ABSTRACT

Autophagy is an evolutionary conserved cellular process found in all eukaryotic cells to play roles in cell growth, development, homeostasis and innate immunity. In autophagy, sequestration of cytosolic contents initiates with the development of a phagophore that grows into an autophagosome while enclosing a portion of the cytosol. The completed autophagosome fuses with a lysosome to form autolysosomes. Lysosomal enzymes in autolysosome degrade the sequestered substrates for recycling back into the cytoplasm. Autophagy is involved in the innate immune response by delivering the intracellular pathogens to lysosomes for their elimination and contributes in adaptive immunity by promoting effective presentation of an antigenic peptide by MHC-II and MHC-I molecules. Autophagy facilitates a presentation of both exogenous and endogenous antigen by MHC-II molecule as well as by MHC-I molecule. Evidence supporting the role of autophagy in upregulating antigen presentation by CD1 molecule are also presented. Hence, autophagy in antigen presenting cells (APC), either in vivo or ex vivo is important for generating highly efficient APC for effectively fight against diseases. Ex vivo autophagy induced APC can be used for successful adoptive immunotherapy. Thus, a potential role of autophagy in antigen presentation, as well as the importance of this cellular process in immunotherapy, is established in this review.

Keywords: Autophagy, Antigen presentation, Adoptive, Immunotherapy, Diseases
INTRODUCTION

Autophagy is an evolutionarily conserved process which is common in all eukaryotic cells from yeast to human and plays roles in cell growth, development, homeostasis and innate immunity(1). During autophagy, a portion of cytoplasm containing damaged organelles, long-lived protein aggregates and/or cytosolic microorganisms are sequestered by double membrane vesicles to form autophagosomes. Then, autophagosomes fuse with lysosomes to form autolysosomes, where their contents are degraded by acidic lysosomal hydrolases (2, 3). Note that in metazoan cells, autophagosomes may receive vesicles from the endocytic pathway to form amphisomes before fusion with the lysosomes (2, 3). Thus, autophagy eliminates unwanted proteins and damaged organelles from cytoplasm through lysosomal proteolysis in order to prevent toxic accumulation and to provide additional nutrients by releasing molecular subunits such as amino acids to be reused by the cells. Hence, autophagy is mainly reported for its role in cell survival by maintaining cellular homeostasis during stress conditions such as starvation, hypoxia, and heat. In addition, autophagy has been shown to play important roles in both innate and adaptive immunity with few exceptions (4, 5). Autophagy is involved in the innate immune response by delivering the intracellular pathogens to the lysosome for their elimination(4) as well as plays a vital role in antigen processing and presentation(6, 7). Thus, autophagy as an innate immune response in professional antigen presenting cells contributes in adaptive immunity by promoting effective antigen presentation by MHC-II and MHC-I molecules(8-14). There is also emerging evidence that supports its role in antigen presentation by CD1 molecule(6, 15, 16). In the following sections, I will review the roles of autophagy in antigen presentation pathways and explore its clinical importance for immunotherapy of infectious diseases and cancers.

