Immunity
Review

Autophagy and Cellular Immune Responses

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Autophagy constitutes a mechanism for the sequestration and lysosomal degradation of various cytoplasmic structures, including damaged organelles and invading microorganisms. Autophagy not only represents an essential cell-intrinsic mechanism to protect against internal and external stress conditions but also shapes cellular immunity. Recent evidence indicates that autophagic responses in antigen-donor cells affect the release of several cytokines and "danger signals." Thus, especially when it precedes cell death, autophagy alerts innate immune effectors to elicit cognate immune responses. Autophagy is also important for the differentiation, survival, and activation of myeloid and lymphoid cells. Accordingly, inherited mutations in autophagy-relevant genes are associated with immune diseases, whereas oncogenesis-associated autophagic defects promote the escape of developing tumors from immunosurveillance. Here, we discuss the regulation of autophagy in the course of cellular immune responses and emphasize its impact on the immunogenicity of antigen-donor cells and on the activity of antigen-presenting cells and T lymphocytes.

Introduction

Macroautophagy (hereafter referred to as "autophagy") initiates with the sequestration of organelles or portions of the cytoplasm within double-membraned vesicles, so-called autophagosomes. Autophagosomes then fuse with lysosomes to generate autolysosomes, and their luminal content is degraded (Mizushima et al., 2011; Yang and Klionsky, 2010). Autophagy represents a phylogenetically ancient response that was first dissected at the genetic level in unicellular eukaryotes (notably yeast) and was later found to be important in the adaptation of cells to endogenous and exogenous stress (Kroemer et al., 2010; Levine and Kroemer, 2008).

Autophagy not only preserves cellular homeostasis in conditions of endogenous distress (Kroemer et al., 2010) but also plays a primordial role in controlling intracellular pathogens in evolutionarily distant species, ranging from unicellular organisms to humans (Levine et al., 2011). Thus, together with the endoplasmic reticulum (ER) stress response, autophagy represents one of the most primitive examples of innate immune responses. In animals, this cell-autonomous defense mechanism also facilitates the recognition of infected cells by innate immune effectors, especially when infection leads to cell death (Galluzzi et al., 2008a), setting off an elaborate inflammatory or immune response. The importance of autophagy in the host defense against infection is underscored by the fact that bacteria and viruses have developed a myriad of strategies for subverting or harnessing the autophagic machinery. The complex crosstalk between host and microbe and their intimate coevolution are reviewed elsewhere (Kuballa et al., 2012; Levine et al., 2011) and will not be discussed here.

Accumulating evidence suggests that autophagy influences cellular immune responses well beyond its role as a cell-intrinsic mechanism of defense against invading pathogens. In particular, autophagy has recently been shown to influence not only the antigenic profile of antigen-donor cells (ADCs) and their ability to release immunogenic signals (Caron et al., 2011; Michaud et al., 2011) but also the survival, differentiation, and function of antigen-presenting cells (APCs) and T lymphocytes (Fiegl et al., 2013; Jia and He, 2011; Pua et al., 2007; Wildenberg et al., 2012). Here, we discuss the host-intrinsic regulation of autophagy in the course of cellular immune responses and examine how autophagy impacts (1) ADCs, whose phenotypic and/or behavioral features are modified upon infection or oncogenic mutations; (2) APCs, mainly dendritic cells (DCs), which capture antigens from ADCs and present antigenic peptides in complex with MHC molecules on their own surface; and (3) T lymphocytes, which upon activation by APCs finally attack infected or transformed cells in an antigen-restricted fashion. The pathophysiological implications of autophagy in this context are discussed.

Autophagy: A General Stress Response

Constitutive autophagy is required for cellular "housekeeping," for example to eliminate occasionally damaged organelles such as depolarized mitochondria that cannot rejoin the mitochondrial network (Green et al., 2011). In addition, autophagy is upregulated when cells are confronted with potentially dangerous environmental cues, be they physical (thermal stress, irradiation) (Apel et al., 2008), chemical (changes in pH, osmolarity) (Xu et al., 2011), or metabolic (shortage in nutrients or oxygen) (Boya et al., 2005) and thus constitutes an almost universal

response to stress (Kroemer et al., 2010). In mammals, the core autophagic pathway starts with the formation of an isolation membrane (also known as a phagophore), most often at contact sites between mitochondria and the endoplasmic reticulum (ER) (Hamasaki et al., 2013), although other sources of autophagic membranes have been reported (Ravikumar et al., 2010). Autophagy involves multiple molecular components, including (1) the unc-51-like kinase 1 (ULK1) complex, which is coupled to the autophagy suppressor TOR complex 1 (TORC1) (Egan et al., 2011); (2) the Beclin 1 (BECN1)/class III phosphoinositide-3-kinase (PI3K) complex, which is usually inhibited by interactions with proteins of the Golgi apparatus, antiapoptotic proteins of the BCL-2 family, and other signal transducers (He and Levine, 2010); (3) two transmembrane proteins, ATG9 and vacuole membrane protein 1 (VMP1) (Molejon et al., 2013; Reggiori and Klionsky, 2006); (4) two ubiquitin-like conjugation systems, operating on ATG12 and microtubule-associated protein 1 light chain 3 (MAP1LC3, the mammalian homolog of yeast Atg8, best known as LC3) (Mizushima et al., 1998); (5) several proteins that mediate the fusion between autophagosomes and lysosomes (Tumbarello et al., 2012); and (6) a large panel of lysosomal hydrolases, which digest proteins, lipids, and nucleic acids in an acidic microenvironment (Kroemer and Jäättelä, 2005). These molecular systems drive autophagy only when activated in a highly coordinated manner and are directly connected to cell-intrinsic stress-response mechanisms (Kroemer et al., 2010; Mizushima et al., 2011; Yang and Klionsky, 2010).

Autophagy might occur as a general response during which distinct portions of the cytoplasm are sequestered and digested in an apparently nonspecific fashion. The autophagic response of eukaryotic cells to amino acid deprivation is viewed as a case of general autophagy, although accumulating evidence challenges this model (Dengjel et al., 2008; Gomes et al., 2011). Alternatively, autophagy might target specific portions of the cytoplasm that are marked for destruction, generally by ubiquitination. Under specific circumstances, a series of ubiquitinases covalently add to cellular structures K63-linked ubiquitin chains, which are bound by various adaptors containing an LC3-interacting region (LIR) and hence are recruited to closing autophagosomes (Fimia et al., 2013; van Wijk et al., 2012). According to this principle, distinct organelles or intracellular entities (e.g., mitochondria, ER, peroxisomes, ribosomes, protein aggregates) can be selectively targeted for autophagic destruction, resulting in organelle-specific instances of autophagy that are referred to as "mitophagy," "reticulophagy," "pexophagy," "ribophagy," or "aggrephagy," for example.

General and organelle-specific cases of autophagy are stimulated by demand and supply, respectively. The demand for autophagic turnover is increased, for example, by (1) activation of sensors, such as sirtuin 1, AMP-activated protein kinase (AMPK), and mammalian target of rapamycin (mTOR), that respond to a reduction in nutrient availability, (2) activation of stress-responsive kinases, including c-Jun N-terminal kinase 1 (JNK1); protein kinase, RNA-activated (PKR); PKR-like ER kinase (PERK); TGF-β-activated kinase 1 (TAK1); death-associated protein kinase 1 (DAPK1); and the inhibitor of κB kinase (IKK) complex, (3) translocation of stress-activated transcription factors such as p53 and signal transducer and activator of transcription 3 (STAT3) from the cytoplasm to the nucleus, and (4) release of the chromatin-binding factor high-mobility group box 1 (HMGB1) into the cytosol (Criollo et al., 2011; Kroemer et al., 2010; Shen et al., 2012; Tang et al., 2012). Conversely, the supply of autophagic substrates is increased by ubiquitination of damaged organelles (such as mitochondria, which can be ubiquitinated by parkin) or protein aggregates. Moreover, the affinity of the adaptors that bridge ubiquitinated proteins to LC3, the so-called sequestosome 1 (SQSTM1)-like receptors (SLRs), for their substrate can increase in response to phosphorylation by TANK-binding kinase 1 (TBK1), as shown for SQSTM1 (best known as p62) and optineurin (Pilli et al., 2012; Wild et al., 2011). In this context, any increase in the autophagic demand stimulates the preferential consumption of supplied substrates, thus preferentially ridding the cells of damaged organelles or protein aggregates.

One major connection between autophagy and other stress-response pathways is determined by the subcellular structure at which phagophores form. This structure is in the close proximity of (or corresponds to) so-called mitochondria-associated ER membranes (MAMs), sites of anatomical and functional interconnection between mitochondria and the ER (Hamasaki et al., 2013). MAMs are required for nutrient-deprivation-induced autophagy in mammalian cells, presumably as a result of the ability of the ER protein syntaxin 17 to bind ATG14 and initiate the recruitment of the ATG5-ATG12 complex and several other components of the autophagic machinery, including ATG16L1 (Hamasaki et al., 2013; Lei et al., 2012). In addition, MAMs constitute a preferential location for lipid metabolism (including the generation of phosphatidylethanolamine, the lipid that is conjugated to LC3 during autophagy), the regulation of mitochondrial membrane dynamics (fusion and fission), Ca^{2+} signaling, and the execution of lethal molecular cascades culminating either in mitochondrial membrane permeabilization or local caspase-8 activation (Chan, 2012; Iwasawa et al., 2011). At MAMs, multiple immune-relevant signal transducers physically interact. These include supramolecular complexes organized around the NLR family; pyrin domain containing 3 (NLRP3) inflammasome (a platform for the activation of proinflammatory caspase-1) (Subramanian et al., 2013; Zitvogel et al., 2012), mitochondrial antiviral signaling (MAVS), which interacts with other mitochondrial proteins, such as NLR family member X1 (NLRX1) and Tu translation elongation factor (TUFM); sensors of viral RNA, such as retinoic acid inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5); and stimulator of interferon genes (STING) (Galluzzi et al., 2012a). These interactions are important for the elicitation of both innate and adaptive immune responses.

