Immunologic manifestations of autophagy

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The broad immunologic roles of autophagy span innate and adaptive immunity and are often manifested in inflammatory diseases. The immune effects of autophagy partially overlap with its roles in metabolism and cytoplasmic quality control but typically expand further afield to encompass unique immunologic adaptations. One of the best-appreciated manifestations of autophagy is protection against microbial invasion, but this is by no means limited to direct elimination of intracellular pathogens and includes a stratified array of nearly all principal immunologic processes. This Review summarizes the broad immunologic roles of autophagy. Furthermore, it uses the autophagic control of Mycobacterium tuberculosis as a paradigm to illustrate the breadth and complexity of the immune effects of autophagy.

Introduction

The term autophagy in its broadest sense refers to a set of diverse processes that deliver cytoplasmic constituents to lysosomes for degradation. In this Review, we focus only on the sensu stricto autophagy as a well-delineated pathway controlled by autophagy-related gene–encoded (ATG-encoded) factors (1). Since general roles of autophagy in immunity have been extensively covered recently (2, 3), here we primarily give a summary with an update and extend this to a focus on one of the early paradigms of autophagy in immune defense — control of Mycobacterium tuberculosis (4). Our understanding of this model system has continued to evolve since the initial reports that autophagy can eliminate intracellular bacteria (4, 5) and helps to illustrate a number of general immunologic manifestations of autophagy.

A cardinal structural and functional feature of autophagy is the formation of organelles called autophagosomes. The formation of autophagosomes is under the control of the ATG factors Unc-51 like autophagy activating kinase 1/2 (ULK1/2; Atg1 in yeast), beclin 1 (Atg6 in yeast), and mammalian paralogs of yeast Atg8 (light chain 3A [LC3A], LC3B, LC3C, GABARAP, GABARAPL1, and GABARAPL2) (ref. 1 and Figure 1A). A cascade of events controlled by these and additional ATG factors leads to the formation of a phagophore from several membrane sources including ER (6) and the endosomal system (7). Recently, additional contributions of phospholipid precursors and signals from lipid droplets have been recognized (8). The phagophore elongates, captures cytoplasmic targets earmarked for autophagic degradation, and following closure, delivers them to lysosomes (1).

Evolutionarily, autophagy may be the earliest form of eukaryotic innate defense against invading microorganisms. Competition for intracellular nutrients might have been one of the most primordial danger signals available to the eukaryotic cell to detect microbial invasion and eliminate microbes through autophagy. This is manifested in present-day relationships. For example, metabolic signaling downstream of starvation is associated with antimicrobial autophagy in response to bacterial invasion (9). Starvation can induce autophagy to kill virulent M. tuberculosis in macrophages (4). Thus, the classical starvation signals for autophagy should also be considered as signals for immune defenses. The nutritional signals leading up to autophagy activation are transduced by mTOR and AMPK. mTOR inhibits ULK1 by phosphorylating residues at inactivating sites (e.g., Ser757), whereas AMPK stimulates ULK1 by phosphorylating ULK1 at activating residues (e.g., Ser317 and Ser777) (10, 11). Activated ULK1 phosphorylates beclin 1 at Ser15 (12). Additionally, AMPK directly phosphorylates beclin 1 at Ser91/Ser94 and helps activate it (13). Furthermore, K63 ubiquitination events lead to stabilization of the autophagy regulatory complexes (14, 15). These events set off a complex cascade of membrane trafficking transactions governing initiation of autophagy, elongation of phagophores, and maturation of autophagic organelles into autolysosomes.

