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Review

Role of autophagy in MHC class I-restricted antigen presentation

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ABSTRACT

Major histocompatibility complex (MHC) class I molecules present peptide antigens to MHC class I-restricted $CD8^+$ T lymphocytes. The peptides loaded onto MHC class I molecules are typically derived from cytosolic antigens, which includes both self and foreign proteins. In addition to this classical MHC class I antigen presentation pathway, some cell types, especially dendritic cells can present antigens from exogenous sources to MHC class I-restricted $CD8^+$ T cells, in a process called cross-presentation. A variety of cellular processes, including endocytosis, vesicle trafficking, and autophagy, play critical roles in these antigen presentation pathways. In this review article, we discuss the role of autophagy, an intracellular degradation system that delivers cytoplasmic constituents to lysosomes, in MHC class I-restricted antigen presentation. A mechanistic understanding of the role of autophagy-related proteins in MHC class I restricted antigen presentation may guide future efforts in manipulating autophagy to prevent or treat human disease.

1. Introduction

Major histocompatibility complex (MHC) proteins present self and foreign peptide antigens to T lymphocytes (Blum et al., 2013). The peptide antigens bound by MHC class I molecules are typically derived from intracellular sources and are presented to $CD8^+$ T cells with cytotoxic effector functions. In contrast, the peptide antigens bound by MHC class II molecules are predominantly derived from extracellular sources and are presented to $CD4^+$ T cells that release cytokines and assist other immune cells in eliminating foreign invaders. A key step in the generation of effective T cell responses is the presentation of antigens to naïve T lymphocytes in peripheral lymphoid organs by professional antigen-presenting cells (APCs), especially dendritic cells (DCs). Activated DCs can deliver co-stimulatory signals to naïve T lymphocytes that synergize with signals received via the T cell receptor (Steinman, 1991). DCs and other professional APCs have co-opted a variety of cellular processes, including endocytosis, phagocytosis, vesicle trafficking and autophagy, to facilitate the generation and loading of antigenic peptides onto MHC molecules. Here we review recent studies on the role of autophagy, a self-degradation process, in MHC class I-restricted antigen presentation.

2. A brief primer on autophagy

Autophagy is an evolutionarily conserved nutrient sensing system that induces the degradation of cytoplasmic proteins and damaged organelles by lysosomes (Ktistakis and Tooze, 2016; Mizushima et al., 2011). The resulting degradation products are then used to generate energy and new cellular components. Three different mechanisms have been described in mammals for the delivery of autophagic cargo to lysosomes: macroautophagy, microautophagy and chaperone-mediated autophagy. Macroautophagy involves eradication of damaged organelles or unused proteins, microautophagy involves direct engulfment of small amounts of cytoplasmic material into the lysosome, and chaperone-mediated autophagy involves targeting of proteins to the lysosome via binding to a complex that includes the chaperone hsc70 (heat shock cognate 71 kDa protein). Macroautophagy, which is the focus of this review and hereafter referred to simply as autophagy, involves a variety of protein complexes composed of autophagy-related gene (Atg) products that were originally identified in yeast but are largely conserved in mammals. Nutrient deprivation and other stresses initiate autophagy by inducing the dissociation of the mammalian/mechanistic target of rapamycin complex 1 (mTORC1) from the Atg1/ULK complex. Release of the Atg1/ULK complex from mTORC1 triggers

Abbreviations: APC, antigen-presenting cell; Atg, autophagy-related gene; DC, dendritic cell; DRiP, defective ribosomal product; GCN2, general control nonderepressible 2; LC3, microtubule-associated protein 1A/1B-light chain 3; MHC, major histocompatibility complex; mTORC1, mammalian/mechanistic target of rapamycin complex 1; OVA, chicken ovalbumin; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol 3-phosphate; TAP, transporter associated with antigen processing; TIM-4, T cell immunoglobulin mucin-4; Vps34, vacuolar protein sorting 34; YF, yellow fever

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autophagosome nucleation and elongation. The latter process requires Atg6/Beclin-1, which recruits the class III phosphatidylinositol 3-kinase (PI3K) Vps34 to generate phosphatidylinositol 3-phosphate (PI3P). The generation of PI3P leads to recruitment of two ubiquitin-like proteins, Atg12 and Atg8 (the latter is called microtubule-associated protein 1A/1B-light chain 3 [LC3] in mammals) and their associated conjugation systems, the E1-like conjugation system Atg7, and the E2-like conjugation system Atg10. Activation of these conjugation systems results in the covalent binding of Atg12 with Atg5, which together bind Atg16L1 to form pre-autophagosome structures. In a second ubiquitin conjugation reaction, Atg4 cleaves LC3 to LC3-I, which is then lipidated by Atg7 and Atg3 to generate LC3-phosphatidylethanolamine (LC3-II). LC3-II associates with newly formed autophagosome membranes until they fuse with lysosomes. The generation of LC3-II is often employed to monitor autophagy (Klionsky et al., 2016). Following fusion with lysosomes the contents of autophagosomes are degraded and the resulting products are released into the cytosol by lysosomal permeases and recycled to generate new cellular constituents and energy.

