In macroautophagy, the cytoplasm is sequestered into double-membraned structures known as autophagosomes which fuse with lysosomes. In chaperone-mediated autophagy (CMA), specific cytosolic proteins are transported into lysosomes via a molecular chaperone/receptor complex composed of hsc70 and LAMP-2A.

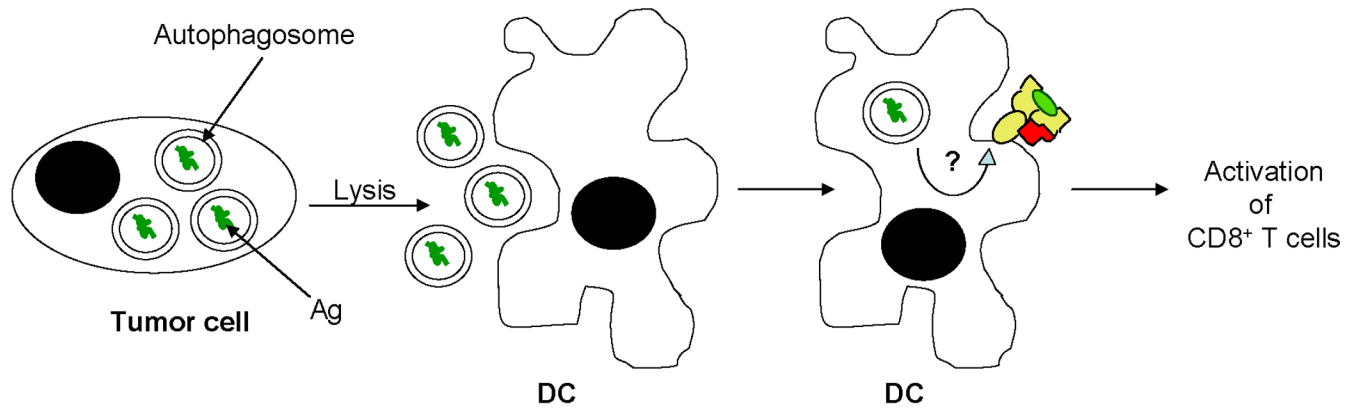


FIGURE 2. Autophagy as a potential mechanism for MHC class I-mediated presentation of tumor Ag

Macroautophagy can be induced in tumor cells undergoing cellular stress as a result of nutrient depletion or chemotherapy. Autophagosomes containing tumor Ag may be released into the extracellular environment where they are internalized by DC. How and where tumor Ag in autophagosomes intersect with MHC class I molecules in DC is unknown. DC present tumor peptides in the context of class I for the activation of CD8⁺ T cells.

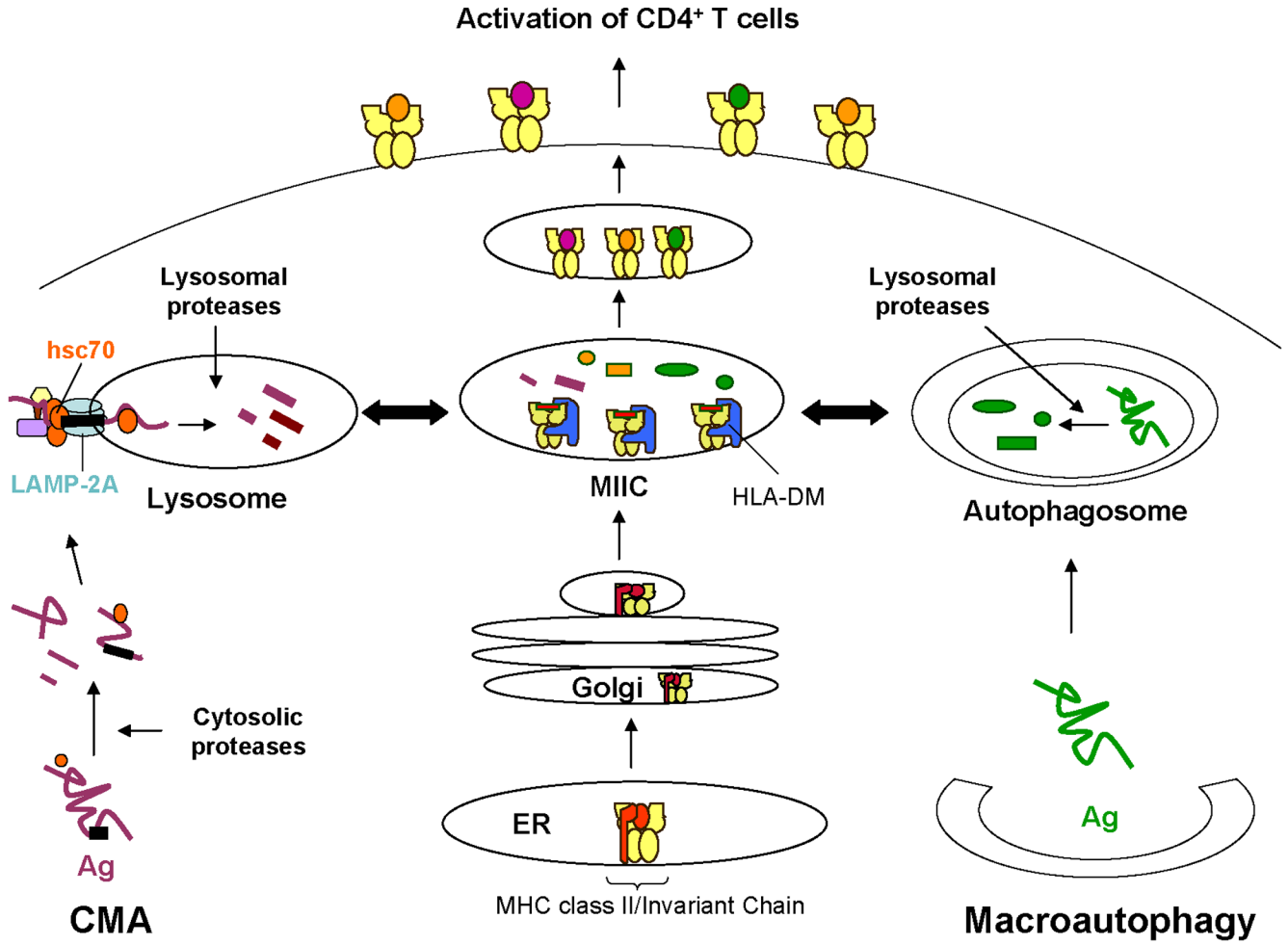


FIGURE 3. Autophagy pathways in the MHC class-mediated presentation of cytoplasmic and nuclear Ag

Approximately 10–25% of MHC class II molecules display peptides from nuclear and cytoplasmic Ag likely accessing autophagy to reach class II. *Left*, Cytoplasmic Ag are first processed by cytosolic proteases and then utilize chaperone-mediated autophagy (CMA) to access lysosomes. These antigenic fragments intersect with MHC class II molecules in a mature endosomal/lysosomal compartment known as the MIIC prior to presentation to CD4⁺ T cells. *Right*, Cytoplasmic or nuclear Ag are sequestered into double-membrane structures known as autophagosomes which continuously fuse with mature endosomes such as the MIIC and lysosomes, thus allowing Ag to associate with MHC class II molecules. Further processing of the cytoplasmic or nuclear Ag by lysosomal proteases may occur prior to binding to class II and presentation to CD4⁺ T cells.