Published in final edited form as:

Curr Opin Immunol. 2009 February ; 21(1): 53–62. doi:10.1016/j.coi.2009.02.002.

Multiple Regulatory and Effector Roles of Autophagy in Immunity

Vojo Deretic

Department of Molecular Genetics and Microbiology, University of New Mexico Health Sciences Center, 915 Camino de Salud NE, Albuquerque, NM 87131, U.S.A

Summary

Autophagy is a cytoplasmic homeostasis pathway, enabling cells to digest their own cytosol, remove toxic protein aggregates, and eliminate defective or surplus organelles. A plenitude of studies have now expanded roles of autophagy to both effector and regulatory functions in innate and adaptive immunity. In its role of an immunological effector, autophagy plays many parts: (i) In its most primeval manifestation, autophagy captures and digests intracellular microbes; (ii) it is an anti-microbial output of Toll-like receptor (TLR) response to pathogen associated molecular patterns (PAMP); and (iii) autophagy is an effector of Th1-Th2 polarization in resistance or susceptibility to intracellular pathogens. As a regulator of immunity, autophagy plays a multitude of functions: (i) It acts as a topological inversion device servicing both innate and adaptive immunity by delivering cytosolic antigens to the lumen of MHC II compartments and cytosolic PAMPs to endosomal TLRs; (ii) autophagy is critical in T cell repertoire selection in the thymus and control of central tolerance; (iii) it plays a role in T and B cell homeostasis; and (iv) autophagy is of significance for inflammatory pathology. A properly functioning autophagy helps prevent autoimmunity and assists in clearing pathogens. When aberrant, it contributes to human inflammatory disorders such as Crohn's disease.

Introduction

Autophagy is a collection of related biomass quantity and quality control systems targeting a range of cytoplasmic components for degradation [1]. Autophagic targets include: (i) individual macromolecules, processed by chaperone-mediated autophagy; and (ii) protein aggregates, sizeable portions of the cytosol, or even whole organelles, all subject to macroautophagy (here and in general referred to as autophagy). A hallmark morphological feature of autophagy is the formation within the cytosol of membrane crescents wrapping around cytoplasmic targets, generating double membrane autophagosomes that convert upon fusion with late endosomal/lysosomal organelles into single delimiting membrane autolysosomes where the captured material is degraded (Fig. 1). Autophagy primarily ensures cellular survival and proper function. For example, cells employ autophagy to remove defective mitochondria or to autodigest their own cytosol to preserve essential anabolic functions during times of nutrient or growth factor deprivation [1]. In contrast to its pro-life function, autophagy can lead to cell death through its molecular and physiological interactions with apoptosis and necrosis and, when excessive, possibly by itself [1]. Since autophagy impacts all cell types, it is not surprising that it broadly affects health, including degenerative diseases and cancer [1]. Here, we will review the immunological roles of autophagy (Box 1) that have grown rapidly

Correspondence to: Vojo Deretic, Ph.D., Professor and Chair, Department of Molecular Genetics and Microbiology, University of New Mexico Health Sciences Center, 915 Camino de Salud, NE, Albuquerque, NM 87131, U.S.A. (505) 272-0291, FAX (505) 272-5309, vderetic@salud.unm.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

within the past several years from a scattering of hints to a full panel of functions interfacing with nearly all aspects of innate and adaptive immunity processes.

Autophagy as a pathway

Facilitated by the work in yeast, where 31 proteins called Atg participate in autophagy, rapid progress is being made in understanding mammalian autophagy [1]. During initiation and elongation autophagosomal crescents (termed phagophores) are formed and enlarged, driven by, in the simplest rendition, the products of two protein conjugation systems: (i) Atg16 (in mammals termed Atg16L1), which “marks the spot” for initiation [2], non-covalently complexed with a covalent conjugate of Atg5-Atg12, and (ii) LC3-II, a derivative of the LC3-I protein lipidated with phosphatidylethanolamine at its C-terminus. The Atg5-12/16L and LC3 lipidation systems interact, and the former complex acts as an E3 enzyme (borrowed nomenclature from the ubiquitin system) to position properly and stimulate LC3 lipidation, resulting in LC3-II conversion and autophagosomal initiation/elongation (Fig. 1). LC3 is only one of several mammalian paralogs of the yeast Atg8, and the full panel in mammals includes LC3A (with two splice isoforms a and b in humans), LC3B, GABARAP, GABARAPL1, and GATE-16 (GABARAPL2). In most studies, it is LC3B that is analyzed, and the roles of other mammalian Atg8 paralogs are far less understood, albeit indications are that at least GATE-16 lipidation may resemble that of LC3 [3]. Mammalian Atg16 contains 3 distinct regions – the N-terminal portion interacting with Atg5, the coiled-coil domain (CCD) necessary for Atg16L oligomerization and complex formation with Atg12-Atg5 conjugate, and the WD repeats domain, absent in yeast, which is the site of the Crohn’s disease risk variant [4]. Further upstream from the conjugation systems, autophagy is negatively controlled by the Ser/Thr kinase Tor (Fig. 2): When Tor is activated by growth factors (e.g. via Akt signaling) and other inputs, autophagy is inhibited (Fig. 2 – inputs in red are activators of Tor and a number of them have been shown to inhibit autophagy). When Tor is inhibited autophagy is induced (Fig. 2; inputs in green upstream of Tor are inhibitors of Tor and known inducers of autophagy). Tor signaling is the point where classical experimental inducers of autophagy come into play, e.g. starvation (which acts as a physiological inhibitor of Tor) or Rapamycin (a pharmacological antagonist of Tor). Starvation and rapamycin are routinely used in laboratory for induction of autophagy.