The molecular machinery of autophagy overlaps with that of other cellular processes such as endocytosis, phagocytosis and vesicle trafficking (Galluzzi et al., 2017). Thus, individual autophagy-related proteins might play a role in multiple cellular processes. Consequently, investigations of individual autophagy-related gene products need to be interpreted with caution.

Autophagy plays a critical role in metabolic homeostasis and cell survival, and defects in this process have been linked to a variety of diseases, including inflammatory bowel disease, metabolic syndrome, cancer, and neurodegeneration (Ktistakis and Tooze, 2016; Mizushima et al., 2011). In the immune system, autophagy has been implicated in both innate and adaptive responses (Kuballa et al., 2012; Shibutani et al., 2015). In innate immunity, pattern recognition receptors such as the Toll- and NOD-like receptors enhance autophagy, which can induce the degradation of pathogens. In the adaptive immune response, autophagy has been implicated in B and T cell homeostasis and in antigen presentation. Here, we focus on the role of autophagy in MHC class I-restricted antigen presentation. Its contribution to MHC class II-restricted antigen presentation has been reviewed elsewhere (Munz, 2016).

3. MHC class I cell surface expression

The surface expression levels of MHC class I molecules are controlled by the release of stable MHC class I-peptide complexes from the ER lumen for egress to the cell surface, and by the subsequent internalization of surface MHC class I proteins into endosomal compartments (Van Kaer, 2002; Wearsch and Cresswell, 2008). Internalized MHC class I proteins may be subsequently degraded in lysosomes or, alternatively, transported back from the endosome to the cell surface in a process referred to as MHC class I recycling. Studies with primary murine DCs genetically deficient in Atg5, Atg7 or Vps34 revealed elevated surface levels of MHC class I as compared with wild-type DCs (Loi et al., 2016; Mintern et al., 2015a; Parekh et al., 2017) (Table 1). For cells deficient in Atg5 or Atg7, increased MHC class I expression was shown to be due to decreased endocytosis and decreased MHC class I degradation (Loi et al., 2016). No alterations in MHC class I recycling from endosomal locations back to the cell surface were observed in these autophagy-deficient cells. This process appeared to involve the assembly polypeptide-2 (AP2)-associated protein kinase 1 (AAK1), which has been implicated in clathrin- and dynamin-mediated endocytosis. Additional studies suggested that LC3 lipidation was important for localization of AAK1 to MHC class I molecules for optimal internalization and degradation (Loi et al., 2016). These findings suggest that autophagy diminishes MHC class I-restricted antigen presentation by enhancing MHC class I endocytosis and facilitating its degradation. Consistent with this conclusion, Atg5-deficient DCs cultured for 1 h with antigenic peptides were more efficient than similarly treated wild-type DCs in activating virus-specific T cells (Loi et al., 2016).

Table 1
MHC class I-restricted antigen presentation defects observed in DCs deficient in autophagy-related proteins.

Process	Autophagy-related protein				
	Atg5	Atg7	Beclin − 1[a]	Vps34	GCN2
Class I surface expression	↑	↑	nd	↑	nd
Classical pathway	↑	nd	nd	↑	nd
Cross-presentation pathway					
Soluble antigen	normal	↓	nd	normal	nd
Receptor-delivered	nd	normal	nd	normal	nd
Bacteria-associated	nd	nd	nd	normal	nd
Apoptotic cell-associated	normal	normal	nd	↓	nd
Cells infected with YF-17D	↓	↓	↓	nd	↓

Abbreviations: Atg, autophagy-related gene; DC, dendritic cell; GCN2, general control nonderepressible 2; n/a, not applicable; nd, not done; Vps34, vacuolar protein sorting 34; YF-17D, yellow fewer-17D vaccine strain.
[a] heterozygous null mutant.

4. Classical MHC class I antigen presentation pathway

The peptide cargo of MHC class I molecules is predominantly derived from newly synthesized cytosolic protein antigens and defective ribosomal products (DRiPs) (Van Kaer, 2002; Wearsch and Cresswell, 2008; Yewdell, 2011). Such antigens are degraded by the proteasome to peptides, which are subsequently transported to the lumen of the ER by the heterodimeric transporter associated with antigen processing (TAP). Within the ER, peptides may be further trimmed at their N-terminus by ER-associated amino peptidases, and then bind with peptide-receptive, ER-resident MHC class I proteins. Fully assembled MHC class I-peptide complexes then egress to the cell membrane via the secretory pathway for surface display and survey by $CD8^+$ T cells.