Autophagy in antigen presentation by MHC-II

In the conventional pathway, peptides processed from exogenous antigens are presented by MHC-II to CD4+ T cells(17). Recently, autophagy has been shown to play crucial roles in MHC-II presentation of peptides derived from not only exogenous but also endogenous antigens. Upon induction of autophagy, exogenous or endogenous antigens either located inside vesicles or free in the cytosol are enclosed by autophagosomes which will ultimately fuse with endosomal MHC-II loading compartments resulting in the increase in MHC-II antigen presentation(8-10). For example, autophagy induction by TLR-2 and NOD2 agonists was reported to enhance the delivery of phagocytosed and endocytosed antigens such as that...
of Salmonella to lysosomes for processing and loading of the antigenic peptides onto MHC-II molecules(18, 19). The increase in MHC-II presentation of Salmonella-antigens could be blocked by siRNA silencing of autophagy genes Atg5, Atg7 and Atg16L1 which further confirmed the contribution of autophagy in MHC-II presentation of these antigens(20). Similarly, Atg5-deficient dendritic cells infected with either OVA-herpes simplex virus-2 or OVA-Listeria monocytogenes are incompetent to prime OVA-specific CD4+ OT-II T cells, suggesting that these autophagy compromised dendritic cells are unable to process ovalbumin for MHC-II antigen presentation(20). Furthermore, by targeting influenza matrix protein 1 (MP1) to autophagosome through its fusion to autophagy protein Atg8/LC3, an enhancement of MHC-II presentation of the peptide derived from MP-1 was observed (10). In addition, autophagy was shown to be critical for the MHC-II presentation of cytosolic phosphotransferase (NeoR) antigen and nuclear antigen 1 (EBNA-1) of Epstein Barr virus (EBV) as demonstrated by the reduction in their presentation to CD4+ T cells when autophagy is inhibited by autophagy inhibitors 3-MA and wortmannin or by siRNA knockdown of an autophagy gene, Atg12 (8, 11). Likewise, EBV latent membrane protein 1 (LMP1) degradation and clearance are impaired when autophagy is inhibited by siRNA knockdown of autophagy genes, beclin 1 and Atg7 (21). LMP1 peptides presented via MHC-II molecules are often recognized by CD4+ T cells, supporting the role of autophagy in the processing of this antigen for MHC-II antigen presentation (21). Moreover, following infection of dendritic cells, HIV can inhibit autophagy for favoring its own replication as well as for preventing MHC-II presentation of its antigens to CD4+ T cells(22) through the interaction of an HIV protein Nef with Atg6/Beclin-1(22). Nevertheless, exogenous induction of autophagy can be employed to overcome this block resulting in an enhanced MHC-II presentation of HIV antigen and this presentation was shown to be diminished by siRNA knockdown of Atg5 and Atg8(23) supporting the crucial role of autophagy in this process. Also, endogenous processing of complement C5 for MHC-II antigen presentation in mouse macrophages and B-cells was shown to be hampered by the treatment with autophagy inhibitors NH4Cl and 3-MA, indicating that the presentation of C5 is reliant on autophagy(24). Autophagy induced by amino acid starvation was also reported to enhance the MHC-II presentation of several self-proteins such as RAD23, cathepsin D, HSP70 and EF1-a1(21). Interestingly, autophagosomes have recently been shown to emerge from MHC-II loading compartments upon induction of autophagy by TLR4 agonist lipopolysaccharide (LPS) in dendritic cells. This unconventional population of autophagosomes also contains antigen processing machinery for MHC-II presentation(25). In the context of adoptive
immunotherapy, it has been demonstrated that MHC-II presentation of mycobacterial antigen Ag85B in infected murine dendritic cells and macrophages is enhanced upon *ex vivo* induction of autophagy by rapamycin treatment resulting in an increased immunity against mycobacteria upon adoptive transfer of these cells into mice(9). This enhancement of MHC-II presentation of Ag85B could be blocked by suppression of autophagy through 3-MA treatment or siRNA knockdown of *beclin 1*(9). Altogether, it can be concluded that autophagy contributes to MHC-II antigen presentation of exogenous and endogenous antigens derived from the virus, bacteria, and cancerous cells resulting in activation and expansion of antigen-specific CD4+ T cells.