Autophagy regulates inflammation

Proinflammatory effects of autophagy. A number of studies describe both pro-inflammatory and anti-inflammatory actions of autophagy. Autophagy assists productive inflammatory processes, including inflammasome activation (Figure 1B). For example, autophagy delivers cytosolic pathogen-associated molecular patterns (PAMPs) to lumenally oriented TLRs in the endosomes, enabling detection of viral replication intermediates and type I IFN production by plasmacytid DCS, as demonstrated for TLR7 (16). Furthermore, when an inflammasome is properly activated in response to a need to clear an irritant, autophagy contributes to the unconventional secretion of IL-1β as well as IL-18 and high-mobility group box 1 (HMGB1) (refs. 17, 18, and Figure 1B). Whereas autophagic processes can amplify productive TLR signaling to enhance antimicrobial defenses, autophagic augmentation of PAMP/pattern recognition receptor (PAMP/PRR) responses may also contribute to autoimmune pathology (19, 20).
Autophagy and inflammasome. Autophagy suppresses inflammasome activation (Figure 1C and refs. 21–25). Loss of autophagy (ATG16L1 deficiency) increases IL-1β levels in a mouse model of gut inflammation (21). Basal autophagy clears sources of endogenous NLRP3 inflammasome agonists such as depolarized mitochondria leaking ROS, mitochondrial DNA, and oxidized mitochondrial DNA (22, 23), thereby preventing spurious inflammasome activation. Furthermore, autophagy degrades several inflammasome components, including NLRP3 (26) and the IFN-inducible protein AIM2 (27). Processed caspase-1 (p20) is reduced in cell extracts upon stimulation of autophagy (27). As described above, once the inflammasome is activated, autophagy contributes to the unconventional secretion of IL-1β as well as IL-18 and HMGB1 (17, 18). An alternative view suggests that pro–IL-1β may be degraded through autophagy (28). Thus, autophagy rations extracellular IL-1β following productive responses, whereas it prevents the unnecessary and excessive tissue-damaging inflammasome activation by clearing endogenous danger-associated molecular patterns (DAMPs). Autophagy can be further induced or propagated by extracellular IL-1β (29) in an autocrine and paracrine fashion following inflammasome activation; however, it is inhibited by activated caspase-1, which degrades adaptor molecules necessary for autophagy induction (30). These circuits represent regulatory loops with feed-forward (IL-1β) and inhibitory (caspase-1) effects on autophagy. Thus, autophagy prevents unscheduled or excessive inflammasome activation but supports normal inflammasome output.
can come directly from intracellular bacteria (38, 39). STING controls spatial activation of TBK1, leading to IFN regulatory factor 3 (IRF3) phosphorylation and type I IFN production downstream of nucleic acid sensing and cGAMP synthesis. ATG9 inhibits the assembly of STING and TBK1, which has to occur at an appropriate intracellular location where IRF3 and IRF7 are phosphorylated, thus suppressing type I IFN activation in response to cytosolic dsDNA (34). The most upstream autophagy activator protein kinase, ULK1, phosphorylates STING at Ser366 and inhibits sustained type I IFN activation in response to dsDNA (40). Beclin 1, another key regulator of autophagy initiation and maturation, also suppresses the type I IFN response by direct binding and inhibition of cGAS in response to viral DNA (41).

The autophagic process as a whole also controls type I IFN activation. A negative relationship between autophagy and type I IFN production is evident in autophagy-deficient cells. In the absence of autophagy, RLR signaling is increased due to amplified MAVS levels secondary to the accumulation of mitochondria (where MAVS resides) in the absence of mitophagy, a process of autophagic elimination of mitochondria (42). Moreover, increasing pools of depolarized mitochondria resulting from the absence of autophagy contribute ROS, further enhancing RLR outputs (42). In the context of the dsDNA/cGAS/cGAMP/STING axis that promotes type I IFN expression, cGAS binding to beclin 1 dissociates its negative regulator Rubicon. This facilitates autophagic removal of cytosolic dsDNA and curtails excessive type I IFN responses (41). Thus, multiple autophagy...
Autophagy downregulates NF-κB signaling. Autophagy modulates NF-κB responses to microbial agonists. For example, autophagy downregulates the NF-κB response to fungal pathogens that occurs through the immunoreceptor tyrosine-based activation motif receptor dectin 1, which requires the CARD/BCL10/MALT1 (CBM) signalingosome. First, Rubicon, a negative regulator of beclin 1, directly inhibits the CBM signalosome by binding to CARD9 (43). Second, autophagy, disinhibited by the translocation of Rubicon from the beclin 1 to CARD9 of the CBM complex, directly degrades BCL10 (44). Thus, these two linked processes downregulate pro-inflammatory NF-κB signaling downstream of dectin 1.