The activation of cell death also represents a mechanism of adaptation to stress because it preserves organismal homeostasis once cellular damage is irreparable (Fuchs and Steller, 2011). Some cell-death events are accompanied by a massive autophagic response, a circumstance referred to "autophagic cell death" (Galluzzi et al., 2012b). However, autophagy often is a cytoprotective, rather than a cytotoxic, mechanism, thus reducing the propensity of stressed cells to die (Maiuri et al., 2007). Conversely, lethal activation of caspases and other proteases (e.g., calpains) results in the digestion of several essential mediators of autophagy, including BECN1 and ATG5, hence inactivating the autophagic machinery (Djavaheri-Mergny et al., 2010; Yousefi et al., 2006). Hence, the autophagic and the

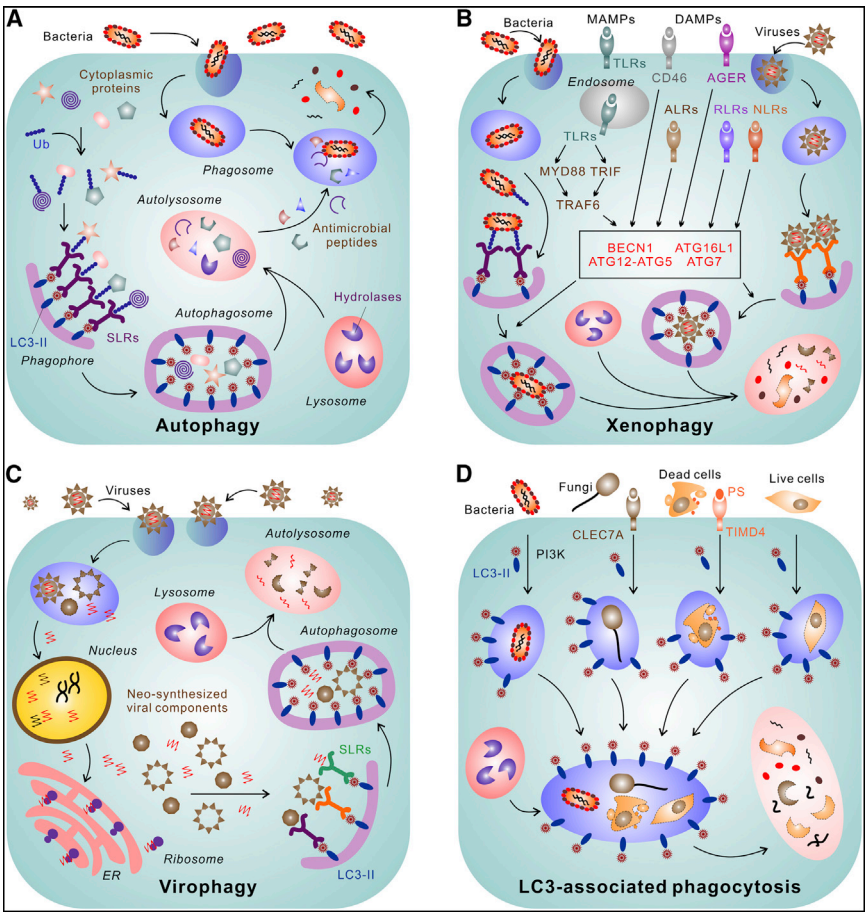

Figure 1. Autophagy, Xenophagy, Virophagy and LC3-Associated Phagocytosis in the Control of Intracellular Pathogens

(A) Autophagy. Specific ribosomal components and ubiquitinated cytosolic proteins can be delivered by p62 or other sequestosome 1 (SQSTM1)-like receptors (SLRs) to autolysosomes, where they are converted into antimicrobial peptides. These peptides operate as endogenous antibiotics upon the fusion of autolysosomes with bacteria-containing phagosomes. Abbreviations are as follows: LC3-II, lipidated LC3; and Ub, ubiquitin.

(B) Xenophagy. Microbe-associated molecular patterns (MAMPs) or damage-associated molecular patterns (DAMPs) engage surface or intracellular pattern-recognition receptors (PRRs) and hence activate the autophagic machinery, which targets intracellular pathogens for lysosomal degradation. Abbreviations are as follows: AGER, advanced glycosylation end product-specific receptor; ALR, AIM2-like receptor; BECN1, Beclin 1; NLR, NOD-like receptor; RLR, RIG-I-like receptor; and TLR, Toll-like receptor.

(C) Virophagy. Viruses hijack the host machinery to synthesize nucleic acids (DNA or RNA) and other components that are required for the assembly of new viral particles. Such neosynthesized viral components can be recognized by various SLRs and directed to lysosomal degradation.

(D) LC3-associated phagocytosis. Phagocytic vesicles that contain internalized pathogens as well as dead or live cells can be transiently decorated with LC3. Upon the vessicles' fusion with lysosomes, the cargo of these single-membraned organelles is degraded. Abbreviations are as follows: CLEC7A, C-type lectin domain family 7, member A; PI3K, phosphoinositide-3-kinase; PS, phosphatidylserine; and TIMD4, T cell immunoglobulin and mucin domain containing 4.

apoptotic programs appear to inhibit each other (Galluzzi et al., 2008b), presumably reflecting the fact that the former generally attempts to recover cellular, as opposed to organismal, homeostasis. Aging is frequently accompanied by a general autophagy defect that is commensurate with the reduced capacity of aged organisms to adapt to stress (López-Otín et al., 2013). Several maneuvers that reinstate normal function of the autophagic machinery, such as the administration of the mTOR inhibitor rapamycin, might actually decelerate the acquisition of a senescent phenotype (Harrison et al., 2009).

Autophagy, Xenophagy, Virophagy, and Phagocytosis

By definition, autophagy degrades endogenous components of the cell. In addition, the autophagic pathway or parts of it have been integrated in defense mechanisms that control invading pathogens.

Autophagic vacuoles that contain antimicrobial peptides arising from the degradation of initially innocuous cytoplasmic proteins, such as ubiquitin and ribosomal precursor proteins, can fuse with phagosomes containing bacteria, such as *Mycobacterium tuberculosis*, and kill them (Ponpuak et al., 2010). In this context, autophagy can be viewed as a process that generates endogenous antibiotics to combat invading pathogens (Figure 1A).

In addition, intracellular bacteria can be marked by cellular ubiquitinases for autophagic degradation, a process that is referred to as "xenophagy" (Figure 1B) (Levine, 2005). Xenophagy requires all the molecules that are involved in classical autophagy; in this setting, these molecules orchestrate the recognition, capture, and elimination of intracellular pathogens. In the course of infection, autophagy can be stimulated by prominent pattern-recognition receptors (PRRs), including SLRs, Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), and absent in melanoma 2 (AIM2)-like receptors (ALRs). It can also be stimulated by pathogen receptors such as CD46, as well as by advanced glycosylation-end-product-specific receptor (AGER, best known as receptor for advanced glycosylation end products, RAGE), which together detect a large panel of microbe-associated and danger-associated molecular patterns (MAMPs and DAMPs, respectively) (Deretic, 2011; Tang et al., 2012).

Thanks to one or several LIRs, SLRs act as autophagic adaptors between ubiquitin tags on microbial (or endogenous) targets and Atg8 paralogs such as LC3 itself and $GABA_A$-receptor-associated protein (GABARAP), thus bringing autophagic cargoes to nascent autophagosomes. SLRs include p62, optineurin, neighbor of BRCA1 gene 1 (NBR1), and nuclear dot protein of 52 kDa (NDP52) (Deretic, 2012a). The affinity of SLRs for distinct types of ubiquitin chains, nonubiquitinated proteins, and Atg8 paralogs varies, a fact that may explain why SLRs differ in their specificity for invading pathogens. Indeed, whereas both

NDP52 and p62 have been shown to control *Salmonella enterica* (Thurston et al., 2009), only the latter mediates the elimination of Sindbis virus (Orvedahl et al., 2010; Thurston et al., 2009).

Autophagy can also target individual viral components for degradation, a process termed "virophagy" (Figure 1C) (Orvedahl et al., 2011). Virophagy differs from xenophagy in that it targets neosynthesized viral components rather than entire viral particles as xenophagy does (usually shortly after endocytosis). For instance, p62 has been shown to recognize (and send to degradation) the Sindbis virus capsid in an ubiquitination-independent fashion. Globally, it appears that viral proteins and RNA-protein complexes can be targeted by distinct host factors for autophagic degradation.

Finally, single-membraned phagocytic vesicles that contain engulfed bacteria can be transiently decorated with LC3. This process, which is referred to as "LC3-associated phagocytosis" (LAP), never results in the formation of double-membraned vesicles and depends on both the PI3K complex and the LC3 conjugation system but not on the ULK1 complex, underscoring its biochemical and functional distinction from autophagy (Figure 1D) (Sanjuan et al., 2007). LAP is important in order for macrophages to clear invading *Burkholderia pseudomallei*, the causative agent of melioidosis (Gong et al., 2011). In addition, LAP is involved in the degradation of dead cells and the presentation of fungal antigens by macrophages downstream of the recognition of such entities by T cell immunoglobulin and mucin-domain-containing 4 (TIMD4, best known as TIM4) or C-type lectin domain family 7, member A (CLEC7A, also known as dectin 1), respectively (Ma et al., 2012; Martinez et al., 2011). The internalization of live cells by other cells of the same type, a process called entosis, also involves the transient translocation of LC3 to the engulfing vacuole (Florey et al., 2011), suggesting that entosis—whose relevance as a bona fide cell death remains debated (Galluzzi et al., 2012b) —might constitute a special case of LAP. Of note, BECN1 controls the very first steps of the internalization of apoptotic cells as it localizes to early phagocytic cups together with the small GTPase RAC1, with which it interacts (Konishi et al., 2012). Thus, autophagy-relevant proteins might control the engulfment of cell corpses through an additional mechanism. Overall, LAP exemplifies a biological process that is distinct from, but related to, autophagy and that involves multiple components of the autophagic machinery.