**Autophagy in antigen presentation by MHC-I**

In classical MHC-I antigen presentation pathway, cytosolic antigens from a virus, intracellular bacteria, and cancerous or self-proteins are digested by proteasomes and the generated peptides are transported into the endoplasmic reticulum (ER) through a complex of the transporter associated with antigen processing-1 and -2 (TAP1 and TAP2)(26). Then, the antigenic peptides with a length of 8-10 amino acids in the ER will bind to nascent MHC-I molecules and are ultimately transported to the cell surface via the secretory pathway for their presentation to CD8+ T cells(17). The contribution of autophagy in classical MHC-I antigen presentation is previously believed to be nonvital but there are several recent reports in support of an important role of autophagy in MHC-I antigen presentation in specific conditions. For instance, MHC-I presentation of antigens derived from herpes simplex virus 1 (HSV-1) has been shown to be mediated by autophagy. HSV-1 can inhibit autophagy through the interaction of its protein ICP34.5 with Beclin-1. When mice are infected with HSV-1 mutant lacking ICP34.5, the proliferation of CD8+ T-cells is significantly enhanced when compared to that of mice infected with wild-type HSV-1. These data indicated that autophagy is involved in processing and presentation of HSV-1 antigen via MHC-I(27). In another study, HSV-1 glycoprotein B-specific CD8+ T cell activation was shown to be impaired following inhibition of autophagy with bafilomycin A1, 3-MA or siRNA knockdown of *Atg5*, supporting the important role of autophagy in the processing and MHC-I presentation of this antigen. This is further confirmed by the detection of HSV-1 particles in autophagosomes by both fluorescence and electron microscopy. Glycoprotein B-specific CD8+ T cell response was also shown to be impaired by the inhibition of proteasomal degradation(12). The authors hypothesized that processing and presentation of glycoprotein B
may occur in two steps. First, glycoprotein B is degraded within the autolysosomes by Lysosomal hydrolases into peptides but these are not small enough in size to be presented by MHC-I molecules. As a result, in the second step, these peptides then escape from autolysosomes into the cytosol for further processing by the proteasome and are ultimately entered into the classical pathway of MHC-I antigen presentation in the ER(12). Similarly to glycoprotein B, MHC-I presentation in dendritic cells of two peptides M1.1 and M1.2 derived from a tumor protein mucin 1 was shown to be dependent on autophagy as demonstrated by the reduced response of antigen-specific CD8$^+$ T cells upon autophagy inhibition(28). Like that of glycoprotein B, MHC-I presentation of M1.1 and M1.2 peptides also relies on proteasomal degradation (28). On the contrary, however, MHC-I antigen presentation of human cytomegalovirus (HCMV) latency associated protein pUL138 depends on autophagy but not on proteasome or TAP(13). In this study, pUL138-specific CD8$^+$ T cell response is enhanced by heat shock-induced autophagy and is diminished by inhibition of autophagy with chloroquine, 3-MA or siRNA knockdown of Atg12. In contrast, the T cell response persists and is unaffected after inhibition of the proteasome with epoxomicin, lactacystin, or ER aminopeptidase inhibitor (13). Therefore, the authors hypothesized that both processing of pUL138 and successive loading of the peptide onto MHC-I occur in the autophagosomes without any aid of any classical pathway components. This hypothesis is further supported by the detection of recycling MHC-I molecules and measles virus F protein in a lysosome-like compartment where an exchange of previously-loaded peptides on recycling MHC-I molecules to newly-generated peptides takes place (29). In mild acidic condition, peptides previously loaded on the recycling MHC-I molecules are detached which allows the loading of newly generated peptides with higher affinity onto the empty MHC-I molecules for subsequent presentation (29). Furthermore, Johnstone et al. have recently shown that the MHC-I presentation of an epitope of respiratory syncytial virus is mediated by autophagy(30). Moreover, a study in B16 melanoma cells also revealed that the induction of autophagy with synergistic action of interferon-gamma (IFN-$\gamma$) and rapamycin can enhance the surface presentation of tumor antigens with MHC-I which is decreased after inhibition of autophagy with siRNA knockdown of beclin 1(31). In addition, in implanted melanoma cells, MHC-I presentation of tumor-derived antigens is significantly increased after induction of autophagy resulting in the proliferation of CD8$^+$ T cells and reduction of the tumor growth(14). Altogether, the aforementioned data pointed to a crucial role of autophagy in the MHC-I presentation of specific endogenous antigens.
In addition to autophagy role in the MHC-I presentation of endogenous antigens, autophagy is also reported to function in the MHC-I presentation of exogenous antigens which is known as cross-presentation. For instance, induction of autophagy in HEK293T cells by starvation or rapamycin treatment has been reported to enhance the cross presentation of OVA antigen to epitope-specific CD8\(^+\) T cells (32) and this autophagy-mediated cross-presentation is reduced upon autophagy inhibition with 3-MA in vitro(33). In another study, induction of autophagy in bone marrow-derived dendritic cells were shown to enhance OT-I specific T cell activation both in vitro and in vivo when the αAl\(_2\)O\(_3\) nanoparticle is used as an adjuvant for OVA. The αAl\(_2\)O\(_3\) nanoparticle-associated OVA is delivered to autophagosomes in dendritic cells for processing and cross-presentation of antigen to CD8\(^+\) T cells and this cross-presentation can be suppressed by autophagy inhibition with 3-MA, wortmannin or siRNA knockdown of beclin-1(34). Similarly, cross-presentation of tumor antigen gp100 in OVA-expressing HEK294T cells or in melanoma cells is increased by induction of autophagy and this can be diminished upon autophagy inhibition with 3-MA, wortmannin or siRNA knockdown of Atg6, Atg12 or beclin-1(35). Furthermore, autophagy was found to be enhanced in dying embryonic fibroblasts of box/Bak-deficient mouse. When these autophagy-enhanced dying cells are infected with Influenza A virus and are phagocytosed by antigen presenting cells, an increase in cross-presentation of viral antigen to CD8\(^+\) T cells was observed. In addition, this increase in cross-presentation of viral antigen is abolished by autophagy inhibition with siRNA knockdown of Atg5(36). These data indicated that autophagy-enriched dying cells act as antigen processing cells and then donate the processed antigens to the living antigen-presenting cells for cross-presentation. This idea is further supported by a study showing that the purified autophagosomes containing exogenous antigens act as antigen donating vesicles to dendritic cells for cross-presentation(37).

