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Autophagy as an immune effector against tuberculosis

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Summary

The now well-accepted innate immunity paradigm that autophagy acts as a cell-autonomous defense against intracellular bacteria has its key origins in studies with *Mycobacterium tuberculosis*, an important human pathogen and a model microorganism infecting macrophages. A number of different factors have been identified that play into the anti-mycobacterial functions of autophagy, and recent in vivo studies in the mouse model of tuberculosis have uncovered additional anti-inflammatory and tissue-sparing functions of autophagy. Complementing these observations, genome wide association studies indicate a considerable overlap between autophagy, human susceptibility to mycobacterial infections, and predisposition loci for inflammatory bowel disease. Finally, recent studies show that autophagy is an important regulator and effector of IL-1 responses, and that autophagy intersects with type I interferon pathology-modulating responses.

Autophagy pathway as a general antimicrobial defense

Macroautophagy (or simply autophagy) is an evolutionarily conserved pathway controlling quality and quantity of eukaryotic organelles and the cytoplasmic biomass [1]. The autophagy pathway is depicted in an abbreviated form in Fig. 1, showing the minimal core components relevant for the discussion in this article. The key features are that physiological, pharmacological and immunological stimuli can induce autophagy via elements such as mTOR (mammalian target of rapamycin, a negative regulator of autophagy), AMP kinase (a positive regulator of autophagy), Ulk1 (mammalian Atg1, the most upstream autophagy-specific kinase), and Beclin 1 (mammalian Atg6) [1]. Downstream of Ulk1 and Beclin 1, a conjugation cascade (with steps of the pathway covered in detail by Boyle and Randow in this thematic issue) is initiated with the Atg5-Atg12/Atg16L1 complex serving as an E3 ligase guiding the conjugation of phosphatidylethanolamine to the C-terminus of LC3 (Atg8). This generates the LC3-II form associated with autophagic membranes. The generation of LC3-II is an emblematic event associated with autophagy and allows formation of double membrane autophagosomes and later on leads to their fusion with lysosomes. The fusion event generates autolysosomes, the highly lytic degradative organelles (Fig. 1). Another feature of LC3-II is that it can be recruited, under various circumstances, to conventional phagosomes upon induction of autophagy, enhancing the fusogenicity of phagosomes with lysosomal organelles, again resulting in a mixed product akin to autolysosomes (Fig. 1). This process has been referred to as LC3-associated phagocytosis (LAP) [2].

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Autophagy is uniquely suited for degradation of sizeable cytoplasmic targets, and represents a recently recognized cell-autonomous defense against intracellular pathogens [3,4], including bacteria (such as *M. tuberculosis* [5], *Shigella* [6-9], *Salmonella* [10-12], *Listeria* [8,13], and *Streptococci* [14]), or viruses [15]. In order for bacteria to be captured and destroyed by autophagy, they or host macromolecules or membranes have to be earmarked with tags such as ubiquitin [7,10,12,16,17] or galectins [11,18] that are recognized by autophagic adaptors (covered by Boyle and Randow in this thematic issue). Emphasizing the importance of autophagy in controlling pathogen survival and replication, many pathogens defend themselves against autophagy [19] using specific factors and processes [6,13,15,20-29]. Subsequent layers of host countermeasures are exemplified in the case of host septins, a family of factors that can overcome some of the bacterial autophagy-evading capabilities such as actin-based motility [8].