Altogether, these observations exemplify the importance of autophagy for the cell-intrinsic control of invading microbes. As discussed below, autophagy also plays a key role in the regulation of cellular immune responses.

Soluble Mediators and Autophagy

Although autophagy constitutes a cell-autonomous mechanism for the control of noninfectious stress and microbial pathogens, multiple soluble factors stimulate or inhibit autophagic responses within cell populations, hence assuring their spatial and temporal coordination. In addition, the production of cytokines is modulated by autophagy. This mutual relationship underpins multiple mechanisms through which the cell-intrinsic regulation of autophagy is connected to cell-extrinsic stress-response pathways (Figure 2).

One primordial response to viral infection is the secretion of type 1 interferons (IFNs), a series of partially redundant and pleiotropic cytokines that act on the IFN (α, β, and ω) receptor 1 (IFNAR1). Type 1 IFNs stimulate autophagic responses in several cell lines (Schmeisser et al., 2013). Other soluble mediators that promote autophagy include the T_H1 cytokines tumor necrosis factor α (TNF-α) and IFN-γ, the pro-inflammatory factor interleukin (IL)-1β, and a large panel of DAMPs, such as HMGB1, S100 proteins, ATP and histone-DNA complexes. These signals are perceived by specific cytokine receptors or by a series of extracellular or intracellular PRRs including TLRs, AGER, purinergic P2RX7 receptors, and AIM2 (Deretic, 2011; Tang et al., 2012). In sharp contrast, T_H2 cytokines, including IL-4 and IL-13, as well as the anti-inflammatory mediator IL-10, inhibit autophagy (Su et al., 2012). Because DAMPs and cytokines operate in a spatially and temporally restricted fashion (Zitvogel et al., 2010), these observations suggest that autophagy is regulated by soluble mediators in a context-dependent fashion (Figure 2A). Nonetheless, it is tempting to correlate the T_H1/T_H2 polarization of immune responses to the autophagy-mediated control of mycobacteria, a process that appears to be favored by T_H1 and to be inhibited by T_H2 cytokines (Doherty, 2012). Moreover, autophagy is stimulated by multiple cell-death-associated DAMPs, perhaps with the scope of promoting adaptive responses (and hence minimizing cellular demise) and stimulating innate immune effectors in tissue areas adjacent to where cells are dying.

Autophagy favors the release of several proteins, including cytokines, through processes of "unconventional" secretion (i.e., the delivery of cytosolic proteins to the extracellular milieu via a mechanism that does not rely on the conventional secretory pathway's proceeding through the Golgi apparatus) (Figure 2B). These proteins include acyl-CoA-binding protein (ACBP) (Bruns et al., 2011), HMGB1 (Thorburn et al., 2009), and—at least under specific (and presumably transient) circumstances—IL-1β and IL-18 (Jiang et al., 2013). Conversely, autophagy limits the secretion of proinflammatory cytokines, notably IL-1β, by virtue of its capacity to dampen the activation of the inflammasome (Nakahira et al., 2011). This could reflect the ability of autophagy to remove damaged mitochondria, which release inflammasome activators such as reactive oxygen species (ROS) and mitochondrial DNA (mtDNA) (Nakahira et al., 2011; Zhou et al., 2011), and/or to target ubiquitinated AIM2 and NLRP3 inflammasomes for destruction (Shi et al., 2012). Moreover, at least in macrophages and DCs, autophagy controls IL-1β secretion by mediating the degradation of pro-IL-1β (Harris et al., 2011). Of note, autophagy-related processes such as LAP can also regulate cytokine production. In the absence of LAP, the engulfment of dead cells by macrophages is accompanied by an increased secretion of proinflammatory cytokines such as IL-1β and IL-6, as well as by a decreased production of anti-inflammatory mediators such as IL-10 and TGF-β (Martinez et al., 2011). Beyond its negative impact on the secretion of some cytokines, autophagy might exert additional anti-inflammatory actions by negatively regulating RLRs (Jounai et al., 2007). This effect could involve the autophagy-mediated elimination of ROS-producing mitochondria (Tal et al., 2009), as well as direct interactions between the ATG5-ATG12 conjugate or ATG16L1 with the mitochondrial proteins NLRX1 and TUFM, which operate in RLR-ignited signal transduction pathways (Lei et al., 2012).

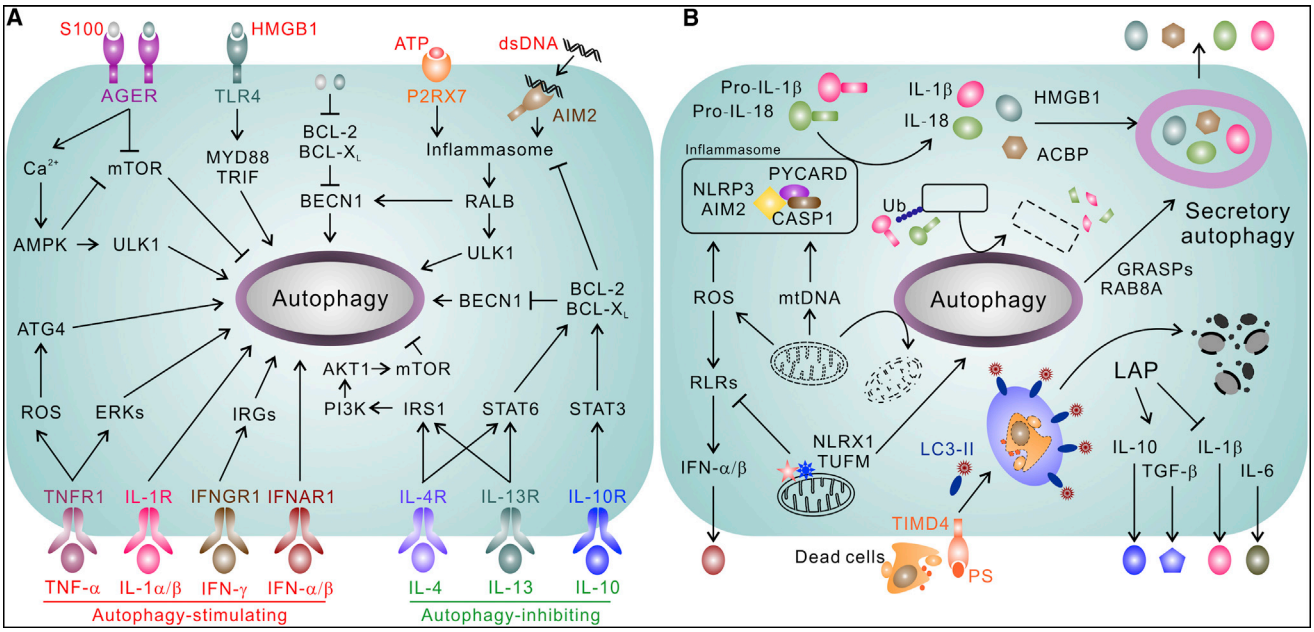

Figure 2. Connection between Extracellular Mediators and Autophagy

(A) Impact of extracellular mediators on autophagy. The binding of various damage-associated molecular patterns (DAMPs) to pattern-recognition receptors (PRRs) stimulates autophagy. High-mobility group box 1 (HMGB1) and S100 proteins, upon binding to advanced glycosylation-end-product-specific receptor (AGER), can either reduce the phosphorylation of mammalian target of rapamycin (mTOR, the main negative regulator of autophagy) or activate AMP-activated protein kinase (AMPK), which in turn inhibits mTOR and activates Unc-51-like kinase 1 (ULK1), a key initiator of the autophagic flux. HMGB1 and S100 proteins also induce autophagy by dissociating Beclin 1 (BECN1) from inhibitory interactions with BCL-2 and BCL-X_L or by signaling via the Toll-like receptor (TLR) adaptors myeloid differentiation primary response gene 88 (MYD88) or TIR-domain-containing adaptor-inducing interferon-β (TRIF). The detection of extracellular ATP and foreign double-stranded DNA (dsDNA) by the purinergic receptor P2RX7 and DNA sensor absent in melanoma 2 (AIM2), respectively, activates the inflammasome, which in turn can stimulate the formation of autophagosomes via a signaling cascade involving the small GTPase RAS-like protein B (RALB), ULK1, and BECN1. Interferon (IFN)-γ induces autophagy via immunity-related GTPases (IRGs), whereas tumor necrosis factor α (TNF-α) does so via an extracellular signal-regulated kinase (ERK)-dependent signaling pathway or through the generation of reactive oxygen species (ROS), which facilitate the ATG4-dependent lipidation of LC3. Interleukin (IL-1)α/β and IFN-α/β also stimulate autophagy, although the underlying mechanisms remain to be fully elucidated. Conversely, IL-4, IL-10, and IL-13 exert autophagy-suppressive functions by stimulating an insulin receptor substrate 1 (IRS1)- or phosphoinositide-3-kinase (PI3K)- dependent pathway leading to the AKT1-mediated activation of mammalian target of rapamycin (mTOR). Alternatively, the autophagy inhibitory effects of IL-4, IL-10, and IL-13 depend on signal transducer and activator of transcription 3 (STAT3) and STAT6, which disrupt the inhibitory interaction between BECN1 and either BCL-2 or BCL-X_L. Extracellular factors exerting autophagy-stimulating or autophagy-inhibiting effects have been labeled in red and green, respectively. Abbreviations are as follows: IFNAR1, IFN (α, β and ω) receptor 1; IFNGR1, IFN-γ receptor 1; and TNFR1, TNF receptor 1.

