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Review

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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 plasmacytoid 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).

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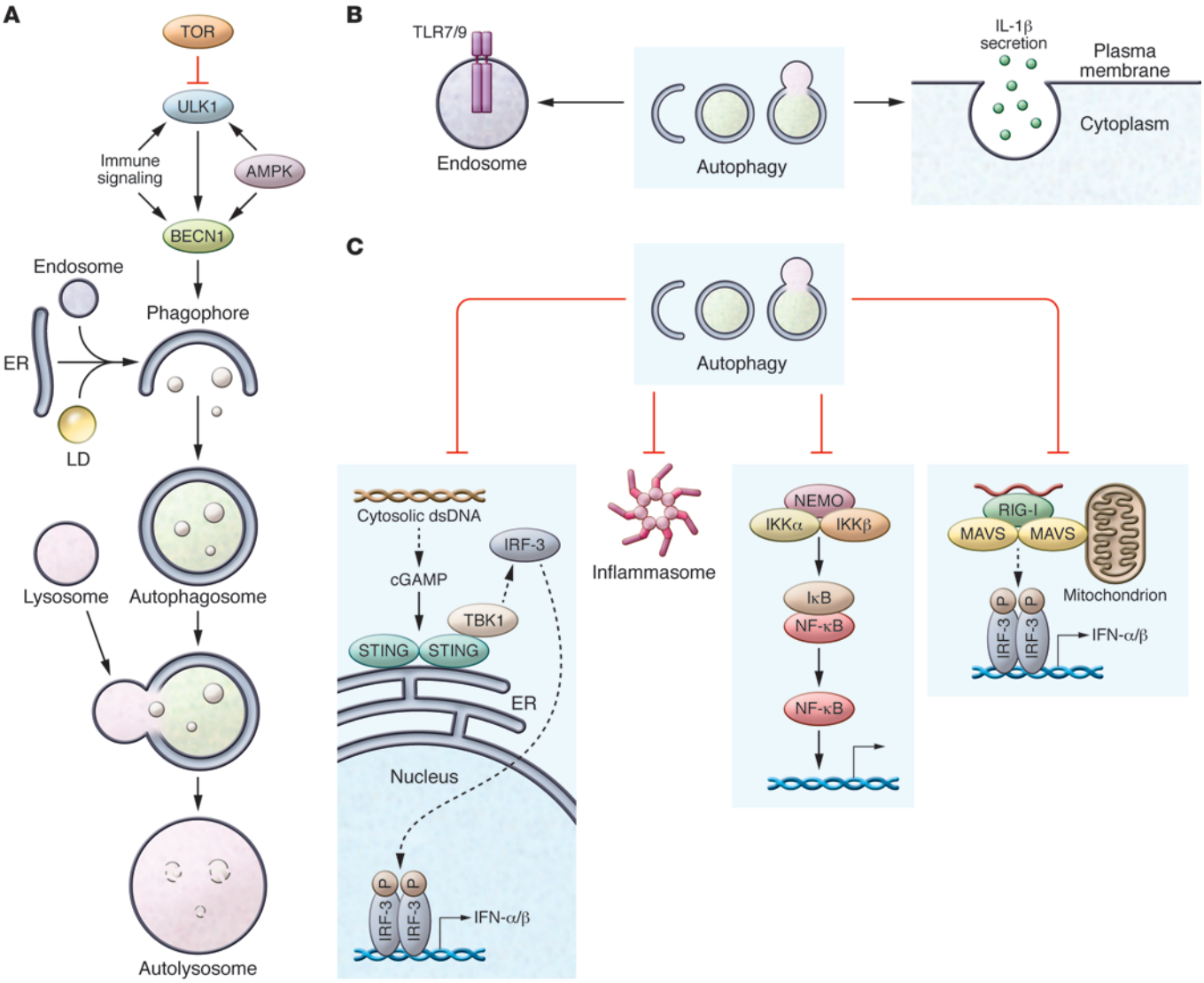

Figure 1. Autophagy modulates inflammation. (**A**) Autophagy – a simplified pathway. TOR, AMPK, and immune signaling control activation of ULK1 and beclin 1, the central regulators of autophagy, which in turn bring about autophagic membrane formation (crescent represents a nascent phagophore) from ER with contributions from endosomes and lipid droplets (LD). Completed autophagosomes (double membrane) fuse with lysosomes to form autolysosomes or autophagolysosomes, as described in the text. (**B**) Autophagy promotes delivery of PAMPs and activation of endosomal TLRs (TLR7 and TLR9) and assists the unconventional secretion of IL-1β upon inflammasome activation. (**C**) Autophagy inhibits spurious or excessive inflammasome activation and interferes (directly or indirectly) with signaling via cGAS (generating cGAMP upon dsDNA binding), MAVS, and RIG-I to downregulate type I IFN responses, and can suppress NF-κB activation.

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.