Genetic links between autophagy and tuberculosis

There are genetic links between autophagy and predisposition for tuberculosis disease. A very recent genome-wide association study (GWAS) shows an unexpectedly broad overlap between genetic predisposition for chronic inflammatory bowel disease (IBD) and mycobacterial disease, including 3 genes involved in tuberculosis [30], making of particular interest the polymorphisms in the autophagy genes that affect both IBD and tuberculosis. Additional polymorphisms were found in *ATG16L1* and *IRGM*, initially identified through GWAS as autophagy risk loci for Crohn's disease [31], a common form of IBD. The published evidence from human genetic studies indicates that the *IRGM* locus, encoding an autophagy-modulating factor [32,33], is a risk factor for tuberculosis in different populations [34-37]. Indeed, mice lacking LRG47, the mouse orthologue of *IRGM*, fail to control *M. tuberculosis* and rapidly succumb to infection [38]. Further studies are needed to examine whether the expanding number of autophagy loci affecting IBD and other inflammatory diseases may also correlate with tuberculosis. This does not necessarily imply that a correlation will always be direct (e.g. increase in autophagy results in increase in protection), as there is evidence that a defect (decrease) in autophagy may paradoxically confer protection against certain bacterial infections, with characteristics of inflammation underlying the phenomenon [39]. Finally, genetic polymorphisms in *VDR* (encoding vitamin D receptor) are associated with susceptibility to tuberculosis when combined with low serum levels of calcidiol, a precursor to calcitriol (1,25-dihydroxyvitamin D3), the active form of vitamin D [40]. Functionally, this has now been connected to the role of calcitriol in autophagic elimination of *M. tuberculosis* [41-47] as elaborated in the subsequent section. Similarly, the association of lower vitamin D levels with Crohn's disease [48] suggests a connection with autophagy.

Autophagy as an anti-*M. tuberculosis* mechanism

Germane to the focus of this review, several of the seminal observations regarding the antimicrobial role of autophagy have been made using *Mycobacterium tuberculosis* [5,32]. This was followed by gradual increase in studies on the topic of autophagy as a cell-autonomous, pharmacologically, physiologically and immunologically inducible anti-*M. tuberculosis* or anti-BCG process [33,49-57]. Some of the examples of immunological and physiological induction of mycobactericidal autophagy are (Fig. 2): (i) induction of anti-*M. tuberculosis* autophagy in macrophages through direct contacts between *M. tuberculosis*-specific T cells and infected macrophages [55]; (ii) ATP/P2X7-mediated stimulation of autophagy that can kill intracellular *M. tuberculosis* [52]; (iii) stimulation of infected macrophages with Th1 cytokines such as IFN- γ in a process that can be antagonized by Th2 cytokines including IL-4 and IL-13 [50,53]; and (iv) enhancement of IFN- γ -induced

autophagy by 1,25-dihydroxyvitamin D3 (calcitriol), correlating with levels of the calcitriol precursors in human sera and susceptibility to tuberculosis in human populations [46].

A systematic study of all steps of autophagy has established that the entire pathway is important for anti-mycobacterial action of autophagy [51]. Autophagy kills intracellular mycobacteria based on the strong degradative and other antimicrobial properties unique to autolysosomes [51]. Additional specifics of how autophagy eliminates mycobacteria include (Fig. 2): (i) induction of autophagy indirectly promotes maturation of *M. tuberculosis* phagosomes into degradative organelles [5,46,50], overcoming the well known *M. tuberculosis* phagosome maturation block; (ii) autophagosomes directly capture a subset of intracellular *M. tuberculosis* that then progress into degradative autolysosomes [5,58]; (iii) autophagy delivers to *M. tuberculosis* conventional anti-*M. tuberculosis* antimicrobial peptides such as cathelicidin through fusion with lysosomes where cathelicidin is stored [41,46]; and (iv) autophagy generates and delivers to *M. tuberculosis* a mixture of neo-antimicrobial peptides known as cryptides produced through autophagic proteolysis of innocuous cytosolic proteins such as ubiquitin [49] and ribosomal proteins [51]. In summary, autophagy can be induced by immunological and physiological stimuli enabling cells to kill intracellular *M. tuberculosis*.

Vitamin D metabolite calcitriol is a co-inducer of autophagy in human macrophages