(B) Effects of autophagy on cytokine secretion. By removing damaged mitochondria, which otherwise would release inflammasome activators including ROS and mitochondrial DNA (mtDNA), autophagy limits the secretion of mature IL-1β and IL-18. Moreover, autophagy appears to target assembled inflammasomes for degradation. Mitochondrial NLR family member X1 (NLRX1) inhibits the RIG-I-like receptor (RLR)-dependent production of type I IFN but promotes autophagy by interacting with the ATG5-ATG12 complex or with ATG16L1 via its binding partner, Tu translation elongation factor (TUFM). Autophagy controls the secretion of various other soluble factors via Golgi reassembly and stacking proteins (GRASPs) and the RAS-related protein RAB8A. Along similar lines, the LC3-associated phagocytosis (LAP) of dead cells engulfed though T cell immunoglobulin and mucin-domain-containing 4 (TIMD4) modulates the release of several cytokines, including IL-1β, IL-6, IL-10, and transforming growth factor β (TGF-β). Abbreviations are as follows: AIM2, absent in melanoma 2; CASP1, caspase-1; NLRP3, NLR family, pyrin domain containing 3; PS, phosphatidylserine; PYCARD, PYD and CARD domain containing; and Ub, ubiquitin.

Altogether, these observations suggest that the systemic or local induction of autophagy as coordinated by cytokines or DAMPs is part of a negative-feedback loop that restrains the excessive generation of proinflammatory factors.

Autophagy in Antigen-Donor Cells

Although it contributes to the cell-autonomous lines of defense against pathogens, autophagy can also stimulate immune responses against microbe- or tumor-associated antigens, especially if ADCs die. Conceptually, this might constitute a major checkpoint (Figure 3A). Autophagic responses that are not ensued by cell death are likely to privilege self-limiting, cell-autonomous defense mechanisms. Conversely, when autophagy is unable to re-establish homeostasis and is followed by cell death, an immune response is elicited. There are multiple mechanisms whereby premortem autophagy can influence the propensity of ADCs or portions thereof to be engulfed by APCs for presentation to T cells in a productive fashion (Figure 3B).

First, at least in the context of developmental cell death, autophagy appears to increase the emission of potent chemotactic "find-me" signals, such as ATP and lysophosphatidylcholine (Elliott et al., 2009; Lauber et al., 2003; Ma et al., 2013), as well as the exposure of phagocytic "eat-me" signals, such as phosphatidylserine (Fadok et al., 2000), on the plasma membrane. Through these chemotactic and phagocytic cues, premortem autophagy facilitates the heterophagic clearance of apoptotic corpses by neighboring cells and thus prevents inflammatory reactions (Qu et al., 2007). In line with this notion, embryonic tissues of both *Atg5*$^{-/-}$ and *Becn1*$^{+/-}$ mice, which exhibit low intracellular ATP amounts that can be reversed by the provision of the metabolic substrate methylpyruvate, manifest an inflammatory response that is driven by the accumulation of cell

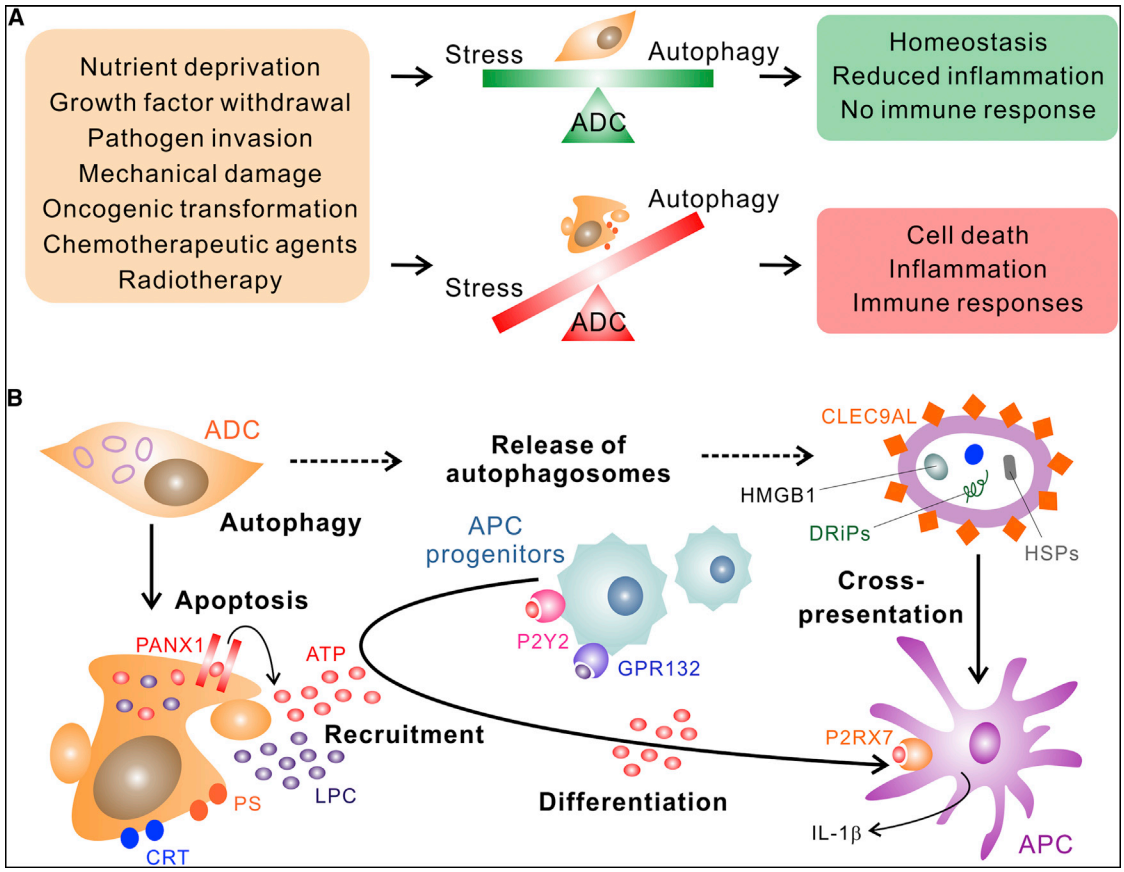

Figure 3. Impact of Autophagy on Antigen Donor Cells

(A) The life/death switch. In response to stressful conditions, including nutrient deprivation, growth-factor withdrawal, pathogen invasion, mechanical damage, oncogenic transformation, and exposure to chemotherapeutic agents or radiotherapy, autophagy is activated as a means of reestablishing cellular homeostasis and preventing inflammatory or immune responses. However, when stress conditions are too harsh or prolonged, the autophagic machinery is overwhelmed and thus fails to reestablish homeostasis. In this setting, the death of antigen donor cells (ADCs) is inevitable and is accompanied by the elicitation of inflammatory or immune responses.

(B) Autophagy in ADCs and its impact on immune responses. Premortem autophagic responses in ADCs enhance the release of "find-me" signals, such as ATP and lysophosphatidylcholine (LPC), as well as of "eat me" signals, such as calreticulin (CRT) and phosphatidylserine (PS), which attract antigen-presenting cell (APC) progenitors and facilitate antigen uptake, respectively. ATP also stimulates the local differentiation of APCs as well as the activation of their inflammasome, resulting in the secretion of interleukin-1β. Dying ADCs can also release intact autophagosomes that contain not only multiple antigens but also heat-shock proteins (HSPs), CRT, high-mobility group box 1 (HMGB1), and defective ribosomal initiation products (DRiPs). Because C-type lectin domain family 9, member A (CLEC9A) ligand (CLEC9AL) is expressedon their surface, autophagosomes can be taken up by APCs, resulting in the crosspresentation of their antigenic cargo. Abbreviations are as follows: GPR132, G protein-coupled receptor 132; and PANX1, pannexin 1.

corpses (Qu et al., 2007). Pre-mortem autophagic responses are also required for the optimal release of ATP by dying cancer cells (Michaud et al., 2011). In this context, autophagy participates in the trafficking of ATP from specific intracellular compartments that bear lysosomal markers to a hitherto undefined secretory compartment, and caspase-dependent, pannexin 1-mediated secretion of ATP into the extracellular milieu then follows during the blebbing phase of apoptosis (Chekeni et al., 2010; Martins et al., 2013). Autophagy-deficient malignant cells, which fail to release ATP in response to chemotherapy, are unable to recruit myeloid cells into the tumor bed and hence cannot elicit an anticancer immune response. This defect can be reversed by ecto-ATPase inhibitors, preserving extracellular ATP levels and hence allowing for the recruitment of successive waves of myeloid and lymphoid cells into the tumor in response to chemotherapy (Ma et al., 2013). Such an ATP-driven chemotactic response requires the expression of metabotropic P2Y2 receptors on immune cells (Elliott et al., 2009; Ma et al., 2013). In addition, extracellular ATP not only can stimulate granulocyte myeloid precursors to differentiate into inflammatory DCs rather than into neutrophil granulocytes (the default pathway) (Michaud et al., 2011) but also can bind ionotropic P2RX7 receptors on DCs, and thereby stimulate them to secrete IL-1β (Ghiringhelli et al., 2009).