NF-κB signaling may also be downregulated by effects on IKK through targeting of NEMO for autophagic degradation (45). NDP52, which is an adaptor protein acting in autophagic elimination of bacteria (46), assists degradation of TLR adaptors TRAF6 and TRIF and attenuates NF-κB signaling (47, 48). The recently described autophagy connections with heat shock response may also be linked to NF-κB downregulation (49). Thus, autophagy interferes with NF-κB-driven inflammation.

Genetic polymorphisms link autophagy and immune disorders. Autophagic control of inflammation and immunity is underscored by the genetic links between immunologic or inflammatory disorders and polymorphisms in human orthologs and paralogs (e.g., ULKI, ATG2A, ATG16LI, etc.) of the yeast Atg genes as well as in human autophagy genes absent in yeast (e.g., IRGM, NDP52, etc.). Polymorphisms in ATG16LI (21) and IRGM (50) confer risk for Crohn’s disease, a common form of inflammatory bowel disease (IBD) (51, 52). An extensive genome-wide association study (GWAS) analysis of 75,000 cases indicated a general overlap between IBD and mycobacterial disease susceptibility loci (53), expanding on the theme that IRGM polymorphisms play a role in both Crohn’s disease and tuberculosis. IRGM polymorphisms may also be a risk factor in systemic lupus erythematosus (SLE) (54). Polymorphisms in ULKI (55), NDP52 (48), and ATG2A, ATG4A, and ATG4D (56) have also been linked with Crohn’s disease. GWAS analyses have linked ATG5 variants with SLE (57–59) and asthma (60). Rheumatoid arthritis has been linked to variants in the PRDMD1-ATG5 intergenic region (61). A human autophagy locus required for autophagosomal maturation has been linked to Vici syndrome, conferring immunodeficiency (62). Thus, ample genetic evidence indicates a role for autophagy in inflammatory and immune disorders in human populations.

Autophagy affects adaptive immunity

Autophagy affects T, memory B, and plasma cells. Autophagy influences development, repertoire selection, maturation, homeostasis, function, and polarization of T cells (Figure 2). In the HSC compartments, autophagy plays a cytoprotective role for self-renewing HSCs (63). Autophagy is important for a proper balance between myeloid and lymphoid progenitors, and conditional deletion of Atg7 in HSCs causes severe myeloproliferation (64). Moreover, lymphocyte, but not the myeloid lineage, is reduced in the absence of autophagy (16, 65). Thymic selection is affected by autophagy (66). Upon exit from the thymus, naïve T cells in the periphery require the autophagy mediator VPS34 (PIK3C3) for proper IL-7 signaling, (67). The naïve T cells depend on autophagy for proper maturation, which entails reduction of mitochondrial content through mitophagy (68) and reduction of mitochondrial content through mitophagy (68) and...
trimming of the ER in order to sustain proper Ca^{2+} homeostasis (69). Autophagy promotes survival of activated T cells by counteracting the proapoptotic effects of FAS/FASL signaling secondary to TCR stimulation (70). Autophagy factors such as beclin 1 may play a role in responsiveness of effector T cells to suppression by regulatory T cells (71). Autophagy also influences T cell polarization. Excessive cell-autonomous release of IL-1α and IL-1β from autophagy-deficient macrophages enhances polarization and duration of Th17 responses (72). The Th17 response is further promoted through abnormal persistence of immunologic synapses formed between autophagy-defective DCs and T cells (73).

Autophagy is necessary for maintenance of memory B cells (74). Autophagy-dependent ER maintenance in plasma cells is necessary to balance immunoglobulin secretion, and loss of autophagy results in abnormal hypersecretion of immunoglobulins (ref. 75 and Figure 2). Autophagy is also important for survival and homeostasis of the bone marrow plasma cell pool and long-lasting humoral immunity (75).