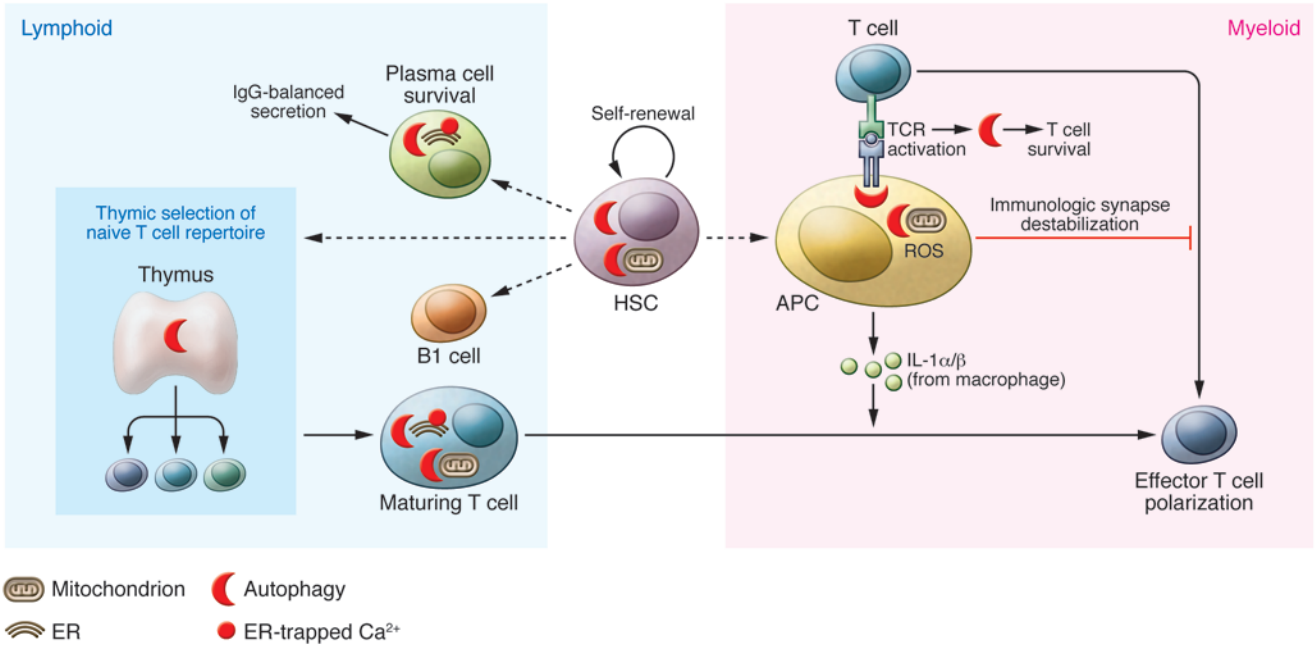

Figure 2. Autophagy affects lymphocyte development and function. Autophagy affects self-renewal of HSCs, plasma cell survival, IgG secretion, survival of a special type of B cell, B1, and maintenance of memory B cells (not shown). Autophagy affects T cell survival following TCR activation and controls stability of immunologic synapses; it also controls innate immune cell (e.g., macrophage) signaling by suppressing ROS via the removal of depolarized mitochondria and inhibition of IL-1 release that influences, along with the durability of immunologic synapses, polarization of T cells into a Th17 phenotype. Autophagy also affects selection of naive T cell repertoire in the thymus by self antigen processing and presentation and survival and functionality of maturing T cells by trimming mitochondria and ER, thus ensuring Ca^{2+} homeostasis. Red crescents symbolize the entire autophagy pathway.

Additionally, autophagy and inflammasomes cooperate during conventional protein secretion, especially in the process of regulated secretion. Regulated secretion differs from the aforementioned unconventional secretion of cytosolic proteins such as IL-1β. Regulated secretion follows the canonical secretory pathway from the ER to the Golgi and then to cytoplasmic storage granules or secretory vesicles, from which the lumenal cargo is eventually secreted to the extracellular space. This is exemplified by mucin secretion from mucin-containing secretory granules in goblet cells that is dependent upon both autophagy and the nonhematopoietic inflammasome NLRP6 (31). Such secretion has been suggested to regulate colonic microbiota composition (31), as may also be the case with the role of autophagy in regulated secretion from intestinal Paneth cells (32).