Second, antigen-sequestering autophagosomes can be directly transferred from cell corpses to DCs for optimal cross-presentation. These organelles are particularly efficient at stimulating specific immune responses if they are purified from cells that are treated with proteasome inhibitors because this increases the p62-dependent uptake of various autophagosomal substrates (Twitty et al., 2011). Indeed, purified autophagosomes harbor not only long-lived proteins but also short-lived polypeptides, including defective ribosomal initiation products (DRiPs) and several DAMPs, such as calreticulin (CRT) and heat-shock proteins (Li et al., 2011). The cross-presentation of

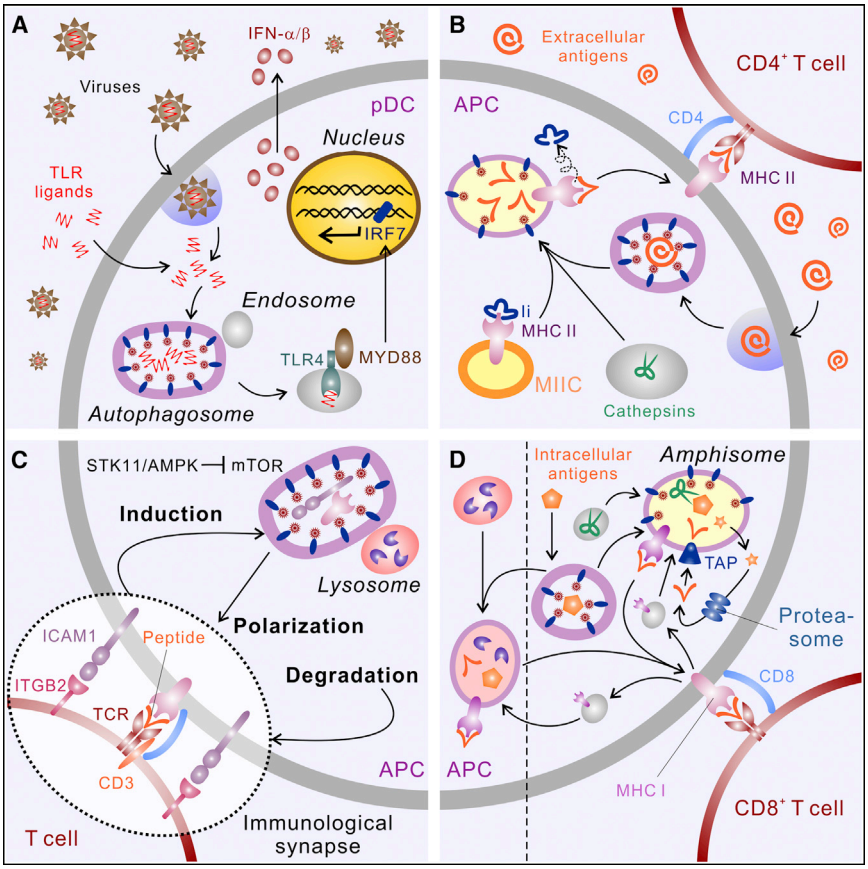

Figure 4. Impact of Autophagy on Antigen-Presenting Cells

(A) Toll-like receptors (TLRs). Natural or synthetic TLR ligands can be sequestered in autophagosomes, which facilitate the binding of cognate endosomal TLRs. This process can stimulate the secretion of type I interferon (IFN) and thus enhance antigen presentation. Abbreviations are as follows: IRF7, IFN-regulatory factor 7; MYD88, myeloid differentiation primary response 88; and pDC, plasmacytoid dendritic cell.

(B) MHC class II presentation. Extracellular antigens captured by APCs are delivered to autophagosomes, which utilize hydrolases from endosomes (such as cathepsins) to generate immunogenic peptides and load them onto MHC class II molecules for presentation to $CD4^+$ T cells. Ii, invariant chain; MIIC, MHC class II loading compartment.

(C) Immunological synapse. The formation of an IS between APCs and T lymphocytes leads to the activation of serine threonine kinase 11 (STK11) and AMP-activated protein kinase (AMPK), which inhibit mammalian target of rapamycin and hence stimulate autophagy. In this setting, autophagosomes are oriented toward the IS and degrade synaptic components, eventually destabilizing it and inhibiting T cell activation. Abbreviations are as follows: ICAM1, intercellular adhesion molecule 1; ITGB2, integrin, β2; and TCR, T cell receptor.

(D) Amphisomes. Intracellular antigens engulfed in autophagosomes can be digested sequentially by amphisomes (formed upon the fusion of autophagosomes with endosomes) and proteasomes. The degradation products of proteasomes can be transported back into amphisomes by transporter associated with antigen processing (TAP) and hence loaded onto recycling MHC class I molecules for antigen presentation to $CD8^+$ T cells. Alternatively, lysosomal hydrolases can digest intracellular antigens independently of proteasomes. These peptides are loaded onto recycling MHC class I molecules (and hence presented to $CD8^+$ T cells) independently of TAP.

autophagosomal antigens depends at least in part on the interaction between the C-type lectin domain family 9, member A (CLEC9A) ligand, which is expressed on the surface of autophagosomes, and CLEC9A (also known as DNGR-1) on DCs, as we well as on the caveolae-mediated endocytic pathway, which routes antigens to nonacidic compartments (Li et al., 2011). Of note, purified autophagosomes can also induce the activation of B cells in a TLR2- and myeloid differentiation primary response 88 (MYD88)-dependent fashion (Li et al., 2013). Although the purification of autophagosomes might yield vaccines that are more efficient than whole-cell lysates, it is not clear whether the transfer of such organelles from dying cells to APCs naturally occurs in vivo, for instance in the context of antimicrobial or anticancer immune responses.

Thus, autophagic responses in virus-infected or transformed ADCs favor the engulfment and presentation of antigens by APCs (Michaud et al., 2011; Twitty et al., 2011; Uhl et al., 2009) (Figure 3B). The pharmacological induction of autophagy can be exploited as an adjuvant strategy to invigorate anticancer immune responses (Li et al., 2012).

Autophagy in Antigen-Presenting Cells

Basal autophagic activity is elevated in conventional DCs as compared with other cell types. Through a variety of mechanisms, this might contribute to the processing of intracellular and extracellular antigens toward MHC-class-I- or -II-restricted presentation.

Autophagy plays an important role in facilitating the recognition of intracellular danger signals such as MAMPs by APCs, thus allowing bacterial, viral, or pharmaceutical adjuvants to stimulate antigen presentation (Figure 4A). Autophagy can shuttle cytosolic MAMPs to the lumen of endosomes, where they can interact with the ligand-binding domain of TLRs, as demonstrated for TLR7 and TLR9 ligands (Deretic, 2012b). In line with this notion, *Atg5*$^{-/-}$ plasmacytoid DCs fail to detect the vesicular stomatitis virus via TLR7 or the herpes simplex virus 1 via TLR9 and hence fail to produce IFN-α in response to these pathogens (Lee et al., 2007). Similarly, *Atg7*$^{-/-}$ plasmacytoid DCs fail to activate IFN-regulatory factor 7 (IRF7) and to produce IFN-α in response to DNA-containing immune complexes that stimulate TLR9. However, this effect might relate to LAP rather than to conventional autophagy because it was not influenced by the absence of ULK1 (Henault et al., 2012).

Autophagy also plays a major role in the presentation of a subclass of MHC-class-II-restricted peptides (Figure 4B). The stimulation of autophagy promotes the display of peptides derived from cytosolic, mitochondrial, or nuclear (as opposed to membranous) sources, suggesting that the autophagic trafficking facilitates their access to the MHC class II loading compartment (MIIC), which is composed of acidic endosomes containing

cathepsins (Dengjel et al., 2005). Indeed, a particular type of autophagy known as endosome-mediated autophagy prevails in DCs. In this setting, autophagosomes emerge from MIICs and bear both the molecular machinery for antigen presentation and autophagosomal markers such as LC3 and ATG16L1. Endosome-mediated autophagy may be responsible for the engulfment of DC aggresome-like lipopolysaccharide-induced structures (DALISs), which are marked by ubiquitin and p62 (Kondylis et al., 2013). *Atg5*$^{-/-}$ DCs are impaired in their ability to present soluble and cell-associated antigens on MHC class II molecules, and they thus trigger suboptimal CD4^{+} T cell responses against herpes simplex virus type 2 components (Lee et al., 2010). Moreover, the presentation of citrullinated peptides on MHC class II molecules depends on ATG5 expression and can be inhibited by the PI3K inhibitor 3-methyladenine (Ireland and Unanue, 2011). This process could be relevant to the pathogenesis of rheumatoid arthritis, in which citrullinated self-antigens are prominent (Klareskog et al., 2008). Studies in which *Atg5*$^{-/-}$ thymi were transplanted into wild-type recipients illustrate that autophagy is required for the MHC-class-II-restricted presentation of some peptides involved in the positive and negative selection of CD4^{+} T cells (Nedjic et al., 2008). Thus, in thymic medullary epithelial cells, self-antigens gain access to MHC class II presentation at least in part via autophagy, and this process assists T cell selection. The requirement for autophagy in the course of thymic selection is particularly strong for scarce antigens and for antigens that access the autophagic compartment (but not for plasma membrane proteins) (Aichinger et al., 2013). It is not clear whether similar observations apply to antigen presentation by peripheral DCs.

When DCs form conjugates with T cells, their autophagosomes are oriented toward the immunological synapse secondary to the activation of AMPK by serine threonine kinase 11 (STK11, best known as liver kinase B1, LKB1) (Figure 4C) (Wildenberg et al., 2012). The inhibition of autophagy in DCs by the RNA-interference (RNAi)-mediated depletion of ATG16L1 or immunity-related GTPase family M (IRGM) increases the duration of the synaptic interaction between DCs and T cells, hence stimulating T cell activation while favoring the generation of T_H17 responses (Wildenberg et al., 2012). These results suggest that autophagy might impinge on the dialog between APCs and T cells, although they do not unravel the mechanisms that underlie this phenomenon.

Autophagy might also contribute to the cross-presentation of MHC-class-I-restricted antigens, mainly because it participates in the intracellular trafficking and handling of microbial components (Figure 4D). For example, DCs control chlamydial infections within small inclusions that disintegrate upon DC activation, allowing for the release of bacteria into the cytosol. These cytosolic bacteria are then captured by autophagosomes and degraded upon the recruitment of cathepsin-containing amphisomes. Finally, preprocessed antigens access the cytosol compartment and are processed by proteasomes, reimported into the endosomal pathway, and loaded into recycling MHC class I molecules (Fiegl et al., 2013). In other circumstances, autophagy can contribute to the generation of microbial peptides that are loaded into recycling MHC class I molecules within the vacuolar compartment. This pathway does not require the transporter associated with antigen processing (TAP) complex or the trimming of antigenic peptides at the N-terminus but instead relies on the trafficking of newly formed MHC class I peptide complexes through the endocytic pathway to the cell surface (Tey and Khanna, 2012).