More proximally, autophagy is positively controlled by the autophagy-specific phosphatidylinositol 3-kinase (PI3K) complex hVPS34-Atg14-Beclin 1 (Atg6). This PI3K is the target for a classical pharmacological inhibitor of autophagy, 3-methyl adenine. As a participant of the hVPS34 tri-partite complex driving the initiation of autophagy, Atg14 marks the earliest form of phagophore [5] (Fig. 1), possibly equivalent to its precursor known in yeast as the pre-autophagosomal structure. Recent studies have identified Bcl-2 [6] as a critical Beclin 1-interacting partner acting as an inhibitor of autophagy. In response to autophagy agonists such as starvation, Bcl-2 is phosphorylated by the MAP kinase JNK, releases its grip on Beclin 1, and leads to autophagy induction [7]. Following initiation, phagophore is enlarged during the elongation stage, and in the end of the elongation process seals to form an autophagosome. During the final stage of maturation, autophagosomes fuse with lysosomes to form autolysosomes, degradative compartments where the sequestered cytoplasmic cargo becomes exposed to and degraded by the hydrolytic enzymes. During the maturation stages of autophagy another Beclin 1 interacting partner, UVRAG (functionally equivalent to yeast Vps38 [5]) is important for activation of the protein complex termed HOPS, specifically its subunit Vps39 that acts as a Rab7 guanine-nucleotide exchange factor. Once Rab7 is activated, this allows recruitment of lysosomal components to autophagosomes leading to their maturation into autolysosomes [8].

Autophagy as a cell-autonomous defense against intracellular microbes

The relationship between autophagy and microbes has remained ill-defined until a recent convergence of studies showing that autophagy is an innate immune defense against bacteria, protozoa and viral pathogens (Box 1, effector functions) [9,10]. A group of previously reviewed studies [11] have demonstrated a role of autophagy in elimination of microorganisms such as *Mycobacterium tuberculosis* and *Salmonella* residing within phagosomes, intracellular pathogens escaping into the cytosol such as *Shigella*, extracellular pathogens when they invade host cells interiors, as shown with group A streptococci, or protozoan parasites. These and additional publications have established a role for autophagy in innate immunity against a variety of microbes [12–15]. Most importantly, two independent studies using animal models with defective Atg genes and different pathogens have now clearly established that autophagy is important in vivo as an anti-microbial defense [13,15]. Autophagy eliminates intracellular pathogens in a process similar to the capture and digestion of damaged or expended intracellular organelles (Fig. 1). Thus, autophagy serves as a mechanism for the removal of intracellular microbes, in keeping with its primary function as a cytoplasmic sanitation process. Interestingly, autophagy was initially observed as a process supporting survival of intracellular pathogens, which can now be explained in the context of evolutionary adaptations of pathogens that have developed protective mechanisms against autophagy [16,17]: (i) Ogawa et al., found that *Shigella* evades via one of its intracellular motility-associated proteins, IscB [18]; (ii) Orvedahl et al., have shown that HSV-1 interferes with autophagy using its viral gene product ICP34.5 [16]; (iii) Birmingham et al., reported that *Listeria* could block completion of autophagic maturation via pore forming toxins preventing efficient acidification of autophagosomes [17].

Investigations of the mechanisms of bacterial elimination following induction of autophagy have provided insights into the mechanisms of killing of intracellular microbes in cells induced for autophagy. Given the reports that autophagy can kill mycobacteria [19–21], it is possible that novel microbicidal components are present in the autolysosome. It is a common misconception that delivery of microbes to a lysosomal environment is sufficient to eliminate most microbes. With some sturdy pathogens, such as *M. tuberculosis*, the bactericidal repertoire of the standard lysosome may not be sufficient to kill the microbe. In contrast, phagolysosomes are enriched for autolysosomal contents and show enhanced ability to kill mycobacteria [22]. A major difference exists in the composition of standard lysosomes and autolysosomes since autophagy captures cytosolic components and digests them into a complex mixture of peptides. For example, ubiquitin fragments generated by ubiquitin digestion in autophagosomes are particularly effective in killing mycobacteria [22]. Autophagy elegantly explains how cytosolic ubiquitin fragments end up in lysosomal compartments, since autophagy captures ubiquitinated protein aggregate and subjects them to proteolysis in autolysosomes.

Autophagy as an effector of PRR signaling

The PRRs provide early detection and elimination of invading microbes via innate immunity mechanisms and modulate subsequent adaptive immune responses to the pathogens [23]. There are four major classes of PRRs: (i) TLRs; (ii) retinoic acid-inducible gene I (RIG-I)-like helicase receptors (RLRs); (iii) nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs); and (iv) C-type lectin receptors (CLRs). PRRs recognize PAMPs and induce pro-inflammatory cytokines as a well appreciated output. Recently, the repertoire of outputs following PRR activation has been expanded to include induction of autophagy downstream of TLR stimulation [19,24–27] (Box 1, Effector and Regulatory Functions). Shi et al [27] have shown that MyD88 and TRIF, the key adapter proteins required for TLR signaling, are found in complexes with Beclin 1 and affect Bcl-2-Beclin 1 interactions upon TLR stimulation (Fig.

1, a). This provides a clear basis for molecular interactions connecting TLRs and autophagy machinery. The TLR4 agonist LPS is able to induce autophagy [19,24,26]. TLR3 and TLR7 agonists induce autophagy, and induction of autophagy in human cells is detectable after infection with HIV, a virus normally causing TLR7/8 activation [24]. Furthermore, autophagy induced by TLRs can eliminate intracellular mycobacteria [19,24]. Since autophagy as an effector mechanism has direct antimicrobial action it differs from the majority of other PRR outputs that require multiple downstream events to take place often making it difficult to discern the actual process that kills the microbe. In this sense, autophagy can be viewed as a true “blue collar worker”, directly eliminating the offending pathogens. A recent study in *Drosophila* [13] has shown that a cytosolic PRR, PGRP-LE, recognizing diaminopimelic acid-type peptidoglycan, induces autophagy and that autophagy protects the fly from *Listeria monocytogenes* infection. This is an important demonstration of in vivo role of PRR induction of autophagy in defense against pathogens (Box 1, Effector Functions).