Autophagy suppresses type I IFN response. Several autophagy factors directly suppress the type I IFN activation pathway (Figure 1C). One mechanism is the inhibition of RIG-I–like receptors (RLR) by direct binding of the ATG5/ATG12 conjugate to the caspase activation and recruitment domain (CARD) domains of RIG-I and mitochondrial antiviral signaling protein (MAVS; also known as VISA, IPS-1, and CARDIF) (33). The sole integral membrane autophagy factor, ATG9 (34), suppresses activation of stimulator of IFN genes (STING). STING is a receptor for host cell-generated cyclic dinucleotides (2'-5' GMP-AMP [cGAMP]) present in the cytosol produced by the host cGAMP synthase (cGAS) upon recognition of bacterial or other aberrant cytosolic DNA by direct binding to cGAS (35–37). Similar STING agonists, 3′-5′ c-di-GMP or c-di-AMP, can come directly from intracellular bacteria (38, 39). STING controls spatial activation of TANK-binding kinase 1 (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

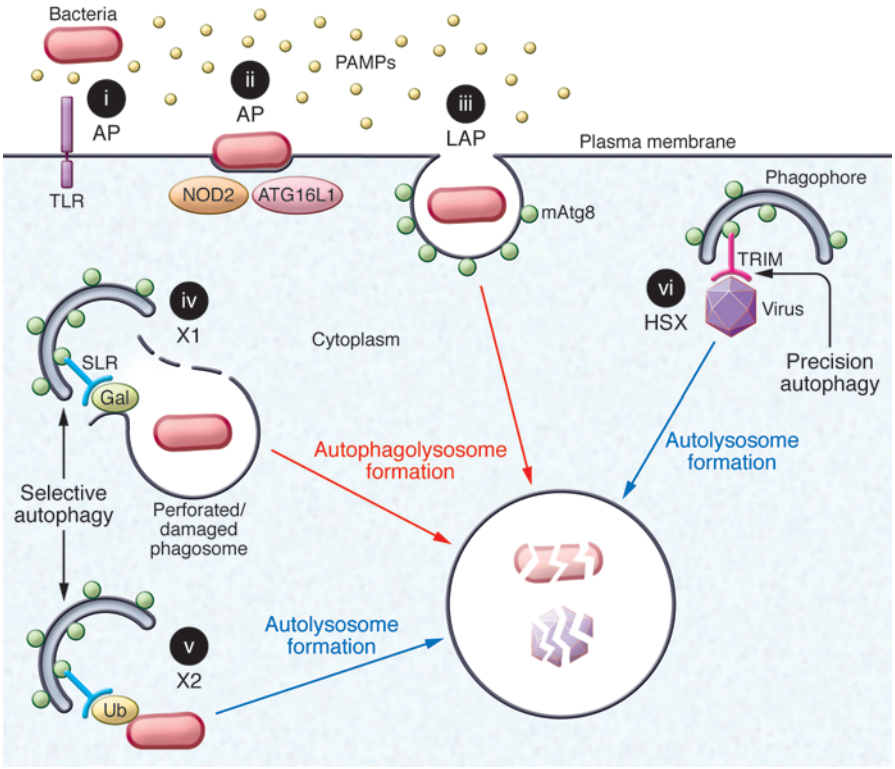

Figure 3. A spectrum of autophagic processes governs elimination of intracellular microbes. Extracellular bacteria (pink) can induce autophagy by stimulating TLRs (i) or NODs (ii) upon shedding of PAMPs or during phagocytosis (iii). The latter induces a hybrid process termed LAP. Intracellular bacteria can be eliminated through canonical autophagy sponsored by SLRs. Some SLRs can recognize galectins (Gal) bound to β-galactoside glycans on pathogen-associated damaged vacuolar membranes (iv), whereas all SLRs identified thus far recognize ubiquitin (Ubq) on cytosolic bacteria or associated host molecules (v). A viral core can be recognized by TRIMs, which act as receptors and inducers of autophagy, thus helping to eliminate retroviral material en route to the nucleus (vi). Fusion with lysosomes leads to formation of autolysosomes (from canonical autophagy acting on cytosolic targets) or autophagolysosomes (from LAP phagosomes or from damaged conventional phagosomes). Elimination of many pathogens involves not one but a spectrum of autophagic processes (processes i–vi correspond to the above descriptions): AP, autophagy; X1, xenophagy mixed with autophagy of host membranes; X2, xenophagy of microbes only; HSX, highly selective xenophagy.

factors directly block type I IFN activation, whereas autophagy as a pathway prevents accumulation of endogenous agonists and reduces the burden of microbial products, leading to type I IFN activation (Figure 1C).

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) signalosome. 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., *ULK1*, *ATG2A*, *ATG16L1*, etc.) of the yeast *Atg* genes as well as in human autophagy genes absent in yeast (e.g., *IRGM*, *NDP52*, etc.). Polymorphisms in *ATG16L1* (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 *ULK1* (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 *PRDM1-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, naive T cells in the periphery require the autophagy mediator VPS34 (PIK3C3) for proper IL-7 signaling, (67). The naive T cells depend on autophagy for proper maturation, which entails 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 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).