Although autophagy-mediated antigen presentation via MHC-I was mainly reported for viral and tumor antigens, it has also been described for antigens of intracellular bacteria and protozoa. For instance, induction of autophagy can increase MHC-I antigen presentation in macrophages and dendritic cells infected with \textit{M. tuberculosis} and \textit{M. bovis}(38). Recently, electron microscopic studies revealed that mycobacteria can escape from phagosomes into the cytosol which allows the entrance of mycobacterial antigens into the MHC-I antigen presentation pathway(39). In addition, when dendritic cells are infected with another intracellular bacterium \textit{Chlamydia}, autophagy was found to be induced through the interaction of bacterial components with a host factor called guanylate-binding protein. As a
result, cytosolic *Chlamydia* is enclosed by autophagosomes which in turn fuse with endosomes to produce cathepsin-positive bacteria-containing amphisomes(40). In the amphisomes, chlamydial antigens are partially processed by cathepsins and are then released into the cytosol for further processing by proteasomal degradation. Ultimately, processed antigenic peptides reenter into the amphisomes to be loaded onto MHC-I. Thus, autophagy plays an important role in the MHC-I presentation of chlamydial antigens(40). Likewise, autophagy was found to participate in the MHC-I presentation of antigen derived from an intracellular parasite *Toxoplasma gondii*(17, 41). *T. gondii* is able to survive in macrophages by avoiding the delivery of their vacuoles to lysosomes. However, autophagy induction by CD40 ligation was found to redirect *T. gondii* to lysosomes and *T. gondii* vacuoles were found to colocalize with autophagosome marker LC3 indicating that the fusion of *T. gondii* vacuoles with lysosomes relies on autophagy(41). Moreover, MHC-I antigen presentation machinery such as ER protein, TAP, and MHC-I molecules were detected in the autophagosomes containing *T. gondii*(17). All of these data pointed to the involvement of autophagy in the MHC-I presentation of *T. gondii* antigen. Altogether, it can be summarized that autophagy plays an important role in presentation of endogenous antigens as well as on cross-presentation of exogenous antigens via MHC-I molecules.