Of note, the importance of autophagy for antigen presentation is not limited to DCs but also extends to other APCs including B cells and macrophages. For instance, both macrophages and B cells treated with pharmacological inhibitors of autophagy are impaired in their ability to present antigens on MHC class II molecules (Brazil et al., 1997; Nimmerjahn et al., 2003).

In summary, autophagy plays a major role in how antigens from infected or transformed cells are taken up by APCs, digested to form peptides that can be loaded onto MHC class I or II molecules, and finally presented to T cells.

Autophagy in T Lymphocytes

Autophagy is induced in both T and B cells upon stimulation of their T cell and B cell receptors, respectively. ATG3, ATG5, and ATG7 are dispensable for the development of thymocytes, but their absence impairs the survival and proliferation of peripheral T cells. (Table 1) (Pua et al., 2007). Thus, ATG3, ATG5, and ATG7 contribute to mature T cell homeostasis and are essential for the extra-thymic survival of T lymphocytes. At least in part, this might reflect the critical role of autophagy in the restriction of the mitochondrial and ER compartments that accompanies T cell maturation (Jia et al., 2011; Pua et al., 2009; Stephenson et al., 2009). Along similar lines, *Atg5*$^{-/-}$ B cell progenitors exhibit developmental defects at the pro- to pre-B transition, resulting in a dramatic reduction of peritoneal B-1 B cells (Miller et al., 2008). Autophagy also appears to be required for the development and function of plasma cells, thuse impacting not only cellular but also humoral immune responses (Conway et al., 2013; Miller et al., 2008). This aspect will not discussed further here.

Autophagy might be needed to preserve intracellular ATP concentrations or to supply other metabolic intermediates (such as lipids) that are required for lymphocyte activation and proliferation (Altman and Dang, 2012). Nonetheless, there is some controversy over the actual contribution of autophagy to the adaptation of T cells to changing metabolic demands. Most data in this respect have been gathered by knockout of essential autophagy-related genes during early thymocytic development, a setting in which T cells accumulate undigested autophagic substrates, including dysfunctional mitochondria. Indeed, although the inducible deletion of *Atg3* in mature naive T cells does not augment their mitochondrial mass and does not compromise their survival, T cells that have lacked *Atg3* since thymic development do exhibit an increased mitochondrial content and are relatively vulnerable to cell death (Jia and He, 2011). This suggests not only that autophagy plays distinct roles at different stages of T cell development but also that the short- and long-term consequences of autophagy must be distinguished from each other.

Autophagy is downregulated in T cells from aging mice, as well as in circulating CD8^{+}CD57^{+} senescent cells from healthy human volunteers, and this downregulation correlates with increased DNA damage (Phadwal et al., 2012). Thus, aging-associated defects in autophagy might contribute to the immunosenescence of T cells. Of note, at least in some settings, autophagy

Table 1. Examples of the Critical Role of Autophagy in Immune Cells

Cell Type	Manipulation or Genotype	Phenotype	Reference
Bone-marrow-derived DCs	MAP1LC3A-specific siRNA; *Becn1*$^{+/-}$	Reduced maturation (MHC class II, CD40, CD80 and CD86 expression); reduced production of TNF-α, IL-6, IL-12p40	(Morris et al., 2011)
Monocytes	*Atg7*$^{fl/fl}$ - *Vav-Cre*	Inhibition of CSF1-driven differentiation into macrophages	(Jacquel et al., 2012)
B lymphocytes	Reconstitution with *Atg5*$^{-/-}$ fetal liver cells; *Atg5* $^{fl/fl}$ - *Cd19-Cre*	Defect in B cell development at the pro- to pre-B cell transition; absence of peripheral CD5^{-} B cells	(Miller et al., 2008)
	Atg5 $^{fl/fl}$ - *Cd19-Cre*	Reduced differentiation into plasma cells; deficient production of immunoglobulins	(Pengo et al., 2013)
T lymphocytes	Reconstitution with *Atg5*$^{-/-}$ fetal liver cells	Reduced numbers of thymocytes, peripheral T lymphocytes and B cells Increased death of mature T cells; reduced proliferation of CD4^{+} T cells upon TCR stimulation	(Pua et al., 2007)
	Atg3$^{fl/fl}$ - *ER-Cre* plus exposure of T cells to tamoxifen in vitro; *Atg3*$^{fl/fl}$ - *Lck-Cre*	Defective survival of naive CD4^{+} and CD8^{+} T cells as well as expanded mitochondria and ER	(Jia and He, 2011)
	Atg5$^{fl/fl}$ - *Lck-Cre* and *Atg7*$^{fl/fl}$ - *Lck-Cre*	Defective survival and proliferation of naive CD4^{+} and CD8^{+} T cells as well as increased mitochondrial mass	(Stephenson et al., 2009)
	Atg7$^{fl/fl}$ - *ER-Cre* plus exposure of T cells to tamoxifen in vitro	Defective IL-2 and IFN-γ production by helper T cells; reduced proliferation of helper T cells after stimulation	(Hubbard et al., 2010)
	Atg7$^{fl/fl}$ - *Lck-Cre*	Increased mitochondrial content, ROS production and defective Ca^{2+} handling; increased apoptotic death of peripheral T cells	(Jia et al., 2011) (Pua et al., 2009)
	Atg7$^{fl/fl}$ - *Cd4-Cre*	Increased apoptotic death upon CD3/CD28 crosslinking	(Ch'en et al., 2011)
	Becn1$^{fl/fl}$ - *Cd4-Cre*	Increased levels of BIM and pro-caspase-3 and −8; increased apoptosis of CD4^{+} cells upon TCR stimulation	(Kovacs et al., 2012)
	Pik3c3$^{fl/fl}$ - *Cd4-Cre*	Increased mitochondrial mass and ROS in naive T cells; reduced survival of naive T cells	(Willinger and Flavell, 2012)

Abbreviations are as follows: Becn1, Beclin 1; Cre, CSF1, colony-stimulating factor 1; ER, estrogen receptor; IFN, interferon; Ig, immunoglobulin; IL, interleukin; Lck, lymphocyte protein tyrosine kinase; MAP1LC3A, microtubule-associated protein 1 light chain α; ROS, reactive oxygen species; siRNA, small interfering RNA; TCR, T cell receptor; and TNF-α, tumor necrosis factor α.

can mitigate the activation of effector T cells, for instance by interfering with the T cell receptor (TCR)-induced stimulation of the NF-κB signal transduction cascade. This signaling pathway requires the adaptor protein BCL10, which can be tagged with K63-linked ubiquitin chains, recognized by p62, and hence degraded by autophagy (Paul et al., 2012). Thus, autophagy can avoid the adverse consequences of the unrestricted NF-κB activation that accompanies immunosenescence.

Autophagy-Related Changes in the Antigenicity of Target Cells

Because autophagy impacts ADC, APC, and T lymphocyte functions, it is logical to ask whether and how autophagy affects the recognition of target cells by cytotoxic effectors. Autophagy can indeed limit the susceptibility of infected or oncogene-transformed cells to lysis by cytotoxic T lymphocytes (Akalay et al., 2013), perhaps reflecting its general cytoprotective functions. Beyond this, autophagy has a profound impact on the breadth of the immunopeptidome, i.e., the ensemble of all antigenic peptides that are presented by cell-surface-exposed MHC class I molecules (Admon and Bassani-Sternberg, 2011). The potential mechanisms underpinning such an antigenic effect are manifold.

First, autophagy profoundly affects translation. The induction of autophagy is accompanied by the phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), which blocks 5′ cap-dependent translation while favoring that of mRNAs harboring an internal ribosome entry site (IRES) (Figure 5A) (Thakor and Holcik, 2012). Along similar lines, autophagy frequently ensues the inhibition of TORC1 (Laplante and Sabatini, 2012) and hence mimics rapamycin in its ability to profoundly alter the immunopeptidome, leading to the appearance of multiple neoantigens on the cell surface (Caron et al., 2011). This can be explained by the fact that TORC1 inhibition causes the dephosphorylation