NLRs 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 locks 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, the first characterized SLR (106). SLRs include sequestosome 1/p62, NBR1 (107), NDP52 (of relevance for human cells but truncated in mice) (108), the NDP52-like receptor calcoco3 (also known as Tax1bp1) (109), and optineurin (110). SLRs usually contain one or more cargo recognition domains (2), which bind ubiquitin (108, 110, 111) or galectin (112, 113) tags (Figure 3). The ubiquitin tags are placed either on bacteria or on host cell components associated with bacteria in the cytosol. Galectin recognizes galactose residues exposed upon host membrane tears (e.g., damaged phagosome) (112). SLRs also contain an LC3-interacting region (LIR), which links them directly to the nascent autophagic membrane (114). Ubiquitination of bacterial targets is accomplished by E3 ligases such as LRSAM1 (115) or Parkin (116), the latter best known for the ubiquitination of permanently depolarized and damaged mitochondria destined for mitophagy. A striking parallel between mitophagy and xenophagy of bacteria has been noted and linked to the endosymbiotic bacterial origins of the present-day mitochondria (117). The affinities of SLRs for the ubiquitinated cargo and for LC3 are modulated by several Ser/Thr protein kinases. For exam-

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 mycobactericidal properties (4, 101, 102, 126). Autophagolysosomes are much more robust antimicrobial compartments than are conventional phagolysosomes. The mycobactericidal 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 Ca^{2+} and Ca^{2+}/calmodulin-dependent kinase 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 *Atg5*$^{fl/fl}$ *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* bacillus Calmette–Guérin (BCG) (146). Excessive levels of IL-1 are present in the lungs of *Atg5*$^{fl/fl}$ *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 *Atg5*$^{fl/fl}$ *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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1. Mizushima N, Yoshimori T, Ohsumi Y. The role of atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol*. 2011;27:107–132.
2. Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. *Nat Rev Immunol*. 2013;13(10):722–737.
3. Ma Y, Galluzzi L, Zitvogel L, Kroemer G. Autophagy and cellular immune responses. *Immunity*. 2013;39(2):211–227.
4. 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(6):753–766.
5. Nakagawa I, et al. Autophagy defends cells against invading group A Streptococcus. *Science*. 2004;306(5698):1037–1040.
6. Tooze SA, Yoshimori T. The origin of the autophagosomal membrane. *Nat Cell Biol*. 2010;12(9):831–835.
7. Puri C, Renna M, Bento CF, Moreau K, Rubinsztein DC. Diverse autophagosome membrane sources coalesce in recycling endosomes. *Cell*. 2013;154(6):1285–1299.
8. Dupont N, et al. Neutral lipid stores and lipase PNPLA5 contribute to autophagosome biogenesis. *Curr Biol*. 2014;24(6):609–620.
9. Tattoli I, et al. Amino acid starvation induced by invasive bacterial pathogens triggers an innate host defense program. *Cell Host Microbe*. 2012;11(6):563–575.
10. Egan DF, et al. Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science*. 2011;331(6016):456–461.
11. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol*. 2011;13(2):132–141.
12. Russell RC, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol*. 2013;15(7):741–750.
13. Kim J, et al. Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell*. 2013;152(1–2):290–303.
14. Shi CS, Kehrl JH. TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. *Sci Signal*. 2010;3(123):ra42.
15. Nazio F, et al. mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat Cell Biol*. 2013;15(4):406–416.
16. Lee HK, Lund JM, Ramanathan B, Mizushima