**Autophagy influences antigen presentation.** Autophagy enhances MHC class II presentation of both cytosolic proteins and exogenous antigens. For the former, autophagy delivers cytosolic proteins to the lumen of antigen-processing compartments (66, 76–78). For the latter, autophagic processes enhance MHC class II presentation of extracellular particulate antigens taken up by phagocytosis (65). Autophagy affects MHC class II antigen presentation in antimicrobial defense (77, 79–81). In DCs, autophagy enhances presentation to both CD4+ and CD8+ T cells, a phenomenon that has been associated with the high efficacy of yellow fever vaccination (82). In response to bacterial products, NOD2 stimulation increases autophagy and enhances MHC class II presentation (83). Outside of microbial antigen processing, autophagy affects presentation of self-antigens. For example, autophagy affects self-antigen presentation by thymic gen processing, autophagy affects presentation of self-antigens. For the former, autophagy delivers cytosolic proteins to the lumen of antigen-processing compartments (66, 76–78). For the latter, autophagic processes enhance MHC class II presentation of extracellular particulate antigens taken up by phagocytosis (65). Autophagy affects MHC class II antigen presentation in antimicrobial defense (77, 79–81). In DCs, autophagy enhances presentation to both CD4+ and CD8+ T cells, a phenomenon that has been associated with the high efficacy of yellow fever vaccination (82). In response to bacterial products, NOD2 stimulation increases autophagy and enhances MHC class II presentation (83). Outside of microbial antigen processing, autophagy affects presentation of self-antigens. For example, autophagy affects self-antigen presentation by thymic epithelial cells during positive and negative selection of CD4+ T cell repertoires in the thymus (66, 84). In the context of autoimmunity, autophagy may promote presentation of citrullinated antigens (85). Less is understood regarding how autophagy affects MHC class I presentation, although effects have been noted (86). Thus, autophagy affects antigen presentation in a variety of contexts.

**Autophagy directly eliminates intracellular microbes.**

**Antimicrobial autophagy is a continuum of processes.** Autophagy can interfere with bacterial pathogens at multiple stages of invasion (2). Likewise, autophagy affects viruses at multiple stages of their life cycle (42, 65, 87–90). Depending on the virus, autophagy can promote or restrict viral infection (91–96). Two terms are frequently used to define different forms of direct antimicrobial actions of autophagy (Figure 3): xenophagy, which is the engulfment of cytosolic microbes into double membrane autophagosomes (97), and LC3-associated phagocytosis (LAP) (19, 98). LAP is a process that engages parts of the autophagic machinery when an extracellular cargo is engulfed by phagocytosis. For example, LAP may come into play when an extracellular bacterium is taken up by conventional phagocytosis and remains separated from the cytosol by the delimiting conventional phagosomal membrane. Xenophagy and LAP are useful terms to describe some aspects of a continuum of autophagic machinery engagement with invading microbes. In most instances, even with a single microbe, there is a broad range of mixed events taking place (Figure 3). In most cases, autophagic responses leading up to xenophagy or LAP are guided by several types of PRRs, including a new class of PRRs termed sequestosome 1/p62-like receptors (SLRs), and are further modulated by cytokines and cellular immune networks (see below) (2).

**TLRs and NLRs control autophagy.** TLR stimulation with PAMPs induces autophagy in advance of microbial invasion (99, 100) or concomitant with LAP (65, 98). The detection of microbial presence via TLR sensing (Figure 3) enhances cellular capacity for autophagic elimination of microbes (99, 100). This involves activation of autophagic molecular assemblies, processing and delivery of antimicrobial peptides to the parasitophorous vacuoles (101–103), and focusing of autophagic effectors to the points of microbial entry (98).