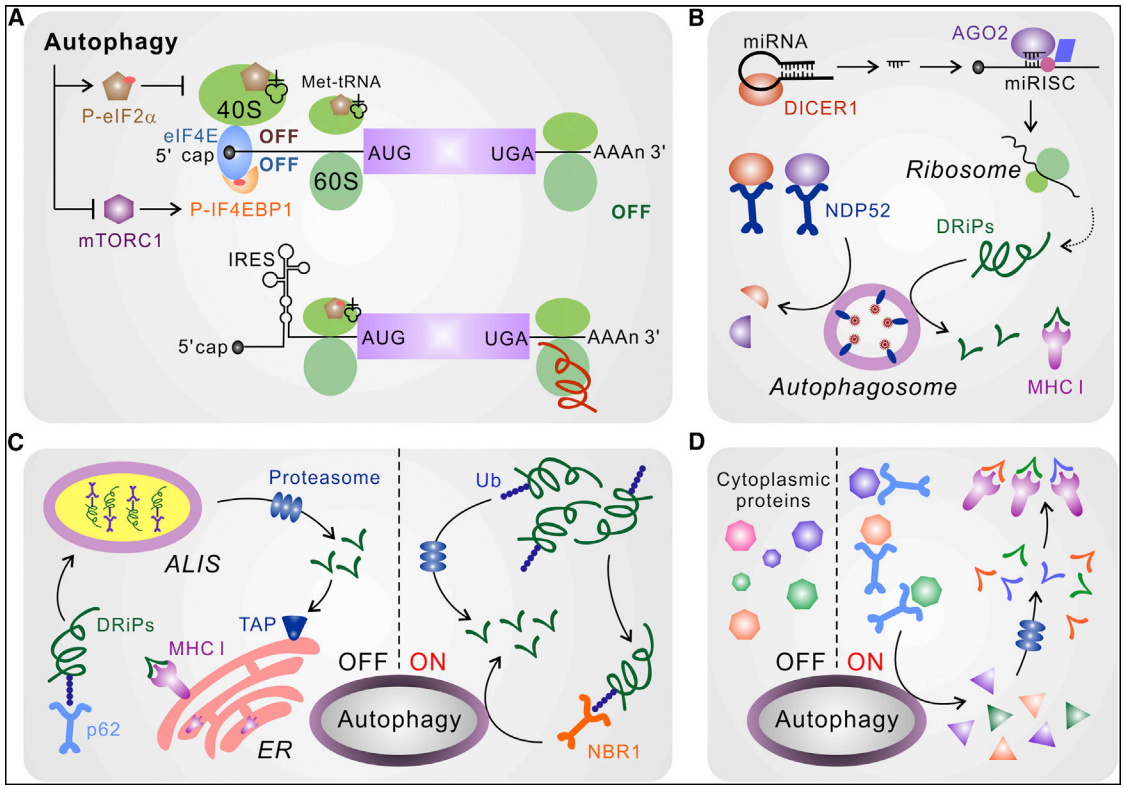

Figure 5. Effects of Autophagy on the Immunopeptidome

(A) Consequences of autophagy on protein translation. The induction of autophagy is accompanied by the phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) and mammalian target of rapamycin complex 1 (mTORC1) inhibition, both of which turn off conventional 5′-cap-dependent transcription by ribosomes. Conversely, the transcription of mRNAs containing internal ribosome entry sites (IRESs) operates independent of 5′-cap molecules and is not inhibited by autophagy. Abbreviations are as follows: eIF4E, eukaryotic translation initiation factor 4E; eIF4EBP1, eIF4E-binding protein 1; and P-eIF2α, phosphorylated eIF2α.

(B) Impact of autophagy on the miRNA core machinery. Two core components of the miRNA-handling machinery, namely, DICER1 and argonaute RNA-induced signaling complex (RISC) catalytic component 2 (AGO2), are captured by the adaptor nuclear dot protein of 52 kDa (NDP52) and degraded by autophagy. mRNAs processed by the miRNA-induced RISC (miRISC) are prone to generate defective ribosomal initiation products (DRiPs), which take precedence over full-length polypeptides as they are loaded onto MHC class I molecules.

(C) DRiP degradation. When autophagy is inhibited, ubiquitinated DRiPs accumulate in aggresome-like-induced structures (ALISs) via a p62-dependent mechanism, are degraded by proteasomes, and enter the classical transporter-associated with antigen processing (TAP)-dependent MHC class I presentation pathway. Conversely, when autophagy is operational, ALISs do not form and ubiquitinated DRiPs are either recognized by neighbor of BRCA1 gene 1 (NBR1) and destined to autophagic degradation or directly processed by proteasomes.

(D) Epitope liberation. Autophagy can mediate the partial degradation of some antigens, thus facilitating their further digestion through proteasomes. This process is known as "epitope liberation."

of eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (eIF4EBP1, best known as 4E-BP1) and ribosomal protein S6 kinase 70 kDa polypeptide 1 (RPS6KB1, best known as $p70^{S6K}$), both of which are involved in 5′ cap-dependent translation (Ma and Blenis, 2009). Experimentally, it has been possible to immunize mice against these novel, rapamycin-induced MHC-class I-restricted peptides and hence to stimulate the development of cytotoxic T lymphocytes that specifically lyse rapamycin-treated, but not untreated, lymphoma cells (Caron et al., 2011). Of note, although it has been proposed that stress-responsive eIF2α kinases such as PKR might indirectly activate TORC1 (and thus stimulate translation) (Kazemi et al., 2007), several authors consider the eIF2α and TORC1 signal transduction cascades to be largely independent from each other (Ma and Blenis, 2009).

Second, autophagy can impact the immunopeptidome by regulating miRNA homeostasis (Figure 5B). NDP52 targets the miRNA-processing enzyme DICER1 as well as the main effector of miRNA-dependent silencing, argonaute RISC catalytic component 2 (AGO2, also known as EIF2C2), for autophagic destruction (Frankel and Lund, 2012). MHC-class I-associated peptides preferentially originate from transcripts bearing miRNA-responsive elements because these are highly susceptible to fail translation and generate DRiPs (Granados et al., 2012), and the abundance of distinct miRNAs has a robust effect on the immunopeptidome.

Third, autophagy directly affects the subcellular fate of DRiPs, which are ubiquitinated and either processed by proteasomes or recognized by NBR1 and destined to autophagic degradation (Figure 5C). In particular, when autophagy is inhibited, DRiPs accumulate in aggresome-like-induced structures (ALISs) via a p62-dependent mechanism, are degraded by the proteasome, and enter the classical MHC class I antigen processing and presentation pathway. As a consequence, a reduced autophagic

clearance of DRiPs might specifically stimulate MHC class I antigen presentation (Wenger et al., 2012).

Fourth, autophagy can directly initiate the degradation of self-antigens, which are subsequently processed by proteasomes and presented in the form of MHC-class-I-bound peptides (Figure 5D). This phenomenon, which has been referred to as "epitope liberation," might confer unique antigenic properties to autophagic cells (Demachi-Okamura et al., 2012).

Altogether, these examples illustrate how autophagy-associated changes can alter the antigenic properties of cells and hence modulate their susceptibility to recognition by the immune system. Although the physiological relevance of these changes has not been investigated in vivo, autophagy might facilitate the recognition of stressed cells by T lymphocytes (Caron et al., 2011). Moreover, the autophagy-associated alterations of the immunopeptidome might affect antigen presentation by DCs upon cross-dressing, i.e., the transfer of preloaded MHC class I molecules from the surface of ADCs to CD8α^+ or CD8α^- DCs (Smyth et al., 2012; Wakim and Bevan, 2011).

Immune-Relevant Perturbations of Autophagy

Genetic alterations in autophagy may be hereditary, predisposing individuals to autoimmune, auto-inflammatory, or infectious diseases. Moreover, autophagy can be inactivated by genetic or epigenetic events in somatic cells, favoring the escape of (pre)malignant lesions from immunosurveillance.

Infectious Diseases

Various single-nucleotide polymorphisms (SNPs) or mutations in genes coding for IFN-inducible GTPases have been linked to an increased susceptibility to bacterial or viral infections in humans. These genes include *IRGM* (which affects the susceptibility to *M. tuberculosis* and *S. enterica* serovar typhimurium) (King et al., 2011), *GBP1* and *GBP2* (influencing the propensity to infection by *Chlamydia trachomatis*, *S. enterica* serovar typhimurium, *Lysteria monocytogeneses*, and adherent invasive *Escherichia coli*) (Kim et al., 2012a), as well as *MX1* (affecting vulnerability to hepatitis B, hepatitis C, measles, and West Nile viruses) (Bigham et al., 2011; Saito et al., 2004). However, although autophagy-deficient mice undoubtedly exhibit a pleiotropic susceptibility to infectious agents, the link between the aforementioned genes and autophagy remains elusive. IFN-inducible GTPases have indeed been postulated to play a broad role in vesicular trafficking by stimulating not just autophagic but also oxidative, membranolytic, and inflammasome-related antimicrobial activities (Kim et al., 2012a).

Systemic Lupus Erythematosus

Genome-wide association studies and meta-analyses have linked genetic polymorphisms in *ATG5* and *DRAM1* to the pathogenesis of systemic lupus erythematosus (SLE) (Harley et al., 2008; Yang et al., 2013). However, the functional consequences of these SNPs on the immune system have not been elucidated yet. T cells from SLE patients or from two distinct SLE-prone mouse strains, namely (NZB × NZW)F_1 and MRL$^{lpr/lpr}$ mice, reportedly contain increased amounts of autophagosomes as compared to T cells from control patients or mice (Gros et al., 2012). This suggests that SLE might be associated with alterations in the autophagic flux, but the underlying molecular defects have not yet been elucidated. According to one study, naive CD4$^+$ T cells from SLE patients are resistant to the induction of autophagy by autologous IgGs (Alessandri et al., 2012). Of note, SNPs in *ATG5* (although different from those associated with SLE) have also been linked to childhood asthma (Martin et al., 2012), although the underlying molecular mechanisms remain elusive.

Crohn's Disease

SNPs in several autophagy-relevant genes (e.g., *ATG16L1*, *NOD2*, and *IRGM*) influence the pathogenesis of Crohn's disease but not that of ulcerative colitis. In particular, ATG16L1 can be affected by an amino acid substitution (T300A) within its conserved WD repeat domain (Hampe et al., 2007), whereas NOD2 might carry multiple distinct mutations in its C terminus (Hampe et al., 2001; Ogura et al., 2001). *IRGM* can be affected by at least two alterations that reduce its expression; namely, these alterations are a 29 Kb deletion upstream of the coding sequence and a synonymous substitution (c.313C > T) that facilitates the interaction of the IRGM-coding mRNA with miR-196, which is expressed in the intestinal epithelium (Patel and Stappenbeck, 2013). NOD2 (as well as its homolog NOD1) can directly interact with ATG16L and is recruited to the plasma membrane of intestinal epithelial cells at bacterial entry sites, from which it channels bacteria to xenophagic degradation (Travassos et al., 2010). Moreover, NOD2 stimulates autophagy in DCs, thus favoring the handling of pathogenic bacteria and the subsequent presentation of bacterial antigens (Cooney et al., 2010). *ATG16L1* loss-of-function mutations that are pathogenic in the context of inflammatory bowel disease could be advantageous in the context of chronic bladder infection by *E. coli.* Thus, the absence of *ATG16L1* from bladder epithelial cells or hematopoietic cells was sufficient to generate a relative resistance against uropathogenic *E. coli* (Wang et al., 2012). In models of Crohn's disease, autophagic defects appear to compromise the antimicrobial defenses of intestinal epithelial cells (in particular Paneth cells) (Cadwell et al., 2008), to subvert the microbicidal function of immune cells, and to lead to an exaggerated secretion of IL-1β, IL-6, and TNF-α (Lapaquette et al., 2012; Saitoh et al., 2008). Although it is tempting to ascribe the proinflammatory effects of ATG16L1^{A197T} on the intestinal epithelium to an autophagic defect, it remains possible that *ATG16L1* mutations affect autophagy-unrelated phenomena. In this respect, an IFN-γ-elicited supramolecular complex involving the ATG5-ATG12 conjugate and ATG16L1 has been suggested to mediate antiviral effects independently from the degradative activity of autophagy (Hwang et al., 2012). Moreover, ATG16L1 reportedly influences hormone secretion by neuroendocrine cells in an autophagy-independent fashion (Ishibashi et al., 2012). This suggests (but does not formally demonstrate) that *ATG16L1* mutations contribute to the pathophysiology of Crohn's disease through pleiotropic effects on multiple cell types.