TLR signaling in the context of autophagy is most certainly cell type dependent. In pDC there is no detectable autophagic increase upon infection with VSV [25]. This can be due to high baseline autophagy levels reported for dendritic cells [28]. Nevertheless, autophagy does play a role in TLR signaling in pDC by delivering cytosolic PRR to endosomally located TLR7 [25]. The reasons for a tighter barrier to autophagy induction via TLRs in primary murine macrophages [29] are not clear. However, the majority of TLR signaling pathways activate NF- κ B, a negative regulator of autophagy [30], and thus a balance between activating and inhibitory pathways may determine whether autophagy will be activated or not. A further complication of autophagy-PRR relationships is that Atg5-Atg12 appears to act as a negative regulation of RIG-I signaling via IPS-1 [31], and that autophagy may act as a negative feedback mechanism to limit proinflammatory signaling [29] (Box 1, Regulatory Functions).

Autophagy as an effector of Th1/Th2 polarization in control of intracellular microbes

Autophagy is regulated by immunologically relevant cytokines (IFN- γ , IL-4, IL-13) and ligands (CD40L-CD40) [32,33]. TNF- α [30,33], activates autophagy but only when NF- κ B is blocked [30]. IFN- γ , a major Th1 cytokine induces autophagy, while the Th2 cytokines IL-4 and IL-13 inhibit autophagy [32] (Box 1). IFN- γ enhances autophagic elimination of intracellular *M. tuberculosis* whereas IL-4 and IL-13 inhibit autophagic control of intracellular mycobacteria [32]. How IFN- γ induces autophagy is presently unknown. IL-4 and IL-13 treatment of macrophages inhibits starvation- or IFN- γ -induced autophagic delivery of mycobacteria into degradative compartments and enhances mycobacterial survival in infected macrophages stimulated for autophagy [32]. Once IL-4 and IL-13 receptors are engaged, this activates insulin receptor substrate (IRS) and stimulates the Akt pathway (note that Akt activates Tor that in turn inhibits autophagy; Fig. 2), which in turn inhibits autophagy induced by starvation [32]. A different signaling pathway, independent of Akt and dependent on STAT-6, is required to suppress IFN- γ -induced autophagy by IL-4. Studies by Harris et al [32] also indicate that Th2 cytokines IL-4 and IL-13 block the protective role of autophagy even when IFN- γ is present, and that in mixed responses Th2 cytokines may override the ability of IFN- γ to induce anti-bacterial autophagy. These findings demonstrate that autophagy is an effector of Th1/Th2 polarization, explaining why Th1 cytokines are protective and Th2 cytokines permissive when it comes to controlling intracellular pathogens (Box 1, Effector Functions).

Autophagy and immunity related GTPases (IRG)

Autophagy has been implicated in the function of immunity related GTPases (IRG) [15,34–38] that comprise a family of GTPases involved in cell-autonomous defense against intracellular

pathogens [39]. In the mouse, the IRG family is abundantly represented by 19 genes (*Irgm1-Irgm3*, *Irgb1-Irgb6*, *Irgb8-Irgb10*, *Irga1-Irga4* and *Irga6-Irga8*) controlled by type I interferon through ISRE elements or IFN- γ via GAS elements, while in humans the IRG family has been reduced to a single member, IRGM [39]. The ostensibly huge differences in the IRG family in mice and humans make comparisons of effects in the mouse and humans somewhat difficult, but not absolutely impossible. The lessons learned in the murine system have enabled studies with the human IRGM. However, now that we know how large the differences are between the murine and human IRG complements, much caution is in order in any attempts to extrapolate murine experiments to related phenomena in humans.

The work on *M. tuberculosis* has led to a connection between *Irgm1* and autophagy in murine cells [34]. Autophagy has furthermore been implicated in the elimination of intracellular *T. gondii* by a series of events dependent on *Irgm3* and culminating in autophagy *Irga6* [36]. The most recent studies using *Atg5^{Flox/Flox} LysM-Cre* mice with *Atg5* null myeloid cells have shown that autophagy plays a role in vivo in the control of *T. gondii* through *Irgma6* action downstream of *Atg5* [15]. The single human IRG member, IRGM, is not directly controlled by IFN- γ and instead is transcribed from the long terminal repeat of an adjacent extinct retroviral element (ERV9) [39]. ERV9 is an endogenous retrovirus repeatedly mobilized during primate evolution millions of years ago, that has left in the human genome a trace of over a hundred provirus remnants and thousands of solitary long terminal repeats (LTRs). Although believed to be independent of IFN- γ for its own expression, IRGM is nevertheless important for autophagy induction in response to IFN- γ in human cells [35]. Furthermore, IRGM plays a role in autophagic control of *M. tuberculosis* in human macrophages [35]. Recently, IRGM, and another autophagy gene *Atg16L1*, have been implicated in human populations as a genetic risk locus in Crohn's disease [4,40,41] (Fig. 2). Although a connection between IRG and autophagy has become clear, additional roles have been proposed for murine IRG members [39], such as proper phagosome acidification and maturation and direct destruction of the parasitophorous vacuole membrane. Recent studies have implicated the mouse *Irgm1* in hemopoietic stem cell function during infection [42] and in protection against autophagic cell death induced by IFN- γ [38]. It is worth noting that the majority of the additional proposed mechanisms are compatible with autophagy and may be best explained by a link with autophagy.

Autophagy as a topological inversion device stoking innate and adaptive immune responses

Endosomal TLR7 exposure to cytosolic viral PAMPs, MHC II-driven T-cell selection for self-tolerant repertoire [43], and MHC II-restricted responses to viral cytosolic antigens [28] have been linked to autophagy. How do MHC II molecules, facing the lumen of the antigen processing and loading organelles, become loaded with cytosolic antigens? It turns out that autophagy can accomplish this since close to 50% of autophagosomes merge with MHC II antigen loading compartments [28]. Thus, cytosolic proteins captured by macroautophagy and fragmented in autophagosomes can be delivered to antigen processing and MHC II loading compartments in antigen presenting cells [10]. Alternatively, individual proteins can be imported one by one directly into the lysosomes by chaperone mediated autophagy [44]. Either way, autophagy is utilized by immune cells as a topological inversion device, bringing cytosolic antigens into the lumen of MHC II compartments (Box 1, Regulatory Functions). This is the basis for a new immunization strategy by coupling antigens to LC3 to augment MHC II presentation of a desired antigen [28].