SLRs influence autophagic antimicrobial responses (83, 104, 105). NLRs, including NODs (83, 104), intersect with the autophagic machinery. NLRP3, NLRP4, NLRP10, and NLRC4 are found in large protein complexes with beclin 1 (105). The exact role of the broad coalescence of autophagy machinery with NLRPs and NLRCs remains to be fully explored. NOD1 and NOD2 respond to specific PAMPs (muramyl peptides) in the cytosol and recruit ATG16L1 to bacterial invasion sites (104) (Figure 3). A truncation of NOD2 that occurs in patients with Crohn’s disease lacks ATG16L1 in the cytosol instead of recruiting it to the bacterial entry site.

The molecular events during PRR-induced autophagy can be gleaned from studies with NOD2 and TLR4. NOD2 activates ULK1 via RIPK2, which phosphorylates ULK1 at an activating Ser residue (24). TLR4 directs the E3 ligase TRAF6 to ubiquitinate beclin 1, thus dissociating beclin 1 from its negative regulators (14). TRAF6 also ubiquitinates and activates ULK1 (15), but this has yet to be established in the context of PRR signaling. Thus, TLRs and NLRs regulate and localize autophagic responses.

**SLRs guide autophagic apparatus against intracellular microbes.** When a bacterium penetrates into the cytosol, autophagy can still capture it by employing specialized PRRs referred to as SLRs (2), a subgroup of autophagic receptors with prominent immunologic functions. SLRs have been named after sequestosome 1/p62-like receptors (SLRs), and are further modulated by cytokines and cellular immune networks (see below) (2), which phosphorylates ULK1 at an activating Ser residue (24). TLR4 directs the E3 ligase TRAF6 to ubiquitinate beclin 1, thus dissociating beclin 1 from its negative regulators (14). TRAF6 also ubiquitinates and activates ULK1 (15), but this has yet to be established in the context of PRR signaling. Thus, TLRs and NLRs regulate and localize autophagic responses.

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ple, TBK1 or casein kinase 2 can phosphorylate LIRs or the cargo binding motifs in SLRs to enhance their action in anti-microbial autophagy (29, 110, 118). Thus, cells employ generic molecular tags (ubiquitin and galectin) recognized by SLRs to direct autophagy at a variety of invading microbes.

TRIMs expand the receptor repertoire for immunologic autophagy. A recent study (119) reported that the tripartite motif (TRIM) family of proteins may represent a new class of autophagy receptors. Several TRIM family members are already known for roles in immunity (120), but the exact functions of most are unknown. There are over 70 TRIMs in human cells, and at least half of them regulate autophagy (119). For example, TRIM5α is a known retroviral restriction factor (121) that serves as an autophagic receptor and directly recognizes (i.e., without a need for ubiquitin tags) the retroviral capsid and delivers it to autophagosomes for degradation (119). Thus, the TRIM family of proteins may drastically expand the number of autophagic receptors.

DAMPs and cytokines modulate autophagy. DAMPs activate autophagy. This includes HMGB1 (122, 123), ATP (124), extracellular self-DNA complexes (20), mitochondrial DNA released into the cytosol (23), and the presence of ROS (125) from sources such as depolarized mitochondria or activated NADPH oxidase. Translocation of HMGB1 from the nucleus to the cytosol directly activates beclin 1 (122). ROS may affect oxidation-sensitive Atg4 (125), a factor that controls the equilibrium between lipidated LC3 (LC3-II), which helps elongate autophagic membranes, and the inactive delipidated form of LC3, LC3-I. Thus, in addition to microbial PAMPs, host alarmins, pathologic metabolism byproducts, intracellular components secreted by live cells or released from dead cells, and environmental DAMPs can also induce autophagy.

A number of key immunomodulatory cytokines are involved in control of autophagy. IL-1β stimulates autophagy through IL-1R (29). The principal Th1 cytokines such as IFN-γ induce autophagy in macrophages (4, 126, 127), whereas TNF-α stimulates SLR-directed autophagy of Shigella and Listeria (128). The Th2 cytokines IL-4 and IL-13 inhibit autophagy. Importantly, their autophagy-suppressive effects trump the autophagy-activating effects of IFN-γ in mixed cytokine responses (126). IL-10 inhibits autophagy (129). IL-6 suppresses autophagy (130), a connection that needs to be explored in the context of various inflammatory conditions. In general, cytokines known to restrict intracellular pathogens induce autophagy, whereas the cytokines permissive to intracellular microbes inhibit autophagy.

