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Antigen processing via autophagy – not only for MHC class II presentation anymore?

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Abstract

T cells monitor intra- and extracellular protein composition via proteolytic products that are displayed to them on major histocompatibility complex (MHC) molecules. For this purpose it has been documented that MHC class II molecules, which were originally thought to just display lysosomal products of endocytosed proteins to CD4⁺ helper T cells, can also present intracellular substrates of autophagic pathways. This has triggered the interest of immunologists into the role of autophagy in antigen processing in general, and recently additional autophagic mechanisms for intra- and extracellular antigen processing onto MHC class I molecules for presentation to CD8⁺ cytolytic T cells have been revealed. Here, I will review the contribution of autophagy for MHC class I and II antigen processing and presentation to T cells.

Keywords

cross-presentation; autophagosome; MHC class II loading compartment; autophagy related protein (Atg); lysosome; T cells; thymus

1. Introduction

T cells dig through proteolytic waste to identify infected or transformed cells, which they need to eliminate during immune responses and for tumor immune surveillance. This proteolytic waste is presented to them in the form of peptides on major histocompatibility complex (MHC) molecules. In this process, MHC class I molecules present primarily proteasomal degradation products to cytolytic CD8⁺ T cells, while MHC class II molecules present peptides that are mainly generated by lysosomal degradation to CD4⁺ T cells. Through this division of labor, MHC presentation monitors the two main proteolytic systems in eukaryotic cells, proteasomal and lysosomal degradation. Both of these systems are involved in the degradation of extra- and intracellular antigens in antigen presenting cells. Proteasomes degrade soluble proteins after ubiquitination [1]. These have either been synthesized in the cytosol or escaped to this location from endosomes by a so far poorly defined mechanism during cross-presentation. In contrast, lysosomes can process endocytosed antigens after endosome fusion with lysosomes or intracellular antigens after

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autophagic delivery from the cytoplasm to lysosomes [2]. By these mechanisms, lysosomes can degrade complex structures like whole organelles or pathogens that are delivered to them via endocytosis or autophagy.

For the import of intracellular antigens into lysosomes at least three distinct pathways of autophagy exist. These are called macroautophagy, microautophagy and chaperone-mediated autophagy (Figure 1) [3]. Microautophagy delivers cytoplasmic material via invagination of the vacuolar membrane to the lysosomal cell organelle in yeast, but has not yet been characterized in higher eukaryotic cells. Chaperone-mediated autophagy (CMA) seems to be a late response to starvation and imports soluble proteins via a signal peptide dependent mechanism into lysosomes [4]. This import is assisted by cytosolic and lysosomal HSC70 proteins as well as Lamp2A, and leads to lysosomal degradation of the imported protein. Finally, macroautophagy occurs constitutively and at increased levels early after starvation in eukaryotic cells including mammalian tissues [5]. It delivers cell organelles and protein aggregates for lysosomal degradation. For this purpose a double membrane, possibly mainly derived from rough ER, engulfs part of the cytoplasm and forms upon closure around its cargo, a double membrane surrounded autophagosome. This then fuses with late endosomes to form amphisomes and with lysosomes to form autolysosomes. In this process, two ubiquitin-like proteins are involved [6]. One, called autophagy related protein (Atg) 12 is coupled in an ubiquitin-like reaction to Atg5, then associates with Atg16L and forms a coat on the outer surface of the forming autophagosome. This complex guides the conjugation of the other ubiquitin-like protein Atg8 to phosphatidylethanolamine in the inner and outer autophagosome membrane. Upon completion all Atgs from the outer membrane are recycled, while Atg8 stays with the inner membrane. Eventually this Atg8 portion and the macroautophagic cargo is degraded by lysosomal hydrolysis. Apart from these functions of autophagy in cellular catabolism, which are utilized by the immune system for innate and adaptive immune responses, particularly macroautophagy seems to assist also in antigen processing of intracellular and extracellular material for MHC class I and II presentation, as well as help in the packaging of antigens for efficient cross-presentation. These different facets of autophagic involvement in antigen processing for MHC presentation to T cells will be discussed in the following.