Autophagy as an immunological topology inversion device participates in the delivery of cytosolic PAMP to endosomally located and lumenally oriented PRR (e.g. TLR7), in a process akin to delivery of cytosolic antigens to MHC II molecules (Box 1, Regulatory Functions)..

For instance, cytosolic intermediates of replicating vesicular stomatitis virus (VSV) recognized by PRR, are delivered by autophagy into the endosomal lumen where TLR7 can recognize viral single stranded RNA [25]. This topological inversion is critical for efficient viral recognition in plasmacytoid dendritic cells (pDC), since this cell type depends exclusively on endosomal TLR7 and TLR9 to detect viral replication intermediates. Another potentially related observation is that autophagy facilitates synergistic B cell receptor (BCR) and TLR9 signaling from an intracellular autophagosome-like compartment where BCR and TLR9 cooperate for optimal MAPK signaling [45]. This is of significance for production of autoantibodies to DNA-containing antigens in systemic autoimmune diseases.

Autophagy in thymic selection, tolerance/autoimmunity, and T cell homeostasis

A recent study [43], following on an observation that thymus, an organ where positive and negative selection of T cells shapes the T cell repertoire of an individual, displays high levels of constitutive autophagy [46], has implicated autophagy in balancing effective immune response and auto-immunity (Box 1, Regulatory Functions). Transplantation of embryonic thymi from *Atg5^{-/-}* mice into athymic *nu/nu* mice resulted in increased frequency of activated CD4⁺ T cells, enlarged lymph nodes, flaky skin patches, enlarged colon, and inflammatory infiltrates in multiple organs including liver, colon and lungs [43]. The specificity for the thymus as dictating the resulting multi-organ inflammation was demonstrated by the absence of effects when *Atg5^{-/-}* liver instead of the thymus was grafted onto *nu/nu* mice, and linkage to CD4⁺ T cells (but not CD8⁺ T cells) was established by adoptive transfers of purified T cells which recapitulated all autoimmunity features. The proposed model [43] for how autophagy influences T cell repertoires accounts for the dual role of thymic epithelial cells (TEC) which play a role via endogenous antigen presentation in the MHC II-restricted fashion in both positive (cortical TEC) and negative (medullary TEC) selection of T cells. Without autophagy to fuel the negative selection of CD4⁺ T cells reactive to endogenous self-antigens presented via MHC II, medullary TEC permit exit from the thymus of autoreactive T cells thus causing multi-organ inflammation (Fig. 2A). Significantly, organs that appear particularly susceptible to autophagy-dependent autoimmunity show high baseline autophagy, which enhances endogenous MHC II antigen presentation, suggesting that increase in MHC II-restricted presentation of self antigens has to be matched by autophagy-dependent tolerogenic selection mechanisms in the thymus. These exciting studies hold promise not only to illuminate autoimmunity from this previously unappreciated angle, but also may offer new modalities in treating autoimmune disease.

Once T cells depart from the thymus, autophagy plays a role in their survival (Box 1, Regulatory Functions). In experiments where lethally irradiated mice were grafted with hematopoietic stem cells from fetal *Atg5^{-/-}* livers, T cells developed fine in the *Atg5⁺* thymus but CD4⁺ and CD8⁺ failed to populate the periphery, most likely due to growth factor withdrawal when cells depend on autophagy in its primary role of cell survival under nutrient/growth factor limitation [47]. Inasmuch as autophagic cell death is still a matter of debate, in contrast to the now well accepted role in cellular survival [48,49], there is a report that T cells showing excessive autophagy undergo cell death with an autophagic component [50].

Autophagy in B cell homeostasis

Using B cell-specific *Atg5* knockout (*ATG5^{fllox/fllox} CD19-Cre*) mice, Miller et al., [51] have provided a telling analysis of how autophagy affects B cell development and maintenance in the periphery (Box 1, Regulatory Functions). The distinct B cell lineages, B-1a, B-1b and B2, were affected differentially by the absence of the key autophagy factor *Atg5*. The production in the bone marrow of B2 cells, which we commonly refer to when we say "B cell", is stalled between

the pro-B to pre-B differentiation stages, although sufficient pre-B cells survive to populate the periphery. B1a cells, which normally have the self-renewal capacity, cannot do so efficiently when they lack Atg5, most likely accounting for their diminished numbers in the periphery [51]. These observations suggest that during development and cellular differentiation there is a role for autophagy possibly as growth factor levels drop or local stromal environment change. This is in keeping with the general notion that autophagy supports cellular survival at certain bottleneck stages in development, possibly most dramatically illustrated in the requirement for Atg5 in the preimplantation development of mammalian embryos [52].

Autophagy in inflammatory disease

Recent genome-wide association (GWA) studies have linked autophagy and Crohn's disease (Fig. 3), a major form of chronic inflammatory bowel disease with both heritable risks and non-genetic components [4]. It is now believed that Crohn's disease is at least in part due to an aberrant or partially ineffective innate immunity response to normal gut flora. This is supported by the fact that Crohn's disease develops primarily at anatomical points (terminal ileum and colon) where normal bacterial presence dramatically increases in mass [4]. This is underscored by the fact that genetic predisposition to Crohn's disease has been linked to the regulators of innate immunity, specifically Nod2 [53]. The latest GWA breakthroughs have uncovered the role for at least two additional immunity pathways: (i) IL12 and IL-23 (IL-23 receptor IL23RArg381Gln) driving the Th17 differentiation of Th1 cells, with the Th17 phenotype frequently associated with organ-specific immunopathology and inflammation; and (ii) the core autophagy protein Atg16L1 [29,54] and an autophagy-linked factor IRGM [35], a process freshly recognized as innate and adaptive immunity effector and regulator [9].