**Autophagy in antigen presentation by CD1**

The contribution of autophagy to CD1 lipid antigen presentation remains undetermined. However, there are several pieces of evidence that showed the link between autophagy and CD1 antigen presentation. For instance, deficiency of saposin C was reported to result in the disrupted autophagic flux and hence the inhibition of autophagosome-lysosome fusion in fibroblasts(42). In turn, saposin C-deficient fibroblasts were shown to be impaired in CD1b and CD1d presentation of exogenous antigens(43). In addition, presentation of lipid antigens by CD1b(44) and CD1d(45) can be suppressed by the treatment of endosomal-acidification inhibitors, such as chloroquine and concanamycin A, both of which are also inhibitors of autophagic flux. Thus, these data indicated that the delivery of lipid antigens to acidic compartments such as late endosomes and lysosomes is a vital step for processing and presentation of the lipid antigens by CD1 family members (46). This idea is further supported by the fact that HSV-1 was reported to inhibit CD1d antigen presentation by preventing transportation of newly synthesized or endocytosed CD1d molecules to the cell surface resulting in CD1d accumulation in LAMP1 positive vacuoles(15). In addition, HSV-1 was
also shown to inhibit autophagic flux by its interaction with Beclin 1(16) and as a result, HSV-1 particles were found to be accumulated in autophagosomes(12). Hence, efficient autophagic flux appears to be crucial for CD1 antigen presentation and points to an important role of autophagy in this process. However, direct evidence are required to make a concrete conclusion on the role of autophagy in CD1 antigen presentation.

Figure 1. Role of autophagy in MHC-I, MHC-II, and CD1 antigen presentation.

For MHC-II antigen presentation, antigen-loaded phagosome may inter classical pathway but after induction of autophagy, antigens either in phagosome or in the cytosol are enclosed by autophagosome and are transferred into MHC-II loading compartments (MIICs) for presentation. Moreover, escaped cytosolic antigenic peptides may also enter into lysosomes via macroautophagy or Chaperone-mediated autophagy and ultimately transport antigens into MIICs. For MHC-I antigen presentation, antigen-loaded autophagosome and amphisome may fuse with the lysosome to form autolysosome where antigens are processed and loaded onto
MHC-I molecules directly. Conversely, antigens may be escaped from autophagosomes or amphisosomes or autolysosome for entrance into the classical pathway of MHC-I antigen presentation. For CD1 antigen presentation, some CD1 molecules such as CD1b and mouse CD1d are entered too late endosomal and lysosomal compartments via sorting endosomes which in turn may fuse with autophagosomes or MICS resulting in available CD1 molecules in those compartments for loading of lipid antigens.

**Clinical importance of autophagy-modulated antigen presentation**

The evidence described above support the crucial role of the autophagy as a talented cellular mechanism for use in the generation of highly-efficient autologous antigen presenting cells. The adoptive transfer of these cells, in turn, will assist in the production of a sufficient number of antigen-specific T cells, of which is a prerequisite for successful adoptive immunotherapy (47, 48). It was reported that T-cell programming and succeeding therapeutic effectiveness are reliant on the signals received from antigen presented by antigen presenting cells(48). Although the use of artificial antigen presenting cells (APC) and systems instead of autologous antigen presenting cells have been developed(49), these artificial systems have drawbacks. For instance, CD3/CD28-based bead and K32 cells, both of which are used as aAPC for nonspecific expansion of antigen-specific T cells, fail to retain antigen specificity in expanded T cells(50). It was reported that CD3/CD28-based aAPC and K32 are not effective for long-lasting expansion of CD8 T cells as it loses their antigen specificity within short-range of expansion(50, 51). Although, other aAPC such as latex magnetic beads or microspheres, HLA-Ig based beads, liposome-based system, and MHC-II tetramer-based beads confer antigen specificity most of these systems are unable to uptake and process antigens for presentation as well as are unable to secrete cytokines or to provide the wide variety of costimulation (52). While *Drosophila*-based cell line used as aAPC can process antigen, these insect cells are not stable at 370 C and cause the release of *Drosophila* antigens because of huge self-destruction during culture with T cells(53). Although acellular aAPC are stable and non-biodegradable resulting in no chance of self-destruction, they may cause microembolism and antigenic reaction if all aAPC are not completely removed from cell culture before adoptive transfer(52). Thus, use of aAPC in adoptive immunotherapy requires an additional step of extensive purification of expanded T cells away from the utilized aAPC to avoid allergic reaction, autoimmunity, and host versus graft disease.
On the contrary, natural autologous APC is equipped with all of the required features for effective antigen presentation, such as antigen uptake and processing ability, antigenic specificity, variable MHC molecules, cytokine secretion and costimulation ability(52). Therefore, the use of natural autologous antigen presenting cells is preferred. However, current knowledge and technologies are still insufficient to control and regulate them successfully for effective antigen presentation resulting in the low quality of antigen presentation, low T-cell priming, and slow expansion of T cells. For instance, the use of autologous APC pulsed with tumor antigens was often unsuccessful to produce potent tumor-specific T cells because of low avidity TCR expression and the inability to recognize tumor cells(49). However, induction of autophagy in autologous APC should resolve the aforementioned problem and improve the quality of antigen presentation resulting in better quality of T-cell priming and expansion. Hence, it can be hypothesized that ex vivo induction of autophagy may be a promising process to solve the above drawbacks in the use of natural autologous APC in adoptive immunotherapy as in vitro and ex vivo induction of autophagy can be used to increase both quantity and quality of antigen presentation which is a prerequisite for successful adoptive immunotherapy (Fig. 2).