Cystic Fibrosis

Cystic fibrosis (CF) is caused by mutations in *CFTR*, encoding cystic fibrosis transmembrane conductance regulator, and is the most common inherited lethal disease among Caucasians. The loss-of-function of CFTR, which is coupled to a progressive autophagic defect presumably as a result of the sequestration of BECN1 in perinuclear aggregates (Luciani et al., 2010), culminates in chronic bacterial infection (and inflammation) of the lungs. The restoration of autophagy by pharmacological agents such as cystamine and rapamycin appears to improve the

residual function of mutant CFTR by increasing its half-life at the plasma membrane, at least in the case of the most frequent pathogenic CFTR mutation, ΔF508 (Villella et al., 2013). Moreover, rapamycin has been shown to exert profound antibacterial and anti-inflammatory effects in murine models of CF and in explanted nasal epithelia from CF patients (Abdulrahman et al., 2011). It remains to be seen whether these preclinical results can be translated to children affected by CF.

Cancer

From a classical point of view, cancer represents a cell-autonomous (epi)genetic disease originating from the accumulation of driver alterations that (1) are accompanied by a plethora of passenger mutations and (2) are coupled to a high degree of genetic and phenotypic heterogeneity (Hanahan and Weinberg, 2011). From an immunological point of view, cancer can only develop when (pre)malignant cells escape from immunosurveillance by losing (or altering) their antigenic properties or by actively suppressing antitumor immune responses (Schreiber et al., 2011). Especially during early oncogenesis, a variety of (epi)genetic events may inactivate autophagy. These include the loss of heterozygosity of *BECN1*; alterations causing constitutive signaling via mTOR, such as activating mutations in the catalytic subunit of PI3K or the loss of *PTEN*; the accumulation of mutant p53, and the overexpression of antiapoptotic proteins from the BCL-2 family (Morselli et al., 2009; White, 2012). This property of incipient cancer lesions favors genomic instability (Mathew et al., 2007), perhaps as a result of redox alterations (Green et al., 2011), and might also be tied to the escape of pre(malignant) cells from immunosurveillance. Autophagy-deficient tumors indeed fail to elicit anticancer immune responses upon exposure to chemotherapy, in line with the essential role of autophagy in the immunogenicity of ADCs (Michaud et al., 2011). It will be important to investigate how these links apply to bioptic specimens from cancer patients and in particular to search for a correlation between natural or therapy-elicited antitumor immunity and autophagic responses, which are often reacquired during tumor progression because they confer to transformed cells an increased resistance against adverse microenvironmental cues (Morselli et al., 2009; White, 2012).

Taken together, these observations suggest that the initiation or progression of various human pathologies with an immunological component is favored by genetic or epigenetic alterations that inhibit autophagy.

Concluding Remarks

Undoubtedly, autophagy plays a critical role in cellular immune responses, in both physiological and pathological settings, raising major expectations on the therapeutic impact of autophagy-modulatory agents. At this point, however, several recurrent problems must be taken into attentive consideration before firm recommendations can be made on the therapeutic inhibition or induction of autophagy.

The conclusion that autophagy is involved in a specific biological process is usually based on interventions targeting one among multiple ATG-coding genes. However, many ATG proteins participate in intracellular trafficking systems beyond autophagy (such systems involve multiple ATG proteins as a module and include, for instance, phagocytosis, LAP, and entosis) or in signal-transduction pathways that crosstalk with PRR-elicited processes (such pathways frequently involve individual ATG proteins). For instance, ATG12 has recently been implicated in both the maintenance of mitochondrial homeostasis (when it is conjugated to ATG3) and mitochondrial apoptosis (Boya et al., 2013; Radoshevich et al., 2010; Rubinstein et al., 2011). Along similar lines, ATG7 reportedly binds p53 and thus controls the ability of the latter to transactivate the gene that encodes cyclin-dependent kinase inhibitor 1A (CDKN1A), a cytoprotective cell cycle inhibitor (Lee et al., 2012). This implies that (1) unequivocal cause-effect relationships between autophagy as a pathway and pathological conditions (or their resolution) have been established in a limited number of instances and (2) much of the data published in this context might have to be reinterpreted in view of the growing number of autophagy-independent functions attributed to ATG proteins (Boya et al., 2013). Thanks to proteomic studies, functional screenings, and robust bioinformatics analyses, multiple autophagy-unrelated physical interactors of ATG proteins have begun to emerge (Behrends et al., 2010; He and Levine, 2010). Further studies, however, are urgently awaited and will need to elucidate the actual pathophysiological relevance of these interactions.

The systemic stimulation or suppression of autophagy can modulate immune responses by affecting ADCs, APCs, or downstream effector cells. This might explain why the clinical manifestations of *ATG5* mutations that are associated with SLE and Crohn's disease in population studies are not so obvious when studied on a patient-by-patient basis; they affect just a fraction of individuals, only once sexual maturity has been attained, and often with an opposite-gender effect. This illustrates the intrinsic difficulty involved in applying linear reasoning to multifactorial autoimmune and autoinflammatory diseases that often develop in a cyclic, nonlinear, and highly context-dependent fashion.

To date, no truly specific modulator of autophagy is broadly available for experimental or clinical exploration. In spite of numerous scientific reports claiming the autophagy-suppressive effects of lysosomotropic molecules such as chloroquine, the actual specificity of these agents is questionable given that lysosomes participate in several cellular processes beyond autophagy. For instance, cloroquine is known to promote lysosomal membrane permeabilization (which triggers apoptotic or necrotic cell death in an autophagy-independent fashion) (Kroemer and Jäättelä, 2005) and to improve antigen presentation by DCs as a result of its effects on lysosomal acidification (Accapezzato et al., 2005). Nonetheless, attractive targets for the development of autophagy inhibitors are several and include the lipid kinase phosphatidylinositol 3-kinase, catalytic subunit type 3 (PIK3C3, best known as VPS34 lipid kinase), the proteolytic enzyme ATG4, and several conjugation systems (Rubinsztein et al., 2012). Moreover, agents that can disrupt inhibitory protein-protein interactions within the BECN1 complex, and thus operate as autophagy inducers, are being developed (Dai et al., 2013; Shoji-Kawata et al., 2013). We surmise that the development of specific autophagy-modulatory drugs will yield invaluable tools for the investigation of the immunological functions of autophagy.

Notwithstanding these multiple caveats, it is plausible that the therapeutic effects of several established drugs might be explained—or at least supported—by their ability to stimulate autophagy. For instance, it appears that 1α,25-dihydroxycholecalciferol (vitamin D3) is rate limiting for the autophagic activity

of human macrophages and that the external supply of this compound potently inhibits HIV-1 replication and clears *M. tuberculosis* in preclinical models (Fabri et al., 2011). This is in line with old epidemiological data revealing the positive impact of UV exposure on the clearance of cutaneous tuberculosis (Van Der Lugt and Rottier, 1958). Importantly, the successful antimycobacterial drugs isoniazid and pyrazinamide potently induce autophagy in *M. tuberculosis*-infected cells, and their antibacterial activity is limited in Atg7-deficient strains of *Drosophila melanogaster* (Kim et al., 2012b). Conversely, azithromycin, a macrolide antibiotic, inhibits autophagy as an unwarranted side effect and thus predisposes CF patients to mycobacterial infections (Renna et al., 2011). These observations illustrate the importance of comprehending the autophagy-modulatory (side) effects of existing drugs.

Many comorbidities of obesity can be explained by the establishment of a systemic inflammatory state, a process that at least in part is mediated by the activation of the so-called "metabolic inflammasome," which also causes insulin resistance. In response to fatty acids, a signaling complex comprising PKR, IKK, eIF2α, and insulin receptor substrate 1 (IRS1) becomes activated, resulting in the corollary PKR-dependent activation of ER stress (Nakamura et al., 2010), inflammasomes (Lu et al., 2012), and autophagy (Shen et al., 2012). Autophagy acts as a negative regulator of this interplay, suggesting that measures, such as fasting, that stimulate the autophagic flux at the whole-body level might be healthy because they limit the activation of the metabolic inflammasome. In this context, it remains to be determined whether there exist any agents that mediate robust anti-inflammatory and health-improving effects by stimulating autophagy; for example, such agents might, include AMPK-activating chemicals such as aspirin, metformin, and methotrexate (O'Neill and Hardie, 2013), which induce a state of pseudo-starvation. Nonetheless, given the potent anti-inflammatory and cytoprotective effects of autophagy and its critical contribution to both innate and adaptive immunity, measures that induce autophagy in many cell types might constitute a means of "sharpening" immune responses. Future studies will have to elucidate to which extent and under which circumstances inducers and inhibitors of autophagy might exert therapeutically relevant immunomodulatory functions.

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