The recent functional work affirms connections between autophagy and calcitriol in human cells (Fig. 2) [41-47]. Calcitriol circulates at very low concentrations in the serum and is generated in the kidney. However, calcitriol (1,25 vitamin D3) can additionally be generated in human macrophages from its precursor 25-hydroxy vitamin D (calcidiol; also present in the serum) by a specific 1- α hydroxylase induced in macrophages in response to TLR activation, stimulation with IFN- γ , or exposure to other stimuli. It has been shown that in human cells calcitriol is required for IFN- γ -induced autophagy (Fig. 2) [46]. Calcitriol is not needed for rapamycin- or starvation-induced autophagy in human macrophages [46], and is not required for induction of autophagy in murine macrophages [5,32,50]. Incidentally, IFN- γ does not stimulate murine macrophages to produce calcitriol, for unknown reasons but possibly related to the nocturnal (mice) vs diurnal (humans) behaviours and the role of sun exposure in the generation of precursors leading to calcidiol [46]. The combined IFN- γ plus calcitriol-induced autophagy in human cells is a canonical form, since it depends on Beclin 1 and Atg5 [46]. As previously shown with other activators of autophagy [5,32,50], the IFN- γ plus calcitriol-induced autophagy in human cells promotes maturation of phagosomes harboring *M. tuberculosis* and facilitates their fusion with lysosomal compartments that contain antimicrobial peptides (Fig. 2). Significantly, vitamin D and autophagy also factor in AIDS and HIV-1 and HIV-*M. tuberculosis* co-infections, a topic covered in this issue in an article by Campbell and Spector.

Autophagy contributes to anti-tuberculosis action of isoniazid and pyrazinamide

Recent studies link action of the classical frontline antituberculosis chemotherapeutics with autophagy [59] (Fig. 2). It has been reported that the key anti-tuberculosis drugs isoniazid and pyrazinamide require autophagy for their full potency against intracellular *M. tuberculosis* through a mechanism based on release of reactive oxygen species (ROS) [59]. Induction of autophagy during pyrazinamide treatment may help explain the sterilizing potency of pyrazinamide and its anti-mycobacterial action primarily being observed with intracellular *M. tuberculosis* in infected macrophages. Isoniazid and pyrazinamide treatment

of *M. tuberculosis* results in release of bacterial products and induces autophagy in infected macrophages, in association with generation of reactive oxygen species by host cell sources (mitochondria and NADPH oxidase) [59]. This is in keeping with the previous observations that ROS from mitochondria and other sources can induce autophagy [60,61]. It is also possible that additional mediators such as released mycobacterial products and cellular alarmins can cause autophagic responses. Importantly, without ability to mount autophagy (e.g. upon Beclin 1 or Atg5 knockdowns), loss of mycobacterial viability in response to therapeutic doses of isoniazid and pyrazinamide was no longer statistically significant, indicating that in macrophages these two frontline drugs are helped by autophagy in their antimicrobial activity [59]. Of further interest is that autophagy induced in response to anti-mycobacterial treatment was playing an additional, anti-inflammatory role [59]. This is in keeping with the general inflammation-suppressing properties of autophagy [62], and with the specific anti-inflammatory role of autophagy during *M. tuberculosis* infection in animal models [58,63]. Complementing these studies, several compounds that can induce autophagy without acting on the bacteria first have been shown to inhibit intracellular *M. tuberculosis* or BCG growth or survival [64–66]. Thus, pharmaceuticals inducing autophagy could be potentially useful as new anti-*M. tuberculosis* chemotherapeutics.

***M. tuberculosis* defends itself against autophagy**

On the flip side, complementary investigations have been examining whether *M. tuberculosis* produces factors antagonizing autophagy. Mycobacterial infection shows indications of both mTOR activation (mTOR inhibits autophagy) and autophagy induction, with a balance being dependent on species, skewed towards autophagy repression when *M. bovis* BCG was tested [57]. Several candidates for specific anti-autophagy factors encoded by *M. tuberculosis* have been reported. Among these are mycobacterial glycolipid lipoarabinomannan [67] and proteins Eis (enhanced intracellular survival) [68–70] and ESAT-6 [71,72]. Eis acts as an e-aminoacyltransferase that can acetylate Lys-55 in a JNK-specific phosphatase [68]. Since JNK action is important for activation of the key autophagy regulator Beclin 1, one of the activity of Eis may be to suppress autophagy [68–70]. Others have shown that ESX-1, a type VII secretion system of *M. tuberculosis*, releases a 6 kDa protein ESAT-6 to inhibit phagosomal maturation into degradative autolysosomal organelles where *M. tuberculosis* is killed [71]. It has also been reported that ESAT-6 (fused to another ESX-1 secretion substrate, CFP-10) inhibits autophagy [72]. This block can be overcome by pharmacological induction of autophagy [71], which clarifies why stimulation of autophagy by starvation or Th1 cytokines [5,50] can lead to autophagic killing of *M. tuberculosis* and at the same time suggests that pharmacological induction of autophagy may be a promising approach to treatment.