However, in vivo induction of autophagy with fasting(54), rapamycin(9) and vitamin D(55) can also be possible but such nonspecific in vivo induction of autophagy in the patient’s body may be clinically harmful and may lead to undesirable effects. For instance, induction of autophagy in cancer cells was shown to cause cytolytic death in the early-stage cancer cells but in contrast, help the late-stage cancer cells to survive in hypoxic condition(56). Likewise, HIV-1 is eliminated by induction of autophagy early after macrophage infection but at the later stage, HIV-1 can inhibit autophagic flux resulting in the generation of non-lytic autophagosomes that favors the synthesis of Gag, a precursor protein for HIV-1 assembly, resulting in enhanced viral replication(22, 57, 58). Therefore, development of a suitable autophagy inducer which will induce autophagy selectively in targeted cells is a prerequisite for using in vivo induction of autophagy in therapy.
In one approach of adoptive immunotherapy, autologous APC can be isolated from peripheral blood by leukapheresis followed by their ex vivo expansion, pulsation with antigen and induction of autophagy in order to generate potent activated APC for direct transfer back into the patient. In another approach, the generated potent activated autologous APC can further be used for activation and proliferation of isolated autologous T cells to generate highly active, avid and antigen-reactive T-cells followed by their adoptive transfer into the patient.

Figure 2. Use of ex vivo autophagy-induced antigen presenting cells for adoptive immunotherapy.
In both approaches, phenotypic and functional test should be performed for assessing the quality before the adoptive transfer.

SUMMARY

In this review, it has been shown that autophagy plays a significant role in antigen presentation via MHC-I, MHC-II, and CD1 molecules. In addition, the clinical importance of autophagy for developing a new approach to treatment of diseases was mentioned. Future investigations may be focused on defining appropriate conditions and supporting factors that will help improve autophagy-mediated antigen presentation and subsequent effective programming of T cells. The fine-tuning of optimum conditions for ex vivo autophagy-induced effective antigen presentation as well as maximizing immunotherapeutic efficacy and minimizing morbidity caused by adoptive transfer may provide a new treatment for slowly progressing diseases such as cancer, tuberculosis, and other chronic infectious diseases, of which better effective treatment is needed. For example, the cell wall of M. tuberculosis is rich in lipid molecules but most of the aAPC is focused on the presentation of antigenic peptides. CD1 presentation of M. tuberculosis lipid antigens is important for activation of lipid antigen-reactive T cells and subsequent control of tuberculosis (59). Hence, establishing a standard process of ex vivo induction of autophagy for effective presentation of mycobacterial lipid antigen via CD1 molecules and their succeeding use in adoptive immunotherapy may be a new way for TB control especially in the emergence of MDR and XDR TB. Similarly, the use of autologous tumor infiltrating lymphocytes (TIL) for adoptive immunotherapy of metastatic melanoma has emerged as an attractive treatment of choice. However, the clinical response rate of TIL-based immunotherapy to metastatic melanoma is still about 50% leading to the expectation of increasing more success rate by improving the quality and quantity of TIL(60). Use of ex vivo autophagy induced APC may be one of the vital factors for improving quality and quantity of TIL which in turn may increase the clinical response rate of TIL-based therapy to melanoma. Hence, the development of successful immunotherapy by using autophagy induced antigen presenting cells for treatment of these diseases may be a new hope of life for millions of people waiting for death.

Conflict of interest

The authors do not have any potential conflicts of interest to declare.
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