Several studies have provided evidence for enhancement of the classical MHC class I antigen presentation pathway by autophagy. Treatment of HeLa cells with the selective PI3 K inhibitor 3-methyladenine led to reduced autophagic degradation of DRiPs and a concomitant enhancement in proteasome degradation and class I antigen presentation (Wenger et al., 2012). Infection of macrophages with herpes simplex virus-1 (HSV-1) induced autophagy and increased the presentation of a peptide derived from HSV-1 glycoprotein B (gB) to $CD8^+$ T cells in a manner that required proteasome function and the secretory pathway (English et al., 2009). Similarly, presentation of the endogenous human cytomegalovirus (HCMV) latency-associated protein pUL138 involved autophagy, but this presentation occurred in a proteasome- and TAP-independent manner that involved MHC class I loading in endosomal compartments (Tey and Khanna, 2012). However, in a study targeting endogenous antigens to autophagosomes via fusion with LC3, antigen presentation was not significantly affected (Schmid et al., 2007).

The best evidence thus far for a role of autophagy in the classical MHC class I antigen presentation pathway comes from studies with DCs deficient in autophagy-related proteins. Atg5-deficient DCs showed enhanced presentation of influenza virus and lymphocytic choriomeningitis virus (LCMV) antigens to $CD8^+$ T cells (Loi et al., 2016), and Vps34-deficient DCs exhibited enhanced presentation of chicken ovalbumin (OVA), influenza virus and LCMV antigens to $CD8^+$ T cells (Parekh et al., 2017) (Table 1).

5. MHC class I cross-presentation pathway

In addition to the presentation of cytosolic antigens, a subset of DCs that express CD8α and CD103 can present exogenous antigens to $CD8^+$ T cells (Cruz et al., 2017). This alternative MHC class I antigen

presentation pathway was originally identified in the context of transplant rejection and is referred to as cross-priming or cross-presentation (Bevan, 2006). Antigens may be targeted for cross-presentation as soluble proteins, when recognized by endocytic receptors or targeted to such receptors with antibodies, bound with small particles such as beads or microbial organisms, or associated with apoptotic cells. Mechanistic aspects of cross-presentation remain incompletely understood and are likely diverse (Grotzke et al., 2017; Mintern et al., 2015b). The predominant cross-presentation pathway involves uptake of the antigen into endocytic compartments followed by release into the cytoplasm, degradation, loading on ER-resident MHC class I molecules and surface display. A less prominent, vacuolar pathway of antigen cross-presentation that involves antigen degradation and MHC class I loading in endocytic compartments has also been identified. For both pathways, versions that include or lack a requirement for proteasomal degradation and/or TAP have been identified.

In the case of cross-presentation of dead cell-associated antigens, autophagy in antigen-containing, apoptotic cells enhanced cross-presentation by DCs to virus or tumor antigen-specific $CD8^+$ T cells (Joubert and Albert, 2012; Li et al., 2008; Uhl et al., 2009). Although mechanisms remain unclear, purified autophagosomes were able to function as efficient antigen carriers for cross-presentation (Li et al., 2008), raising the possibility that autophagosome vesicles might be released by the apoptotic cells and then cross-presented by DCs. Regardless of the mechanisms involved, these findings suggest that autophagosomes may be exploited as antigen carriers for vaccine development and immunotherapy.

Studies have provided evidence both for and against a role of autophagy in antigen cross-presenting APCs (Das et al., 2015). Primary $CD8\alpha^+$ DCs, which are specialized in cross-presenting antigens, exhibit enhanced autophagy as compared with $CD8\alpha^-$ DCs, indicating that autophagy is highly active in the specialized cross-presenting DCs (Mintern et al., 2015a). Consistent with this notion, tumor antigens delivered to autophagosomes by conjugation to nanoparticles were efficiently cross-presented and elicited potent antitumor immune responses (Li et al., 2011). These properties of nanoparticles appeared to be associated with their capacity to be efficiently phagocytosed by DCs and to target their cargo to autophagosomes. Studies with a bone marrow-derived DC cell line further showed a crucial role of autophagy in the cross-presentation of chlamydial antigens to $CD8^+$ T cells, in a manner that involved fusion of autophagosomes with recycling endosomes referred to as amphisomes (Fiegl et al., 2013). Another study showed that the proteasome- but TAP-independent cross-presentation of soluble antigens by Epstein-Barr virus-transformed B cells to $CD8^+$ T cells was inhibited by 3-methyladenine or by knockdown of Atg5 or Atg12 (Dasari et al., 2016). In the latter experimental system, a new cross-presentation pathway was delineated, involving endocytosis of protein antigen by the B cells, followed by processing through an autophagy- and proteasome-dependent pathway, and loading of the peptide antigens onto MHC class I proteins in autophagosomes. While the reasons for some of these discrepancies are unclear, they may be due in part to distinct pathways of cross-presentation involved, differences in the types and activation status of the cells employed, and by the effects of distinct stimuli such as microbial organisms and apoptotic cells on the induction of autophagy.