N, Iwasaki A. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science*. 2007;315(5817):1398–1401.
17. Dupont N, Jiang S, Pilli M, Ornatowski W, Bhattacharya D, Deretic V. Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1β. *EMBO J*. 2011;30(23):4701–4711.
18. Ohman T, et al. Dectin-1 pathway activates robust autophagy-dependent unconventional protein secretion in human macrophages. *J Immunol*. 2014;192(12):5952–5962.
19. Henault J, et al. Noncanonical autophagy is required for type I interferon secretion in response to DNA-immune complexes. *Immunity*. 2012;37(6):986–997.
20. 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(6):799–809.
21. Saitoh T, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production. *Nature*. 2008;456(7219):264–268.
22. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature*. 2011;469(7329):221–225.
23. Nakahira K, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat Immunol*. 2011;12(3):222–230.
24. Lupfer C, et al. Receptor interacting protein kinase 2-mediated mitophagy regulates inflammasome activation during virus infection. *Nat Immunol*. 2013;14(5):480–488.
25. van der Burgh R, et al. Defects in mitochondrial clearance predispose human monocytes to interleukin-1β hypersecretion. *J Biol Chem*. 2014;289(8):5000–5012.
26. Chuang SY, Yang CH, Chou CC, Chiang YP, Chuang TH, Hsu LC. TLR-induced PAI-2 expression suppresses IL-1β processing via increasing autophagy and NLRP3 degradation. *Proc Natl Acad Sci U S A*. 2013;110(40):16079–16084.
27. Shi CS, et al. Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction. *Nat Immunol*. 2012;13(3):255–263.
28. Harris J, et al. Autophagy controls IL-1β secretion by targeting pro-IL-1β for degradation. *J Biol Chem*. 2011;286(11):9587–9597.
29. Pilli M, et al. TBK-1 promotes autophagy-mediated antimicrobial defense by controlling autophagosome maturation. *Immunity*. 2012;37(2):223–234.
30. Jabir MS, et al. Caspase-1 cleavage of the TLR adaptor TRIF inhibits autophagy and β-interferon production during Pseudomonas aeruginosa infection. *Cell Host Microbe*. 2014;15(2):214–227.
31. Wlodarska M, et al. NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell*. 2014;156(5):1045–1059.
32. Cadwell K, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature*. 2008;456(7219):259–263.
33. Jounai N, et al. The Atg5 Atg12 conjugate associates with innate antiviral immune responses. *Proc Natl Acad Sci U S A*. 2007;104(35):14050–14055.
34. Saitoh T, et al. Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. *Proc Natl Acad Sci U S A*. 2009;106(49):20842–20846.
35. Ablasser A, et al. cGAS produces a 2′-5′-linked cyclic dinucleotide second messenger that activates STING. *Nature*. 2013;498(7454):380–384.
36. Civril F, et al. Structural mechanism of cytosolic DNA sensing by cGAS. *Nature*. 2013;498(7454):332–337.
37. Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science*. 2013;339(6121):786–791.
38. Woodward JJ, Iavarone AT, Portnoy DA. c-di-AMP secreted by intracellular Listeria monocytogenes activates a host type I interferon response. *Science*. 2010;328(5986):1703–1705.
39. Burdette DL, et al. STING is a direct innate immune sensor of cyclic di-GMP. *Nature*. 2011;478(7370):515–518.
40. Konno H, Konno K, Barber GN. Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. *Cell*. 2013;155(3):688–698.
41. Liang Q, et al. Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. *Cell Host Microbe*. 2014;15(2):228–238.
42. Tal MC, Sasai M, Lee HK, Yordy B, Shadel GS, Iwasaki A. Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. *Proc Natl Acad Sci U S A*. 2009;106(8):2770–2775.
43. Yang CS, et al. The autophagy regulator Rubicon is a feedback inhibitor of CARD9-mediated host innate immunity. *Cell Host Microbe*. 2012;11(3):277–289.
44. Paul S, Kashyap AK, Jia W, He YW, Schaefer BC. Selective autophagy of the adaptor protein Bcl10 modulates T cell receptor activation of NF-κB. *Immunity*. 2012;36(6):947–958.
45. Fliss PM, et al. Viral mediated redirection of NEMO/IKKgamma to autophagosomes curtails the inflammatory cascade. *PLoS Pathog*. 2012;8(2):e1002517.
46. von Muhlinen N, Thurston T, Ryzhakov G, Bloor S, Randow F. NDP52, a novel autophagy receptor for ubiquitin-decorated cytosolic bacteria. *Autophagy*. 2010;6(2):288–289.
47. Inomata M, Niida S, Shibata K, Into T. Regulation of Toll-like receptor signaling by NDP52-mediated selective autophagy is normally inactivated by A20. *Cell Mol Life Sci*. 2012;69(6):963–979.
48. Ellinghaus D, et al. Association between variants of PRDM1 and NDP52 and Crohn's disease, based on exome sequencing and functional studies. *Gastroenterology*. 2013;145(2):339–347.
49. Dokladny K, et al. Regulatory coordination between two major intracellular homeostatic systems: heat shock response and autophagy. *J Biol Chem*. 2013;288(21):14959–14972.
50. Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science*. 2006;313(5792):1438–1441.
51. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. 2007;447(7145):661–678.
52. Craddock N, et al. Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. *Nature*. 2010;464(7289):713–720.
53. Jostins L, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*. 2012;491(7422):119–124.
54. Ramos PS, et al. A comprehensive analysis of shared loci between systemic lupus erythematosus (SLE) and sixteen autoimmune diseases reveals limited genetic overlap. *PLoS Genet*. 2011;7(12):e1002406.
55. Henckaerts L, et al. Genetic variation in the autophagy gene ULK1 and risk of Crohn's disease. *Inflamm Bowel Dis*. 2011;17(6):1392–1397.
56. Brinar M, et al. Genetic variants in autophagy-related genes and granuloma formation in a cohort of surgically treated Crohn's disease patients. *J Crohns Colitis*. 2012;6(1):43–50.
57. Harley JB, et al. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat Genet*. 2008;40(2):204–210.
58. Han JW, et al. Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. *Nat Genet*. 2009;41(11):1234–1237.
59. Yang W, et al. Meta-analysis followed by replication identifies loci in or near CDKN1B, TET3, CD80, DRAM1, and ARID5B as associated with systemic lupus erythematosus in Asians. *Am J Hum Genet*. 2013;92(1):41–51.
60. Martin LJ, et al. Functional variant in the autophagy-related 5 gene promotor is associated with childhood asthma. *PLoS One*. 2012;7(4):e33454.
61. Raychaudhuri S, et al. Genetic variants at CD28, PRDM1 and CD2/CD58 are associated with rheumatoid arthritis risk. *Nat Genet*. 2009;41(12):1313–1318.
62. Cullup T, et al. Recessive mutations in EPG5 cause Vici syndrome, a multisystem disorder with defective autophagy. *Nat Genet*. 2013;45(1):83–87.
63. Warr MR, et al. FOXO3A directs a protective autophagy program in haematopoietic stem cells. *Nature*. 2013;494(7437):323–327.
64. Mortensen M, et al. The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. *J Exp Med*. 2011;208(3):455–467.
65. Lee HK, et al. In vivo requirement for Atg5 in antigen presentation by dendritic cells. *Immunity*. 2010;32(2):227–239.
66. 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(7211):396–400.
67. McLeod IX, Zhou X, Li QJ, Wang F, He YW. The class III kinase Vps34 promotes T lymphocyte survival through regulating IL-7Rα surface expression. *J Immunol*. 2011;187(10):5051–5061.
68. Pua HH, Guo J, Komatsu M, He YW. Autophagy is essential for mitochondrial clearance in mature T lymphocytes. *J Immunol*. 2009;182(7):4046–4055.
69. Jia W, Pua HH, Li QJ, He YW. Autophagy regu-