M. tuberculosis: a paradigm for immunologic manifestations of autophagy

Autophagy defends against intracellular M. tuberculosis. The role of autophagy in control of M. tuberculosis (4, 50) has been extensively studied by several independent groups (101-103, 116, 124, 126, 127, 131-139) and has become a paradigm for how autophagy controls intracellular pathogens. Stimulation of autophagy, most likely including a combination of LAP and xenophagy as well as a spectrum of other processes akin to those depicted in Figure 3, overcome the classical M. tuberculosis virulence determinant, known as the phagosome-lysosome fusion block. In the absence of autophagy, the pathogen-imposed inhibition of phagosome-lysosome fusion endows M. tuberculosis with the ability to parasitize macrophages (140). Induction of autophagy promotes conversion of M. tuberculosis phagosomes into autophagolysosomes (141) with strong mycobacterial properties (4, 101, 102, 126). Autophagolysosomes are much more robust antimicrobial compartments than are conventional phagolysosomes. The mycobacterial constituents delivered to autophagolysosomes include antimicrobial peptides (101-103, 131).

In DCs, which have the general capacity to utilize autophagic machinery for prolonged MHC class II presentation (81), a measurable fraction of the intracellular M. tuberculosis bacilli is spontaneously susceptible to basal autophagy (139). However, unless autophagy is activated by exogenous stimuli, only a very small fraction of the intracellular bacilli can be eliminated by basal autophagy in macrophages, the cell type readily parasitized by M. tuberculosis (4, 50, 138, 139). This minor susceptible fraction represents the bacilli that escape from conventional phagosomes (142) or otherwise come in contact with the cytosol (138), where they can be subjected to spontaneous elimination by xenophagy. Only induced autophagy, activated by immunologic or physiologic means (4, 50), can render the majority of the intracellular bacilli susceptible to autophagic elimination.

Th1/Th2 and calcitriol in control of M. tuberculosis autophagy. Th1 and Th2 cytokines induce or suppress autophagy, respectively, in keeping with the restriction or permission of M. tuberculosis (4, 126). In human macrophages, calcitriol, also known as 1,25-dihydroxyvitamin D3, is a key hormonal co-inducer required for IFN-γ activation of autophagy (102, 103, 136, 137). Calcitriol is generated in macrophages from calcidiol (25-hydroxy vitamin D3) circulating in the serum by a specific 1-α-hydroxylase that can be induced through TLR signaling, IFN-γ, and other physiologic stimuli. Low levels of calcitriol and its precursor calcidiol in the serum are associated with susceptibility to tuberculosis (143) and exacerbate HIV/M. tuberculosis co-infections ex vivo (136). Calcitriol may help induce autophagy via Ca2+ and Ca2+/calmodulin-dependent kinase-β (CaMKKβ) (144), which in turn activates AMPK (145). An alternative pathway with a role for cathelicidin upstream of autophagy activation has been identified (103), but the underlying mechanisms have not been defined. Thus, IFN-γ and vitamin D metabolites synergize to activate autophagic elimination of M. tuberculosis in human cells.