Studies with primary DCs defective in individual autophagy-related proteins have also provided conflicting results (see Table 1). One study showed that bone marrow-derived DCs deficient in the amino acid starvation sensor kinase GCN2 (general control nonderepressible 2), Atg5 or Atg7, or carrying a heterozygous Beclin-1 null mutation, were defective in cross-presenting antigens from cells infected with a yellow fever virus vaccine strain (Ravindran et al., 2014). Another study showed that Atg7-deficient DCs exhibit a defect in the cross-presentation of soluble antigens but not in the cross-presentation of surface receptor-targeted or dead cell-associated antigens (Mintern et al., 2015a). In sharp contrast, Atg5-deficient DCs exhibited normal cross-presentation of soluble antigens and apoptotic cell-associated antigens (Lee et al., 2010). DCs with a deficiency in Vps34 showed normal cross-presentation of soluble, receptor-targeted and bacteria-associated antigens, but surprisingly, these cells exhibited a defect in the cross-presentation of dead cell-associated antigens (Parekh et al., 2017). The latter defect appeared to be due to impaired uptake of apoptotic cells by the Vps34-deficient DCs. Interestingly, Vps34-deficient DCs expressed reduced levels of T cell immunoglobulin mucin-4 (TIM-4), which recognizes phosphatidylserine on apoptotic cells and functions as one of the main receptors on DCs for uptake of apoptotic cells (Ravichandran and Lorenz, 2007). Surprisingly, however, a prior study reported that tumor-associated phagocytes from TIM-4-deficient mice exhibit enhanced cross-presentation of antigens contained by chemotherapy-killed tumor cells, and that signaling through TIM-4 enhances autophagy to increase phagosomal acidification, causing antigen-over-degradation (Baghdadi et al., 2013). However, in the experimental system used for Vps34-deficient DCs, TIM-4 expression was required for uptake of the irradiated, apoptotic cells (Parekh et al., 2017), whereas in the experimental system used for tumor-associated phagocytes, TIM-4 expression was required for activation of autophagy but not for uptake of the cancer drug-treated tumor cells (Baghdadi et al., 2013). Thus, some of these divergent findings could be explained, at least in part, by differences in the experimental systems employed. Regardless, the effects of Vps34-deficiency on DC function are likely not limited to defective TIM-4-mediated uptake of apoptotic cells. Importantly, DC-specific Vps34-deficient mice also contained a partial defect in the homeostasis of cross-presenting $CD8\alpha^+$ DCs and these cells spontaneously produced a variety of cytokines (Parekh et al., 2017). Because Vps34 plays a role not only in autophagy but also in endocytosis, phagocytosis and vesicle trafficking (Backer, 2016), it remains possible that these effects of Vps34-deficiency are mediated by its role in processes other than autophagy. Nevertheless, Vps34-deficient DCs did not exhibit any major defects in endocytosis or phagocytosis (Parekh et al., 2017), suggesting altered autophagy as the most likely cause of the observed phenotype. At present, the cross-presentation phenotypes reported for DCs defective in distinct autophagy-related genes cannot be easily reconciled.

6. Conclusions and future perspectives

Understanding the mechanisms by which DCs and other professional APCs handle and process antigens for presentation to T cells is critically important for the development of vaccines and immunotherapies against microbial pathogens and tumors, and for devising methods to induce tolerance against self or foreign antigens. The finding that autophagy plays a critical role in MHC class I-restricted antigen presentation opens up the possibility to exploit this pathway for therapeutic purposes, for example when employing DCs in vitro to stimulate and expand T cells for adoptive T cell therapy. Nevertheless, much remains to be learned about the contribution of individual autophagy-related factors in antigen processing. It will be important to perform such studies with relevant APCs, ideally in vivo, and to compare the effects of deficiency in individual autophagy-related genes side-by-side using well-controlled experimental systems.

Conflict of interest

The authors have no conflict of interest to disclose.

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