lates endoplasmic reticulum homeostasis and calcium mobilization in T lymphocytes. *J Immunol*. 2011;186(3):1564–1574.
70. He MX, He YW. A role for c-FLIP(L) in the regulation of apoptosis, autophagy, and necroptosis in T lymphocytes. *Cell Death Differ*. 2013;20(2):188–197.
71. Verghese DA, Yadav A, Bizargity P, Murphy B, Heeger PS, Schroppel B. Costimulatory blockade-induced allograft survival requires Beclin1. *Am J Transplant*. 2014;14(3):545–553.
72. Castillo EF, et al. Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. *Proc Natl Acad Sci U S A*. 2012;109(46):E3168–E3176.
73. Wildenberg ME, et al. Autophagy attenuates the adaptive immune response by destabilizing the immunologic synapse. *Gastroenterology*. 2012;142(7):1493–1503.
74. Chen M, et al. Essential role for autophagy in the maintenance of immunological memory against influenza infection. *Nat Med*. 2014;20(5):503–510.
75. Pengo N, et al. Plasma cells require autophagy for sustainable immunoglobulin production. *Nat Immunol*. 2013;14(3):298–305.
76. Schmid D, Pypaert M, Munz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity*. 2007;26(1):79–92.
77. Blanchet FP, et al. Human immunodeficiency virus-1 inhibition of immunoamphisomes in dendritic cells impairs early innate and adaptive immune responses. *Immunity*. 2010;32(5):654–669.
78. Jin Y, et al. Regulation of SIV antigen-specific $CD4^+$ T cellular immunity via autophagosome-mediated MHC II molecule-targeting antigen presentation in mice. *PLoS One*. 2014;9(3):e93143.
79. Paludan C, et al. Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science*. 2005;307(5709):593–596.
80. Jagannath C, Lindsey DR, Dhandayuthapani S, Xu Y, Hunter RL Jr, Eissa NT. Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. *Nat Med*. 2009;15(3):267–276.
81. Romao S, et al. Autophagy proteins stabilize pathogen-containing phagosomes for prolonged MHC II antigen processing. *J Cell Biol*. 2013;203(5):757–766.
82. Ravindran R, et al. Vaccine activation of the nutrient sensor GCN2 in dendritic cells enhances antigen presentation. *Science*. 2014;343(6168):313–317.
83. Cooney R, et al. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat Med*. 2010;16(1):90–97.
84. Aichinger M, Wu C, Nedjic J, Klein L. Macroautophagy substrates are loaded onto MHC class II of medullary thymic epithelial cells for central tolerance. *J Exp Med*. 2013;210(2):287–300.
85. Ireland JM, Unanue ER. Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. *J Exp Med*. 2011;208(13):2625–2632.
86. Fiegl D, et al. Amphisomal route of MHC class I cross-presentation in bacteria-infected dendritic cells. *J Immunol*. 2013;190(6):2791–2806.
87. Orvedahl A, et al. HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell Host and Microbe*. 2007;1(1):23–35.
88. Kyei GB, et al. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J Cell Biol*. 2009;186(2):255–268.
89. Gannage M, et al. Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. *Cell Host Microbe*. 2009;6(4):367–380.
90. Beale R, Wise H, Stuart A, Ravenhill BJ, Digard P, Randow F. A LC3-interacting motif in the influenza A virus M2 protein is required to subvert autophagy and maintain virion stability. *Cell Host Microbe*. 2014;15(2):239–247.
91. Bouley SJ, et al. Host cell autophagy promotes BK virus infection. *Virology*. 2014;456–457:87–95.
92. Ding B, et al. Phosphoprotein of human parainfluenza virus type 3 blocks autophagosome-lysosome fusion to increase virus production. *Cell Host Microbe*. 2014;15(5):564–577.
93. Pasztor K, Orosz L, Seprenyi G, Megyeri K. Rubella virus perturbs autophagy. *Med Microbiol Immunol*. 2014;203(5):323–331.
94. Moy RH, et al. Antiviral autophagy restricts Rift Valley fever virus infection and is conserved from flies to mammals. *Immunity*. 2014;40(1):51–65.
95. Buckingham EM, Carpenter JE, Jackson W, Grose C. Autophagy and the effects of its inhibition on varicella-zoster virus glycoprotein biosynthesis and infectivity. *J Virol*. 2014;88(2):890–902.
96. Griffin LM, Cicchini L, Pyeon D. Human papillomavirus infection is inhibited by host autophagy in primary human keratinocytes. *Virology*. 2013;437(1):12–19.
97. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature*. 2011;469(7330):323–335.
98. Sanjuan MA, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature*. 2007;450(7173):1253–1257.
99. Xu Y, Jagannath C, Liu XD, Sharafkhaneh A, Kolodziejska KE, Eissa NT. Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. *Immunity*. 2007;27(1):135–144.
100. Delgado MA, Elmaoued RA, Davis AS, Kyei G, Deretic V. Toll-like receptors control autophagy. *EMBO J*. 2008;27(7):1110–1121.
101. Ponpuak M, et al. Delivery of cytosolic components by autophagic adaptor protein p62 endows autophagosomes with unique antimicrobial properties. *Immunity*. 2010;32(3):329–341.
102. Fabri M, et al. Vitamin D is required for IFN-γ-mediated antimicrobial activity of human macrophages. *Sci Transl Med*. 2011;3(104):104ra2.
103. Yuk JM, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. *Cell Host Microbe*. 2009;6(3):231–243.
104. Travassos LH, et al. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat Immunol*. 2010;11(1):55–62.
105. Jounai N, Kobiyama K, Shiina M, Ogata K, Ishii KJ, Takeshita F. NLRP4 negatively regulates autophagic processes through an association with beclin1. *J Immunol*. 2011;186(3):1646–1655.
106. Bjorkoy G, et al. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J Cell Biol*. 2005;171(4):603–614.
107. Kirkin V, et al. A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. *Mol Cell*. 2009;33(4):505–516.
108. Thurston TL, Ryzhakov G, Bloor S, von Muhlinen N, Randow F. The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. *Nat Immunol*. 2009;10(11):1215–1221.
109. Newman AC, et al. TBK1 kinase addiction in lung cancer cells is mediated via autophagy of Tax1bp1/Ndp52 and non-canonical NF-κB signalling. *PLoS One*. 2012;7(11):e50672.
110. Wild P, et al. Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. *Science*. 2011;333(6039):228–233.
111. Dupont N, et al. Shigella phagocytic vacuolar membrane remnants participate in the cellular response to pathogen invasion and are regulated by autophagy. *Cell Host Microbe*. 2009;6(2):137–149.
112. Thurston TL, Wandel MP, von Muhlinen N, Foeglein A, Randow F. Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. *Nature*. 2012;482(7385):414–418.
113. Li S, et al. Sterical hindrance promotes selectivity of the autophagy cargo receptor NDP52 for the danger receptor galectin-8 in antibacterial autophagy. *Sci Signal*. 2013;6(261):ra9.
114. Johansen T, Lamark T. Selective autophagy mediated by autophagic adapter proteins. *Autophagy*. 2011;7(3):279–296.
115. Huett A, et al. The LRR and RING domain protein LRSAM1 is an E3 ligase crucial for ubiquitin-dependent autophagy of intracellular Salmonella Typhimurium. *Cell Host Microbe*. 2012;12(6):778–790.
116. Manzanillo PS, et al. The ubiquitin ligase parkin mediates resistance to intracellular pathogens. *Nature*. 2013;501(7468):512–516.
117. Deretic V. Autophagy of intracellular microbes and mitochondria: two sides of the same coin? *F1000 Biol Rep*. 2010;2:45.
118. Matsumoto G, Wada K, Okuno M, Kurosawa M, Nukina N. Serine 403 phosphorylation of p62/SQSTM1 regulates selective autophagic clearance of ubiquitinated proteins. *Mol Cell*. 2011;44(2):279–289.
119. Mandell MA, et al. TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition. *Dev Cell*. 2014;30(4):394–409.
120. Kawai T, Akira S. Regulation of innate immune signalling pathways by the tripartite motif (TRIM) family proteins. *EMBO Mol Med*. 2011;3(9):513–527.
121. Stremlau M, et al. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5α restriction factor. *Proc Natl Acad Sci U S A*. 2006;103(14):5514–5519.
122. Tang D, et al. Endogenous HMGB1 regulates autophagy. *J Cell Biol*. 2010;190(5):881–892.
123. Yanai H, et al. Conditional ablation of HMGB1 in mice reveals its protective function against endotoxemia and bacterial infection. *Proc Natl Acad Sci U S A*. 2013;110(51):20699–20704.
124. 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.
125. 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(7):1749–1760.
