Abstract: Autophagy delivers cytoplasmic constituents for lysosomal degradation. This catabolic pathway can be used to deliver intracellular antigens for major histocompatibility complex (MHC) class II presentation. In addition, recent evidence suggests that it also facilitates the processing of extracellular antigens for both MHC class I and II presentation.

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**Antigen processing for MHC presentation by autophagy**

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**Abstract**

Autophagy delivers cytoplasmic constituents for lysosomal degradation. This catabolic pathway can be used to deliver intracellular antigens for MHC class II presentation. In addition, recent evidence suggests that it also facilitates the processing of extracellular antigen for both MHC class I and II presentation.

**Introduction and context**

Classically, MHC class I molecules present intracellular antigens to CD8⁺ T cells and MHC class II molecules extracellular antigens to CD4⁺ T cells during adaptive immune responses. However, professional antigen presenting cells, like DCs, can process extracellular antigen for MHC class I presentation by a pathway called cross-presentation [1]. Vice versa, fragments of nuclear and cytosolic antigens have been found among natural MHC class II ligands [2-3], and some antigens have been described to be presented on MHC class II after intracellular processing [4-5]. Some of these use autophagy to gain access to lysosomal degradation in MHC class II loading compartments [6-10].

Primarily one particular autophagic pathway, called macroautophagy, has been implicated in intracellular antigen processing for MHC class II presentation to CD4⁺ T cells.
During macroautophagy an isolation membrane, which can originate from rough endoplasmic reticulum, the outer nuclear envelope membrane or the outer mitochondrial membrane [11-15], engulfs cytoplasmic constituents like damaged organelles, protein aggregates and pathogens. Two ubiquitin-like systems with the Atg8 and Atg12 proteins at their heart are involved in extension of the autophagosomal membrane and substrate recruitment to its interior [16-18]. Upon completion of the resulting double membrane surrounded autophagosome, these proteins are removed from the outer autophagosomal membrane, which allows then the fusion with lysosomes and late endosomes, like multivesicular bodies (MVBs). The inner autophagosomal membrane and the autophagosome cargo are then broken down by lysosomal hydrolases. A subset of MVBs is used in MHC class II expressing cells for antigen loading of these molecules and called MHC class II loading compartments (MIICs). Autophagosomes fuse quite efficiently with these vesicles, resulting in MHC class II presentation of the autophagic cargo [19]. Thus, macroautophagy can deliver cytoplasmic antigens for MHC class II presentation to CD4+ T cells.

**Major recent advances**

However, in addition to the mechanistically fairly plausible intracellular antigen processing onto MHC class II molecules via macroautophagy, recent studies have also suggested that macroautophagy might assist extracellular antigen processing for MHC class I and class II presentation. With respect to cross-presentation, two studies have demonstrated that viral and tumor antigens are more efficiently presented in trans to CD8+ T cells when the antigen donor cell can perform macroautophagy [20-21]. In the first of these studies, apoptosis deficient mouse embryonic fibroblasts (Bax/Bak−/− MEFs) were more efficiently cross-presented after influenza A virus infection than wild-type MEFs, and this cross-presentation was inhibited by siRNA mediated silencing of the essential macroautophagy gene product Atg5 [20]. In a
second study, cross-presentation of the model antigen ovalbumin and the melanocyte differentiation antigen gp100 was diminished when macroautophagy was compromised in the antigen donating epithelial and melanoma cell lines via siRNA against Atg6 and Atg12 [21]. These studies suggest that macroautophagy assists in the packaging of antigens for efficient cross-presentation.

In addition, macroautophagy seems to also facilitate the transport of endocytosed antigen to lysosomal degradation and loading onto MHC class II molecules. Enhanced delivery of phagocytosed material to lysosomes with the assistance of the molecular macroautophagy machinery was first described after TLR2 stimulation of murine macrophages [22]. Furthermore, NOD2 stimulation enhanced macroautophagy, which enhanced lysosomal degradation of *Salmonella* [23]. This pathway also delivered *Salmonella* encoded antigens to MHC class II presentation, and was sensitive to siRNA mediated silencing of Atg5, Atg7 and Atg16L1. Interestingly, mutations in NOD2 and Atg16L1, which predispose for Crohn disease, also compromise both bacterial clearance and MHC class II presentation of bacterial antigens to CD4⁺ T cells. Along the same lines, Atg5 deficient DCs are compromised in priming CD4⁺ T cell responses after herpes simplex virus (HSV) infection and to efficiently process extracellular ovalbumin for MHC class II presentation [24]. At the same time, priming of CD8⁺ T cell responses and cross-presentation on MHC class I are not affected. Finally, human immunodeficiency virus (HIV) infection of DCs seems to inhibit macroautophagy, in order to increase virus production and to prevent viral antigen presentation to CD4⁺ T cells [25]. Macroautophagy stimulation enhances and siRNA mediated silencing of Atg5 and Atg8 decreases HIV antigen presentation on MHC class II, but not on MHC class I molecules. Altogether, these data suggest that macroautophagy facilitates endosome cargo delivery for lysosomal degradation and this results in increased extracellular antigen processing for MHC class II presentation to CD4⁺ T cells.
Future directions

In light of these recent advances it has become clear that macroautophagy regulates antigen presentation by MHC molecules beyond just intracellular antigen processing for CD4+ T cell stimulation. However, the mechanisms of antigen packaging by macroautophagy for cross-presentation and macroautophagy-mediated acceleration of endosome degradation by lysosomes remain elusive. In antigen donor cells macroautophagy could provide the necessary energy to decorate dying cells with ligands for phagocytosis, like for example phosphatidylserine, which needs to be flipped from the inner to the outer cell membrane leaflet in order to become an eat-me signal [26-27]. Alternatively, autophagosome cargo could also be more efficiently released from MVBs via an alternative secretion pathway recently reported for the yeast *Pichia pastoris* and the slime mold *Dictyostelium discoideum* [28-29]. With respect to macroautophagic assistance for endosome fusion with lysosomes, it first needs to be clarified, if this represents a alternative use of Atgs, independent of macroautophagy, as was initially proposed [22], or if amphisomes, the fusion vesicles between autophagosomes and endosomes, get targeted more rapidly to lysosomes. In a second step, the molecular basis for this enhanced targeting then needs to be elucidated. Irrespective of the mechanism, macroautophagic support for endosome fusion with lysosomes could explain why TLR coating increases antigen processing for MHC class II presentation [30]. Although much more needs to be done to characterize the underlying mechanisms, the recent studies, discussed in this report, suggest novel and exciting pathways in immunology and cell biology in general, by which macroautophagy regulates endocytosis.
and exocytosis, in addition to its classical function in the degradation of cytoplasmic constituents by lysosomes.
Abbreviations

Atg, autophagy related gene; MHC, major histocompatibility complex; TLR, toll like receptor; NOD, nucleotide-binding oligomerization domain containing; DC, dendritic cell

Competing interests

The author declares that he has no competing interests.

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FIGURE LEGEND

Figure 1. Macroautophagy regulates cross-presentation and intracellular as well as extracellular antigen presentation on MHC class II molecules.

Autophagosomes, which form as isolation membranes around cytoplasmic constituents, fuse with MHC class II loading compartments (MIIC), which are a subset of multivesicular bodies (MVBs) They do so directly or after fusion with endosomes as so called amphisomes. From the MVBs autophagic cargo can also escape via exocytosis and is then efficiently cross-presented by dendritic cells (DCs).
References


Figure 1

- MIIC
- Autophagosome
- Isolation membrane
- Amphisome
- Endosome

HLA-DM
MHC class II

CD4+ T cell
DC