Of further interest is that *M. tuberculosis* can use host processes to defend against autophagy. One example is the aforementioned “graded” activation of mTOR, which represses autophagy, by more virulent mycobacteria [57]. Furthermore, the host actin-binding protein Coronin is recruited to *M. tuberculosis* phagosomes, and now it has been reported that it inhibits autophagosome formation [26]. This is potentially of more general interest given the emerging significance of the interplay between actin-based factors and bacterial evasion of autophagy [6,8,13]. Finally, “metabolic reprogramming” of host macrophages by *M. tuberculosis* has also been linked to simultaneous increase in lipid droplet content [73,74] and reduction in autophagic capacity sparing intracellular bacilli from autophagic elimination [74]. Lipid droplets are cytoplasmic stores of neutral lipids such as triglycerides and cholesterol, and are visually distinct dynamic organelles [75] that interact with the autophagic apparatus [76,77]. Lipid droplets are also a substrate for autophagic degradation in a process dubbed “lipophagy” [76]. Thus, increase in lipid droplets (causing the foamy macrophage phenotype) observed in *M. tuberculosis* infected

macrophages [74] could be a consequence of or a contributing cause to the inhibition of autophagy. The case for the former is that increase in lipid droplets was linked to ESAT-6 [74], which as we discussed above, can inhibit autophagy [71,72] and thus may impede lipophagy [76]. The case for the latter is that increase in lipid load is known to inhibit autophagy [78]. This may result in a vicious cycle preventing effective anti-mycobacterial autophagy and at the same time providing substrate for *M. tuberculosis* growth.

Autophagy protects against *M. tuberculosis* pathogenesis in vivo

Although *M. tuberculosis* was one of the first microbes to help establish the new paradigm of autophagy as an innate immunity effector [5], an in vivo demonstration for the role of autophagy in a tuberculosis animal model has been lacking until very recently. This has changed with the publication of two studies showing that autophagy protects against bacterial burden, inflammation, lung pathology, and death in a mouse model of tuberculosis [58,63]. Both studies used the Atg5^{fl/fl} LysM-Cre transgenic mice deficient for autophagy in the myeloid lineage, which includes macrophages, the key cell type parasitized by *M. tuberculosis*. Although different strains of virulent *M. tuberculosis* (H37Rv [63] and Erdman [58]) were used, and details of respiratory infection varied, similar effects were observed albeit they differed in magnitude. The study with the Erdman strain showed a substantial increase in bacterial burden and mortality in Atg5^{fl/fl} LysM-Cre⁺ mice [58]. The study with the intranasal inhalation infection with the standard H37Rv virulent strain of *M. tuberculosis* also showed increased bacterial burden and mortality, but the differences were much subtler between the Atg5-defective and Atg5-proficient littermates [63].

Complex roles for autophagy during *M. tuberculosis* infection

The above in vivo studies [58,63] both reported increased levels of pro-inflammatory cytokines and excess necrotic lesions in the lungs of autophagy defective mice infected with *M. tuberculosis*. Both studies agreed in reporting differentially elevated levels of IL-1 α and IL-1 β (in the study with H37Rv the absolute levels of IL-1 β were very low) as opposed to the IFN- γ response that was equal in Atg5^{fl/fl} LysM-Cre⁺ vs Atg5^{fl/fl} LysM-Cre⁻ littermates [58,63]. The researchers who detected a much bigger differences in colony forming units focused on the mechanism of autophagic recognition of intracellular *M. tuberculosis* [58]. They detected ubiquitin as a tag placed on bacteria-associated targets and showed a role for autophagic adaptors p62 and NDP52 in recognizing such tags in clearance of intracellular *M. tuberculosis* Erdman in murine macrophages studied ex vivo [58]. This is in keeping with the well established role of ubiquitin-based recognition of microbial targets through autophagic adaptors recognizing ubiquitin tags, a topic covered extensively in this issue in the chapter by Boyle and Randow.