126. Harris J, et al. T helper 2 cytokines inhibit autophagic control of intracellular Mycobacterium tuberculosis. *Immunity*. 2007;27(3):505–517.
127. Singh SB, et al. Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria. *Nat Cell Biol*. 2010;12(12):1154–1165.
128. Mostowy S, et al. p62 and NDP52 proteins target intracytosolic Shigella and Listeria to different autophagy pathways. *J Biol Chem*. 2011;286(30):26987–26995.
129. Park HJ, et al. IL-10 inhibits the starvation induced autophagy in macrophages via class I phosphatidylinositol 3-kinase (PI3K) pathway. *Mol Immunol*. 2011;48(4):720–727.
130. Shen S, et al. Cytoplasmic STAT3 represses autophagy by inhibiting PKR activity. *Mol Cell*. 2012;48(5):667–680.
131. 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(14):6031–6036.
132. Kumar D, et al. Genome-wide analysis of the host intracellular network that regulates survival of Mycobacterium tuberculosis. *Cell*. 2010;140(5):731–743.
133. Petruccioli E, et al. Specific T cells restore the autophagic flux inhibited by Mycobacterium tuberculosis in human primary macrophages. *J Infect Dis*. 2012;205(9):1425–1435.
134. Juarez E, et al. NOD2 enhances the innate response of alveolar macrophages to Mycobacterium tuberculosis in humans. *Eur J Immunol*. 2012;42(4):880–889.
135. Zullo AJ, Lee S. Mycobacterial induction of autophagy varies by species and occurs independently of mammalian target of rapamycin inhibition. *J Biol Chem*. 2012;287(16):12668–12678.
136. Campbell GR, Spector SA. Vitamin D inhibits human immunodeficiency virus type 1 and Mycobacterium tuberculosis infection in macrophages through the induction of autophagy. *PLoS Pathog*. 2012;8(5):e1002689.
137. Anandaiah A, et al. Vitamin D rescues impaired Mycobacterium tuberculosis-mediated tumor necrosis factor release in macrophages of HIV-seropositive individuals through an enhanced Toll-like receptor signaling pathway in vitro. *Infect Immun*. 2013;81(1):2–10.
138. Watson RO, Manzanillo PS, Cox JS. Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell*. 2012;150(4):803–815.
139. Seto S, Tsujimura K, Horii T, Koide Y. Autophagy adaptor protein p62/SQSTM1 and autophagy-related gene Atg5 mediate autophagosome formation in response to Mycobacterium tuberculosis infection in dendritic cells. *PLoS One*. 2013;8(12):e86017.
140. Vergne I, Chua J, Singh SB, Deretic V. Cell biology of mycobacterium tuberculosis phagosome. *Annu Rev Cell Dev Biol*. 2004;20:367–394.
141. Klionsky DJ, Eskelinen EL, Deretic V. Autophagosomes, phagosomes, autolysosomes, phagolysosomes, autophagolysosomes... wait, I'm confused. *Autophagy*. 2014;10(4):549–551.
142. van der Wel N, et al. M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. *Cell*. 2007;129(7):1287–1298.
143. Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. *Int J Epidemiol*. 2008;37(1):113–119.
144. Hoyer-Hansen M, et al. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-β, and Bcl-2. *Mol Cell*. 2007;25(2):193–205.
145. Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol*. 2011;13(9):1016–1023.
146. Bonilla DL, et al. Autophagy regulates phagocytosis by modulating the expression of scavenger receptors. *Immunity*. 2013;39(3):537–547.
147. Singh V, Jamwal S, Jain R, Verma P, Gokhale R, Rao KV. Mycobacterium tuberculosis-driven targeted recalibration of macrophage lipid homeostasis promotes the foamy phenotype. *Cell Host Microbe*. 2012;12(5):669–681.
148. Shin DM, et al. Mycobacterium tuberculosis eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. *PLoS Pathog*. 2010;6(12):e1001230.
149. Shui W, et al. Organelle membrane proteomics reveals differential influence of mycobacterial lipoglycans on macrophage phagosome maturation and autophagosome accumulation. *J Proteome Res*. 2011;10(1):339–348.
150. Romagnoli A, et al. ESX-1 dependent impairment of autophagic flux by Mycobacterium tuberculosis in human dendritic cells. *Autophagy*. 2012;8(9):1357–1370.
151. Xu X, et al. Autophagy is essential for effector CD8(+) T cell survival and memory formation. *Nat Immunol*. 2014;15(12):1152–1161.