In contrast to the extensively studied Nod2 pathway [53], until the reports by Saitoh et al., [29] and Cadwell et al., [54] little was known about the role of autophagy in Crohn's disease. At present, the role of IRGM is not known, but it may influence a number of immunological processes and cells, including thymic selection, T cell homeostasis, direct elimination of invasive enteric bacteria (Fig. 3A), or it may regulate cell death and associated inflammation in the intestinal tissues. In contrast, more has been learned about Atg16L1 (Fig. 3B). Saitoh et al., [29] generated Atg16L1 Δ CCD mice, which turned out to die within 1 day of birth just like the Atg5^{-/-} knockout mice. Motivated by reports that autophagy plays a role in PRR responses, Saitoh et al., tested Atg16L1 Δ CCD macrophages for proinflammatory cytokine production in response to LPS and found elevated IL-1 β . Comparing mouse chimeras repopulated with Atg16L1 Δ CCD or wild type stem cells, Saitoh et al., observed 100% mortality of the Atg16L1 Δ CCD mouse chimeras in experimentally induced colitis. This was reversible by IL-1 β neutralizing antibodies. Elevated parameters of IL-1 signaling have been detected in intestinal tissues from patients with inflammatory bowel disease and this in turn might increase microbial product translocation due to IL-1 β -induced permeability across the epithelial barrier [55]. Independently of Akira's group, Cadwell et al., generated hypomorphic Atg16L1 (Atg16L1^{HM}) mice, expressing about a third of normal Atg16L1 levels [54] and showed that Paneth cells in the crypt of the ileum are abnormal in these mice, resonating with the effect of the Nod2 mutations in Crohn's disease causing reduced expression of α -defensins in Paneth cells [53]. The actual Atg16L1 Crohn's disease risk allele (Atg16L1*300A) contains an Ala residue at the position 300 in place of Thr found in the protective allele Atg16L1*300T and is unlikely to have a null function. However, Atg16L1*300A is less stable upon infection of epithelial cells in vitro with invasive bacteria relative to the protective Atg16L1*300T allele, in essence having an infection-induced hypomorphic phenotype [56], lending additional credence to the Atg16L1^{HM} mouse model. Nevertheless, it is likely that IL-1 β increase [29], Paneth cell issues [54], and inability to autophagically control invasive enteric bacteria [56], are all contributing to a complex breakdown of innate immunity defenses and enhancement of

compensatory pro-inflammatory mechanisms leading to the immunopathogenesis of Crohn's disease.

Conclusions

Autophagy was initially appreciated primarily for its cell survival and (still controversial) cell death functions. Autophagy has been recognized only recently as a pathway broadly associated with immunity. Autophagy eliminates intracellular pathogens [9] contributes to MHC II restricted endogenous antigen presentation [10,28], is an effectors of Th1/Th2 polarization [32], affects B and T cell homeostasis and repertoire selection [43,47,50,51], delivers cytosolic PAMP or danger associated molecular patterns to endosomal Toll-like receptors (TLR) [25, 45], and acts as an innate immunity effector downstream of TLR and other PRR [13,19,24, 26,27]. The multi-tiered connections between autophagy and immunity uncovered to date may be surprising if one looks upon autophagy primarily as a specialized cytoplasmic maintenance pathway. However, if one considers a model whereby autophagy may have evolved in early eukaryotes as a primitive defense against microbes, additions of different layers of immunological regulation and the ever increasing complexity with phylogeny of the interplay between immunity and autophagy appears only natural. Thus we propose that autophagy is not only peripherally associated with immune defenses but that it likely has been one of the initial if not the first cell autonomous defense against remaining at the heart of immunity.

Box 1

Immune functions of autophagy

The known immunological roles of autophagy are classified as effector or regulatory functions. IRG, immunity related GTPases; PAMP, pathogen associated molecular patterns; PRR, Pattern recognition receptors; TLR, Toll-like receptors.

Effector functions	Regulatory functions
Capture & digestion of intracellular microbes	Endogenous antigen presentation: delivery of cytosolic Ag to MHC II loading compartments
Effector output of TLR & other PRR responses to PAMPs	Central tolerance: positive & negative T cell selection (thymic epithelial cells)
Effector or co-effector of IRG (human IRGM & murine Irgm1, Irgm3, Irga6)	T cell survival & homeostasis in the periphery
Effector of Th1 polarization in control of intracellular pathogens	B cell development & homeostasis
Effector of Th2 polarization in susceptibility to intracellular pathogens	Immune activation: delivery of cytosolic PAMPs to endosomal TLRs
Function of specialized cells contributing to innate defenses (e.g. intestinal Paneth cells)	Reducing damaging immune activation: downregulation of inflammasome action

Acknowledgments

This work was supported by grants AI069345, AI45148, AI42999 from National Institutes of Health, 107160-44-RGRL from amfAR, a Bill and Melinda Gates Grand Challenge Explorations grant and a grant from Crohn's & Colitis Foundation of America

References

1. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 2008;451:1069–1075. [PubMed: 18305538]
- 2*. Fujita N, Itoh T, Omori H, Fukuda M, Noda T, Yoshimori T. The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol Biol Cell* 2008;19:2092–2100. [PubMed: 18321988]Shows that Atg16 “marks the spot” for autophagosome formation
- 3*. Scherz-Shouval R, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. *Embo J* 2007;26:1749–1760. [PubMed: 17347651]Shows that reactive oxygen species released from mitochondria induce autophagy by inactivating Atg4, stabilizing lipidated LC3
4. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007;448:427–434. [PubMed: 17653185]
- 5*. Itakura E, Kishi C, Inoue K, Mizushima N. Beclin 1 Forms Two Distinct Phosphatidylinositol 3-Kinase Complexes with Mammalian Atg14 and UVRAG. *Mol Biol Cell*. 2008Identifies mammalian Atg14 ortholog and defines the pivotal autophagy initiation Atg14-Beclin 1-hVPS34 complex.

6. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 2005;122:927–939. [PubMed: 16179260]
- 7**. Wei Y, Pattingre S, Sinha S, Bassik M, Levine B. JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol Cell* 2008;30:678–688. [PubMed: 18570871]Elegant demonstration of how starvation induces autophagy by causing JNK-1-dependent phosphorylation of Bcl-2, which releases Beclin-1 from the inhibitory complex with Bcl-2, thus initiating autophagy.
- 8*. Liang C, Lee JS, Inn KS, Gack MU, Li Q, Roberts EA, Vergne I, Deretic V, Feng P, Akazawa C, et al. Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. *Nat Cell Biol* 2008;10:776–787. [PubMed: 18552835]Defines UVRAG as a positive regulator of autophagic maturation and reveals its mechanism of action.
9. Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. *Nat Rev Immunol* 2007;7:767–777. [PubMed: 17767194]
10. Schmid D, Munz C. Innate and adaptive immunity through autophagy. *Immunity* 2007;27:11–21. [PubMed: 17663981]
11. Deretic V. Autophagy as an immune defense mechanism. *Curr Opin Immunol* 2006;18:375–382. [PubMed: 16782319]
12. Py BF, Lipinski MM, Yuan J. Autophagy Limits *Listeria monocytogenes* Intracellular Growth in the Early Phase of Primary Infection. *Autophagy* 2007;3:117–125. [PubMed: 17204850]
- 13**. Yano T, Mita S, Ohmori H, Oshima Y, Fujimoto Y, Ueda R, Takada H, Goldman WE, Fukase K, Silverman N, et al. Autophagic control of *Listeria* through intracellular innate immune recognition in *Drosophila*. *Nat Immunol* 2008;9:908–916. [PubMed: 18604211]A first demonstration of the in vivo role of autophagy as an innate defense mechanism against bacterial infection using *Drosophila* as an animal model
14. Cullinane M, Gong L, Li X, Lazar-Adler N, Tra T, Wolvetang E, Prescott M, Boyce JD, Devenish RJ, Adler B. Stimulation of autophagy suppresses the intracellular survival of *Burkholderia pseudomallei* in mammalian cell lines. *Autophagy* 2008;4:744–753. [PubMed: 18483470]
- 15**. Zhao Z, Fux B, Goodwin M, Dunay IR, Strong D, Miller BC, Cadwell K, Delgado MA, Ponpuak M, Green KG, et al. Autophagosome-independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens. *Cell Host Microbe* 2008;4:458–469. [PubMed: 18996346]A demonstration of in vivo role of autophagy in defense against bacterial and protozoan pathogens using an Atg5^{Flox/Flox} LysM-Cre mice.
16. Orvedahl A, Alexander D, Tallóczy Z, Sun Q, Wei Y, Zhang W, Burns D, Leib D, Levine B. HSV-1 ICP34. 5 Confers Neurovirulence by Targeting the Beclin 1 Autophagy Protein. *Cell Host and Microbe* 2007;1:23–35. [PubMed: 18005679]
17. Birmingham CL, Canadien V, Kaniuk NA, Steinberg BE, Higgins DE, Brumell JH. Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. *Nature* 2008;451:350–354. [PubMed: 18202661]
18. Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. Escape of intracellular *Shigella* from autophagy. *Science* 2005;307:727–731. [PubMed: 15576571]
- 19*. Xu Y, Jagannath C, Liu XD, Sharafkhaneh A, Kolodziejaska KE, Eissa NT. Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. *Immunity* 2007;27:135–144. [PubMed: 17658277]The first report on TLR4 induction of autophagy
20. Floto RA, Sarkar S, Perlstein EO, Kampmann B, Schreiber SL, Rubinsztein DC. Small molecule enhancers of rapamycin-induced TOR inhibition promote autophagy, reduce toxicity in Huntington's disease models and enhance killing of mycobacteria by macrophages. *Autophagy* 2007;3:620–622. [PubMed: 17786022]
21. Biswas D, Qureshi OS, Lee WY, Croudace JE, Mura M, Lammas DA. ATP-induced autophagy is associated with rapid killing of intracellular mycobacteria within human monocytes/macrophages. *BMC Immunol* 2008;9:35. [PubMed: 18627610]
- 22*. Alonso S, Pethe K, Russell DG, Purdy GE. Lysosomal killing of *Mycobacterium* mediated by ubiquitin-derived peptides is enhanced by autophagy. *Proc Natl Acad Sci U S A* 2007;104:6031–6036. [PubMed: 17389386]A report suggesting unique bactericidal properties of autophagic organelles.

23. Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S. Host innate immune receptors and beyond: making sense of microbial infections. *Cell Host Microbe* 2008;3:352–363. [PubMed: 18541212]
- 24*. Delgado MA, Elmaoued RA, Davis AS, Kyei G, Deretic V. Toll-like receptors control autophagy. *Embo J* 2008;27:1110–1121. [PubMed: 18337753]A full screen of TLR agonists in induction of autophagy, showing that multiple TLR agonists can induce autophagy
- 25*. Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science* 2007;315:1398–1401. [PubMed: 17272685]A study showing that autophagy can facilitate delivery of PAMPs to endosomal TLRs
- 26*. Sanjuan MA, Dillon CP, Tait SW, Moshiah S, Dorsey F, Connell S, Komatsu M, Tanaka K, Cleveland JL, Withoff S, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* 2007;450:1253–1257. [PubMed: 18097414]A study suggesting that phagosomes can be decorated by LC3, expanding the role of autophagy proteins beyond the canonical autophagosomes in mammalian cells
- 27**. Shi CS, Kehrl JH. MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. *J Biol Chem*. 2008A report on how TLRs induce autophagy - by MyD88 and TRIF being in molecular complexes with Beclin 1
- 28**. Schmid D, Pypaert M, Munz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* 2007;26:79–92. [PubMed: 17182262]An elegant demonstration of how autophagy feeds cytosolic antigens to endosomally located MHC II molecules
- 29**. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1 β production. *Nature* 2008;456:264–268. [PubMed: 18849965]A demonstration in a transgenic Atg16L knockout mouse that autophagy plays a role in intestinal inflammatory disease
30. Djavaheri-Mergny M, Amelotti M, Mathieu J, Besancon F, Bauvy C, Souquere S, Pierron G, Codogno P. NF- κ B activation represses tumor necrosis factor- α -induced autophagy. *J Biol Chem* 2006;281:30373–30382. [PubMed: 16857678]
31. Takeshita F, Kobiyama K, Miyawaki A, Jounai N, Okuda K. The non-canonical role of Atg family members as suppressors of innate antiviral immune signaling. *Autophagy* 2008;4:67–69. [PubMed: 17921696]
- 32**. Harris J, De Haro SA, Master SS, Keane J, Roberts EA, Delgado M, Deretic V. T helper 2 cytokines inhibit autophagic control of intracellular Mycobacterium tuberculosis. *Immunity* 2007;27:505–517. [PubMed: 17892853]A demonstration that Th1 vs Th2 polarization leads to activation or inhibition of autophagy and control or permissiveness to intracellular pathogens
33. Sabauste C, Andrade R, Wessendarp M. CD40-TRAF6 and autophagy-dependent anti-microbial activity in macrophages. *Autophagy*. 2007Ahead of print
34. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 2004;119:753–766. [PubMed: 15607973]
35. Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 2006;313:1438–1441. [PubMed: 16888103]
36. Ling YM, Shaw MH, Ayala C, Coppens I, Taylor GA, Ferguson DJ, Yap GS. Vacuolar and plasma membrane stripping and autophagic elimination of Toxoplasma gondii in primed effector macrophages. *J Exp Med* 2006;203:2063–2071. [PubMed: 16940170]
37. Andrade RM, Wessendarp M, Gubbels MJ, Striepen B, Sabauste CS. CD40 induces macrophage anti-Toxoplasma gondii activity by triggering autophagy-dependent fusion of pathogen-containing vacuoles and lysosomes. *J Clin Invest* 2006;116:2366–2377. [PubMed: 16955139]
- 38**. Feng CG, Zheng L, Jankovic D, Bafica A, Cannons JL, Watford WT, Chaussabel D, Hieny S, Caspar P, Schwartzberg PL, et al. The immunity-related GTPase Irgm1 promotes the expansion of activated CD4 $^{+}$ T cell populations by preventing interferon- γ -induced cell death. *Nat Immunol* 2008;9:1279–1287. [PubMed: 18806793]The murine Irgm1 appears to play a role in protecting T cells from cell death upon stimulation with IFN- γ
39. Howard J. The IRG proteins: a function in search of a mechanism. *Immunobiology* 2008;213:367–375. [PubMed: 18406381]

40. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–678. [PubMed: 17554300]
- 41**. McCarroll SA, Huett A, Kuballa P, Chlewicki SD, Landry A, Goyette P, Zody MC, Hall JL, Brant SR, Cho JH, et al. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat Genet.* 2008A study showing that human IRGM noncoding polymorphism associated with predisposition to Crohn's disease is a part of a large promoter deletion haplotype
42. Feng CG, Weksberg DC, Taylor GA, Sher A, Goodell MA. The p47 GTPase Lrg-47 (*Irgm1*) links host defense and hematopoietic stem cell proliferation. *Cell Stem Cell* 2008;2:83–89. [PubMed: 18371424]
- 43*. Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* 2008;455:396–400. [PubMed: 18701890]
An absolutely critical study demonstrating that autophagy and MHC restricted endogenous antigen presentation by thymic epithelial cells plays a key role in central tolerance in mammals
44. Crotzer VL, Blum JS. Cytosol to lysosome transport of intracellular antigens during immune surveillance. *Traffic* 2008;9:10–16. [PubMed: 17916226]
45. Chaturvedi A, Dorward D, Pierce SK. The B cell receptor governs the subcellular location of Toll-like receptor 9 leading to hyperresponses to DNA-containing antigens. *Immunity* 2008;28:799–809. [PubMed: 18513998]
46. Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol Biol Cell* 2004;15:1101–1111. [PubMed: 14699058]
47. Pua HH, Dzhagalov I, Chuck M, Mizushima N, He YW. A critical role for the autophagy gene *Atg5* in T cell survival and proliferation. *J Exp Med* 2007;204:25–31. [PubMed: 17190837]
48. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* 2007;8:741–752. [PubMed: 17717517]
49. Levine B, Kroemer G. Autophagy in the Pathogenesis of Disease. *Cell* 2008;132:27–42. [PubMed: 18191218]
50. Li C, Capan E, Zhao Y, Zhao J, Stolz D, Watkins SC, Jin S, Lu B. Autophagy is induced in CD4+ T cells and important for the growth factor-withdrawal cell death. *J Immunol* 2006;177:5163–5168. [PubMed: 17015701]
- 51*. Miller BC, Zhao Z, Stephenson LM, Cadwell K, Pua HH, Lee HK, Mizushima NN, Iwasaki A, He YW, Swat W, et al. The autophagy gene *ATG5* plays an essential role in B lymphocyte development. *Autophagy* 2008;4:309–314. [PubMed: 18188005]A very elegant study using B-cell specific deletion of *Atg5* in mice to show that autophagy plays a role in B cell development and homeostasis
52. Tsukamoto S, Kuma A, Murakami M, Kishi C, Yamamoto A, Mizushima N. Autophagy is essential for preimplantation development of mouse embryos. *Science* 2008;321:117–120. [PubMed: 18599786]
53. Kanneganti TD, Lamkanfi M, Nunez G. Intracellular NOD-like receptors in host defense and disease. *Immunity* 2007;27:549–559. [PubMed: 17967410]
54. Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Kc W, Carrero JA, Hunt S, et al. A key role for autophagy and the autophagy gene *Atg16l1* in mouse and human intestinal Paneth cells. *Nature* 2008;456:259–263. [PubMed: 18849966]
- 55*. Al-Sadi R, Ye D, Dokladny K, Ma TY. Mechanism of IL-1 β -induced increase in intestinal epithelial tight junction permeability. *J Immunol* 2008;180:5653–5661. [PubMed: 18390750]A transgenic mouse model indicating that autophagy affects Paneth cell function in the context of Crohn's disease
- 56*. Kuballa P, Huett A, Rioux JD, Daly MJ, Xavier RJ. Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated *ATG16L1* variant. *PLoS ONE* 2008;3:e3391. [PubMed: 18852889]An in vitro analysis using expression of Crohn's disease *ATG16L1* alleles in epithelial cells demonstrating that *ATG16L1* is destabilized following infection and cannot support formation of autophagosomes specific for invasive enteric pathogens