Autophagy protects against M. tuberculosis pathogenesis in vivo. In murine models of tuberculosis, autophagy reduces bacillary burden, suppresses inflammation, and protects against lung pathology (72, 138). Virulent M. tuberculosis strains H37Rv (72) and Erdman (138) cause increased lung necrosis and mortality in transgenic Atg5εε LysM-Cre mice, which have autophagy selectively inactivated in myeloid cells, including macrophages, the principal cell type parasitized by M. tuberculosis. Mice with autophagy defects in myeloid cells show increased pathology when challenged with M. bovis bacilli Calmette–Guérin (BCG) (146). Excessive levels of IL-1 are present in the lungs of Atg5εε LysM-Cre mice infected with M. tuberculosis relative to their autophagy-competent littermates (72, 138), in contrast to no differences in IFN-γ and IL-4 responses (72). The elevated IL-1 parallels the intestinal inflammatory models using autophagy-defective mice (21–23), although there are some differences between IL-1α and IL-1β. In keeping with ele-
vated IL-1, the lung immune cells from Atg5fl/fl LysM-Cre+ mice display a marked increase in IL-17 and elements of Th17 polarization (72). Thus, autophagy guards against overexuberant IL-1 responses and excessive Th17 inflammation in murine models of tuberculosis. This may be of significance in determining whether an initial infection progresses into active disease.

*M. tuberculosis* deploys countermeasures against autophagy. Mycobacterial infections show evidence of mTOR activation (which inhibits autophagy) or autophagy induction, correlating with the pathogenic potential or the species or the virulence of the strain (135). The host cell reprogramming by *M. tuberculosis* reduces autophagic capacity of the cell and protects intracellular *M. tuberculosis* from autophagic elimination (147). A number of candidate anti-autophagy factors encoded by *M. tuberculosis* have been reported. An *M. tuberculosis* protein termed enhanced intracellular survival (Eis) (148) may interact with specific autophagy factors or affect upstream signaling regulators. A mycobacterial glycolipid, lipoarabinomannan, has also been reported to inhibit autophagy (149). ESX-1, a type VII secretion system of *M. tuberculosis*, releases a 6-kDa protein, ESAT-6, to block *M. tuberculosis* phagosomal conversion into degradative autolysosomal organelles (150). Pharmacologic agonists of autophagy can overcome the ESAT-6-based block (150), in keeping with the observations that physiologic or immunologic stimulation of autophagy (e.g., by starvation or IFN-γ) (4, 126) kills *M. tuberculosis* by overpowering its anti-autophagic mechanisms.

**Conclusions**

The immune manifestations of autophagy form a prominent intersection of immunity, cell biological processes, cellular microbiology, and metabolism. These interconnections are informative about key physiologic roles of autophagy and its evolution as a biologic process. The recognition of autophagy as an immune phenomenon has opened a new chapter in the narrative of immunity. The lessons learned from the *M. tuberculosis* model system are important not only because tuberculosis is a highly significant human disease, but also because these studies have helped unveil the multi-tiered connections between autophagy and immunity, exceeding the cell-autonomous removal of intracellular microbes. Nevertheless, the direct elimination of intracellular bacteria and other pathogens is undoubtedly one of the most elegant immune manifestations of autophagy.

Many questions remain: How are autophagy and other stress responses integrated to orchestrate immune and other physiologic adaptations? Are SLRs and ubiquitin or galectin tags the only way to capture intracellular microbes? Are there additional autophagic receptors, such as TRIMs, and can they broaden the repertoire of intracellular targets or confer more specificity? Is autophagy the missing link connecting metabolic syndrome, obesity, and inflammation? Is the capture and killing of microbes the end game, or does autophagy also remove the corpses of dead microbes to prevent continuing inflammation and tissue damage? Is autophagy a purely degradative process, or can it excrete certain cargo from the cell?

It is possible that we are underestimating the antimicrobial power of autophagy. There are only a handful of bacteria fully competent to act as intracellular parasites. Does autophagy silently but tirelessly work to remove a myriad of microbes that may on occasion invade our cells? Does autophagy influence the microbiome of an individual (e.g., intestinal microbiota, as can be inferred from the effects that autophagy imparts on the regulated secretion from Paneth and Goblet cells)? Can autophagy be harnessed for pharmacologic intervention in disorders with significant immunologic components? In the context of infectious diseases, can autophagy be a target for development of host-directed therapies? These are but a few of the many questions that remain, which indicates that thus far we have merely scratched the surface.

**Note added in proof.** A recent study has shown that autophagy is also required for survival of effector CD8+ T cells when they enter the contraction phase and is needed for the formation of memory T cells (151).

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