In addition, authors of the Erdman strain study noticed that *M. tuberculosis* extracellular DNA (Mtb eDNA) was released from mycobacteria and mycobacterial phagosomes, dependent on the type VII secretion system ESX-1 [58]. They proposed that Mtb eDNA may be a target that is recognized by the autophagic apparatus. This is supported by the notion that the *M. tuberculosis* ESX-1 secretion system leads to subtle perforation of *M. tuberculosis* phagosomes, and thus this may allow escape of PAMPs that could initiate autophagy, although it also is a source of known antagonists of autophagic maturation, e.g. ESAT-6 discussed in a previous section [71,72]. Pursuing the idea that DNA released via ESX-1 might recruit autophagic apparatus, the authors of the Erdman study tested whether TBK-1 (known to participate in signal transduction from foreign DNA in the cytosol) was involved in ESX-1-dependent recruitment of autophagic machinery to a fraction of *M. tuberculosis* bacilli occurring spontaneously (i.e. without induction of autophagy by starvation or pharmacological means). Indeed, phosphorylated (activated) TBK-1 was

recruited to *M. tuberculosis* phagosomes in an ESX-1-dependent fashion and overlapped with LC3⁺ phagosomes [58]. Mtb eDNA leaching from the bacilli was found to be responsible for this spontaneous recruitment of autophagic machinery to the fraction of intracellular *M. tuberculosis* with perforated phagosomes. Mtb eDNA induced-autophagy was sensitive to host DNases TREX1 or DNASE IIa [58]. STING was necessary to restrict *M. tuberculosis* through Mtb eDNA-elicited autophagy [58]. The authors concluded that *M. tuberculosis* releases DNA that in turn guides the autophagic apparatus to clear *M. tuberculosis*.

The interesting observations reported in the Erdman study [58] pose several questions. Is the spontaneous release of Mtb eDNA “helping” the bacterium or the host? Based on the proposed role for Mtb eDNA in the autophagic recognition of *M. tuberculosis*, one might expect that this serves the host’s purpose. However, ESX-1 is an acknowledged *M. tuberculosis* virulence factor, and ESX-1 mediated induction of type I IFN is detrimental to the host [79,80]. These two notions appear contradictory. However, it is possible that Mtb eDNA or other products misdirect autophagic machinery and result in a pathogenic type I IFN response. This would be compatible with the data showing that only a fraction of *M. tuberculosis* phagosomes are subject to Mtb eDNA-provoked TBK-1-dependent spontaneous autophagy [58]. Another attractive explanation is that ESX-1 released Mtb eDNA may not serve to clear mycobacteria but may be a target for autophagic clearance of Mtb eDNA as a PAMP, lest it induces a type I interferon response, known to be associated with tuberculosis pathogenesis (Fig. 3). As the role of autophagy in type I IFN responses is complex, further experiments are needed to clarify this interaction in *M. tuberculosis* infection.

Autophagy protects against excessive inflammation during *M. tuberculosis* infection

The authors of the *M. tuberculosis* H37Rv study [63] considered the possibility that a small increase in bacterial burden that they detected may not suffice to explain extensive pathology in autophagy-defective infected mice. They went on to investigate whether other factors, such as excessive inflammation, were responsible for lung pathology and increased mortality [63]. Even in the absence of infection, the lung immune cells of mice with autophagic defect in macrophages showed propensity towards Th17 polarization ex vivo and displayed extended duration of Th17 cytokine production in vivo during infection [63]. This was correlated with the cell-autonomous defect in autophagy-deficient macrophages, which secreted excess levels of IL-1 α [63], the cytokine conspicuously increased in vivo in both H37Rv and Erdman infection studies [58,63]. IL-1 α , just like IL-1 β , can lead to Th17 polarization, as was demonstrated in ex vivo studies [63]. This is in keeping with prolonged IL-17 response detected in vivo during *M. tuberculosis* infection of Atg5^{fl/fl} LysM-Cre⁺ mice [63].