Figure 1. Autophagy regulation and execution stages

Shown are the execution stages of autophagy, controlled by an upstream signaling cascade centered around the Ser/Thr kinase Tor, a metabolic regulator of autophagy, and a cascade of Atg factors, starting with Atg1. **1. Initiation.** Upstream signaling brings about complex changes in autophagic initiation machinery leading to the formation of membranous precursors of autophagosome. **(a)** Signaling: nutrient or growth factor starvation signals via Ser/Thr kinase Tor; immunological stimuli (e.g. TLR activation with PAMPs) act through protein complexes containing MyD88 and Beclin 1 (Atg6), a key regulator of autophagy. **(b)** Beclin 1 is in an inhibitory complex with Bcl-2. Following stimulation, JNK-1 phosphorylates Bcl-2 (Bcl 2- P_i) releasing Beclin 1 from the inhibitory complex with Bcl-2. **(c)** Activated Beclin 1, in a tri-partite complex with type III phosphatidylinositol 3-kinase hVPS34 and Atg14, cooperates with other Atg factors to initiate autophagosome formation. **(d)** Atg16L1 (a genetic risk locus for inflammatory Crohn's disease) is in a noncovalent complex with the Atg5-Atg12 protein conjugate Atg16L1 marks the site of phosphatidylinositol 3-phosphate (PI3P) -dependent initiation of autophagosome formation and sets off phases e, f and g (shown only in elongation phase for clarity). Autophagic isolation membrane (phagophore) wraps around the cytoplasmic target (cytosol, protein aggregates, mitochondria, peroxisomes, microbes, etc.). **2.**

Elongation. Phagophore enlarges and closes to form a double membrane organelle termed the autophagosome. **(e,f)** Atg16L1/Atg5-Atg12 (e) acts as an E3 enzyme to stimulates conversion of LC3 I into the LC3 II form (f) via a protein-protein and protein-lipid conjugation cascade (not shown) LC3 I has a free C-terminal Gly residue, whereas LC3 II C-terminal Gly is lipidated with phosphatidylethanolamine (PE) leading to membrane association. This stage is pre-proteolytic and the lumen is not yet acidified. **(g)** Atg4 reverses LC3 II into LC3 I by removing PE. Atg4 is sensitive to reactive oxygen species (ROS) released from mitochondria and may be one of the signals promoting initiation+elongation stages. **3. Maturation.** Autophagosome matures into autolysosome, where the captured cargo is degraded. **(h)** The maturation phase is controlled by another Beclin 1-interacting factor, UVRAG. A second tri-partite complex with Beclin 1 and hVPS34 has been postulated (indicated by parentheses), containing UVRAG (Vps38) as a subunit in place of the initiation-specific factor Atg14; however, published evidence suggests that UVRAG acts independent of Beclin 1 at this stage. UVRAG interacts with HOPS which acts as a tethering and Rab guanine nucleotide exchange factor (GEF). **(i)** HOPS with Vps39 stimulates activation of Rab7 by loading Rab7 with GTP via the Rab7 Vps39 (GEF) associated with HOPS complexes. Rab7 is a small GTPase controlling trafficking and identity of late endosomal/lysosomal compartments. **(j)** Maturation occurs through fusion

with late endosomal/lysosomal organelles or delivery of trafficking intermediates carrying H^+ ATPase components and lysosomal hydrolytic enzymes. The maturing autophagosome becomes acidified and is converted into a degradative organelle (autolysosome) with single delimiting membrane (the inner of the two membranes is dissolved) containing degraded material including internal membranes originating from the captured cytoplasmic material.

Figure 2. Signaling systems controlling autophagy

Green: positive regulators or agonists of autophagy induction; often, these are negative regulators of Tor. Red: activators of Tor, often acting as antagonists of autophagy. Tor is at the headquarters of the signaling center that decides on whether cells make or reduce biomass. Autophagy is a major mechanism for bulk reduction of cellular biomass. Subscripts: (n), nuclear; (c) or c, cytosolic. Updated and modified from Deretic, V., *Current Opinion in Immunology*, 2006, 18:375–382.

Figure 3. Autophagy role in Crohn's disease

A Normal ileal crypt of Lieberkühn (CL) and vilus (V); AØ, autophagosome; E, enterocyte; E.c., adherent-invasive *E. coli*; G, Goblet cell; SCZ, stem cell zone; P, Paneth cell; TJ, tight junction. **B.** Dotted arrow, microbial translocation. **1.** Possible roles of autophagy in Crohn's disease. Autophagy may affect central tolerance by influencing negative and positive T cell selection in the thymus (for details, see text, section on autophagy in thymic selection). IRGM function in autophagy and Crohn's disease is not known at present (indicated by a question mark). **2–4.** Reported effects of Atg16L1 mutations: 2, reduced autophagy of invasive bacteria (shown using expression of Atg16L1*300A Crohn's disease risk allele and infection of epithelial cells in vitro); 3, increased IL-1β activation (shown with ATG16L1ΔCCD knockout mice); 4, fewer granules or granule contents diffuse in the cytosol of Paneth cells (shown in ATG16L1^{HM} hypomorphic mice expressing 30% of ATG16L1 wild type levels). Modified with permission from Deretic et al., *Dev Cell* 2008, **15**:641–642.