2. Intracellular antigen processing for MHC class II presentation via autophagy

The classical paradigm of antigen processing for MHC class I and II presentation suggested that intracellular antigens get processed onto MHC class I and extracellular ones on class II. However, in peptide elution studies it was noted that 20-30% of natural MHC class II ligands were derived from cytosolic and nuclear antigens [7-9]. Moreover, in pioneering studies Eric Long and colleagues demonstrated that some viral antigens were intracellularly processed for MHC class II presentation [10-13]. Gitta Stockinger and coworkers were then the first to show that 3-methyladenine, an inhibitor of class III PI3 kinases and, therefore, among other vesicular trafficking pathways also macroautophagy, could compromise MHC class II presentation of complement C5, which was used as a model antigen in this study [14]. By also using pharmacological inhibitors two additional antigens could be demonstrated to become presented to CD4⁺ T cells after macroautophagy. These were neomycin phosphotransferase II [15] and mucin 1 [16]. Around the same time we found that nuclear antigen 1 of the Epstein Barr virus (EBNA1) could be found in autophagosomes and siRNA mediated silencing of the essential macroautophagy gene *atg12* inhibited EBNA1 presentation on MHC class II molecules of Epstein Barr virus transformed B cells [17]. We extended these studies to demonstrate that autophagosomes frequently fuse with MHC class II loading compartments in human epithelial and B cell lines as well as dendritic cells [18]. Moreover, coupling of a model antigen to Atg8/LC3 enhanced its presentation on MHC

class II molecules. Consistent with these findings cytosolic and nuclear antigen presentation on MHC class II molecules could be enhanced by macroautophagy induction via starvation, and Peptides derived from the mammalian homologues LC3 and Gabarap of the essential autophagy gene product Atg8 could be eluted from MHC class II molecules [9,19]. These in vitro findings were recently confirmed in two in vivo settings. During thymic T cell education, it was demonstrated that macroautophagy deficient thymi lacking the essential autophagy gene *atg5* were unable to positively select some, but not other CD4⁺ T cell specificities, and gave rise to an autoreactive T cell repertoire, suggesting incomplete negative selection [20]. These data implicated macroautophagy in self-protein processing for MHC class II presentation during central tolerance induction. In addition to tolerance induction, it was demonstrated that vaccination with dendritic cells that had been treated with rapamycin to enhance MHC class II presentation of the mycobacterial antigen Ag85B elicited more potent and protective CD4⁺ T cell responses against this antigen [21]. These studies document a role for macroautophagy in intracellular antigen processing onto MHC class II.

In addition, chaperone-mediated autophagy might constitute a second route, by which cytosolic antigens can be presented to CD4⁺ T cells. MHC class II presentation of the two autoantigens GAD65 and SMA could be enhanced by overexpression of CMA components [22]. Therefore, both autophagic pathways of mammalian cells seem to be used by the immune system to display intracellular antigens on MHC class II for CD4⁺ T cell stimulation.

3. Autophagic assistance for processing of extracellular antigen

In addition to intracellular antigen delivery for MHC class II presentation after autophagy, another role for this catabolic process in extracellular antigen delivery for lysosomal degradation was recently revealed. Toll-like receptor (TLR) signaling via TLR2 enhanced fusion of TLR2 ligand carrying endosomes with lysosomes in a macroautophagy dependent fashion [23]. Indeed, Atg8/LC3 seemed to be transiently recruited to these endosomes, which ultrastructurally did not display double membranes. From these data the authors suggested that the autophagic machinery might also be involved in endosome fusion with lysosomes. However, these findings could be also consistent with amphisome formation prior to fusion with lysosomes. In addition to facilitating fusion with lysosomes, macroautophagy was also found to support the trafficking of endocytosed B cell receptors (BCRs) to TLR9 containing compartments [24]. For this purpose cross-linked BCR was delivered to autophagosomes, which then recruited TLR9 containing endosomes. This mechanism could facilitate TLR9 signaling upon endocytosis of DNA containing complexes, both during immune responses and autoimmunity. These findings suggest that macroautophagy might facilitate antigen processing of endocytosed material. Amphisomes, the fusion vesicles of endosomes and autophagosomes, might seek out lysosomes more efficiently. However, which quality of autophagosomes or amphisomes facilitates fusion with lysosomes needs further investigation.

4. MHC class I presentation of autophagic cargo

Even more surprising than this role in endocytosis, was the recent suggestion that macroautophagy could contribute to Herpes simplex virus (HSV) -1 antigen processing for MHC class I presentation [25]. At late timepoints of HSV-1 infection, this study reported that autophagosomes were formed from the membrane of the outer nuclear leaflet around HSV-1 particles and that inhibition of macroautophagy by siRNA mediated silencing of the essential autophagy gene *atg5* inhibited HSV specific CD8⁺ T cell stimulation. This study suggested that macroautophagic cargo can gain access to MHC class I presentation.

Interestingly, this processing still relied on the proteasome, indicating that HSV-1 antigens can escape from autophagosomes for cytosolic processing. How this might occur and why HSV-1 antigens take the detour via autophagosomes to access proteasomal degradation for MHC class I antigen presentation is unclear to date and requires additional clarification. An additional mechanism, by which macroautophagy seems to influence antigen presentation to CD8⁺ T cells is the degradation of MHC class I molecules themselves [26]. In B16 melanoma cells macroautophagy stimulation decreased MHC class I surface expression, while macroautophagy inhibition had the opposite effect. Both pharmacological inhibitors as well as siRNA mediated silencing of atgs were used in these studies. Consistent with MHC class I down-regulation by macroautophagy, MHC class I molecules could be found in autophagosomes under these conditions. Interestingly, IFN- γ treatment prevented their access to autophagosomes. IFN- γ mediated MHC class I upregulation was then sensitive to macroautophagy inhibition, suggesting that in the presence of this cytokine macroautophagy could contribute to MHC class I loading. These studies suggest that macroautophagy can contribute to antigen presentation to CD8⁺ T cells by either regulating cellular MHC class I levels or even antigen processing.

5. Cross-presentation of autophagy substrates

In addition to intracellular antigen processing for MHC class I presentation after macroautophagy, macroautophagy in the donor cell might also be beneficial for cross-presentation on MHC class I molecules. It was reported that antigens were more efficiently cross-presented by dendritic cells (DCs) from melanoma cells, when these were capable to perform macroautophagy [27]. Optimal packaging of antigens for cross-presentation by macroautophagy was suggested after purified autophagosomes from the tumor cells were found to be highly immunogenic. While it is difficult to envision, how autophagosomes could leave the cell for cross-presentation, autophagosomes fuse with late endosomes to generate multivesicular bodies, from which immunogenic vesicles, called exosomes, originate [28]. Therefore, it is tempting to speculate that macroautophagy could facilitate exosome release, possibly even contributing its inner membrane and cargo to these vesicles. A second study documented that cross-presentation of influenza antigens by DCs occurred more efficiently from infected apoptosis-deficient than from wild-type mouse embryonic fibroblasts [29]. The authors of this study suggested that macroautophagic cell death might be more immunogenic than apoptosis. However, macroautophagy supported antigen packaging similar to what was observed in the previous study could have contributed to cross-presentation from influenza infected cells which survived longer due to the blockade of apoptosis. Therefore, macroautophagy might facilitate antigen packaging for optimal cross-presentation on MHC class I molecules to CD8⁺ T cells. Along these lines, it was shown that under starvation conditions during embryogenesis, energy generation through macroautophagy was necessary to efficiently flip phosphatidylserine to the outer leaflet of apoptotic bodies for efficient phagocytosis by macrophages [30]. Macroautophagy deficiency led to accumulation of apoptotic corpses. Therefore, macroautophagy might facilitate optimal vesicular packaging of antigens for cross-presentation on MHC class I molecules.

6. Conclusions

Cellular protein degradation is used by the immune system to generate peptides for MHC presentation to T cells. Autophagy, as a pathway that delivers cytoplasmic constituents for lysosomal degradation, is also harnessed in this fashion. However, in addition to delivering cytosolic material to late endosomes for MHC class II loading, recent studies have also revealed a role for macroautophagy in enhancing endosome fusion with lysosomes for MHC class II presentation, regulating intracellular antigen processing for MHC class I

presentation, and packaging antigens for optimal cross-presentation on MHC class I molecules. These additional pathways of autophagic antigen delivery require further study to reveal the underlying mechanisms. However, they might be harnessed for vaccine design by targeting antigens for macroautophagy.

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Figure 1. Autophagic pathways

At least three pathways lead to cytoplasmic content degradation in eukaryotic cells. These collectively constitute autophagy and are called macroautophagy, microautophagy and chaperone-mediated autophagy. During macroautophagy, autophagosomes form from isolation membranes and can engulf protein aggregates and cell organelles. They then fuse with lysosomes or late endosomes for degradation of their content and inner membrane. During microautophagy, the lysosomal membrane invaginates and delivers cytoplasmic content into the lysosome lumen, where it is degraded. During chaperone-mediated autophagy, signal peptide (KFERQ) carrying proteins are imported into the lysosome lumen with the assistance of one cytosolic and one lysosomal HSC70 member as well as of Lamp2A.

Figure 2. Autophagy assists antigen processing on MHC class I and II molecules

A) Macroautophagy delivers cytosolic antigens to MHC class II loading compartments (MIIC) for lysosomal degradation and presentation to CD4⁺ T cells. B) Chaperone-mediated autophagy might deliver cytosolic antigens directly into MIICs for loading and presentation on MHC class II molecules. C) Macroautophagy assists endosome fusion with lysosomes, possibly via recruiting autophagosomes to endosomes. This delivers endosomal cargo more efficiently for lysosomal degradation, possibly enhancing MHC class II presentation. D) There might be also a pathway, by which antigen can leave autophagosomes for proteasomal degradation in the cytosol, followed by loading and presentation on MHC class I molecules. E) Macroautophagy seems required in the antigen donor cell for efficient cross-presentation on MHC class I molecules, possibly by assisting exosome generation after autophagic delivery of antigens to multivesicular bodies (MVB).