Autophagy, IL-1, type I IFN, and *M. tuberculosis* pathogenesis

MyD88 signalling is key to early protection against *M. tuberculosis* [81-84], and polymorphisms in the MyD88 signaling pathway is associated with susceptibility to *M. tuberculosis* infection [85]. Searching upstream and downstream of MyD88 for pivotal signals and responses defending against *M. tuberculosis* has proven to be difficult. TLRs, which act upstream of MyD88, appear not to be essential for protection when tested individually or in combined knockout animals [81-84]. In contrast, IL-1 receptor signalling, which also includes MyD88 as an adapter, in response to IL-1 β (and possibly IL-1 α), turned out to be essential for early control of *M. tuberculosis* [86-88], and an IL-1b promoter polymorphism has been linked to tuberculosis infection [89]. However, the exact anti-

mycobacterial effector mechanism induced by IL-1 β has remained elusive [87]. This has changed recently, when it was shown that IL-1 β eliminated *M. tuberculosis* through autophagy (Fig. 2) [90]. IL-1 β induces autophagy in a MyD88-dependent fashion, and promotes autophagosomal maturation into degradative autolysosomes in a TBK-1 dependent manner [90]. IL-1 β levels are regulated by IFN- γ -induced nitric oxide inhibition of the NLRP3 inflammasome [91]. Additional work is necessary to confirm the importance of IL-1 β -mediated induction of autophagy for in vivo protection against *M. tuberculosis* infection.

Both IL-1 β and IL-1 α are increased in autophagy-defective macrophages when tested ex vivo [63]. One explanation for the dominant expression of IL-1 α detected in vivo [63] is that during infection *M. tuberculosis* suppresses inflammasome activation (used to process IL-1 β ; see chapter by Chen and Schroder in this issue for details on inflammasome function) as shown in prior ex vivo studies [92]. The cell-autonomous mechanisms for excessive IL-1 α and IL-1 β activation in autophagy-deficient macrophages actually have similar roots but differ at the stage of pro-IL-1 processing. Autophagy prevents accumulation of defective mitochondria and thus under normal homeostatic conditions reduces ROS and mitochondrial DNA (mtDNA) release (see chapter by Cloonan and Choi in this issue), which otherwise goes up in the absence of autophagy due to accumulation of unkempt mitochondria [63,93,94]. ROS activate NALP3 inflammasome whereas mtDNA activates AIM2 inflammasome, both leading to increased processing and secretion of IL-1 β . For IL-1 α , the dominant component of its activation is inflammasome-independent but still depends on ROS [63]. Excess ROS in autophagy-defective cells activate calpain that in turn processes IL-1 α , resulting in its surplus secretion from autophagy-deficient macrophages [63]. Excess IL-1 α released from Atg5-defective macrophages may lead to a prolonged Th17 response during *M. tuberculosis* infection, contributing to lung tissue necrosis [63]. However, additional studies are needed to confirm the role of potentially dysregulated Th17 responses in the absence of autophagy during *M. tuberculosis* infection.

The above anti-inflammatory action of autophagy may not stop at IL-1, and could extend to the role of autophagy in suppressing type I IFN induction. Elevated Type I IFN response has gained visibility as a factor contributing to tuberculosis pathogenesis. Induction of type I IFN is pathogenic in animal models of *M. tuberculosis*, and increased type I IFN activity correlates with active MTB disease in humans [95,96]. Mice lacking IFNAR or IRF3 survive longer than WT mice after *M. tuberculosis* infection [79,80]. Induction of type I IFN in mice exacerbates disease and increases time-to-death relative to untreated infected mice [97]. *M. tuberculosis*-mediated induction of type I IFN requires the ESX-1 system [79,98-100] and TBK-1 [79,99,100], and this signalling has been shown to be induced by Mtb eDNA through IRF3 [79]. IL-1 α and IL-1 β are both required for protection against *M. tuberculosis* [86,87], and type I IFN has been shown to reduce IL-1 responses through a variety of mechanisms [87,101]. Since IL-1 is required for protection against *M. tuberculosis*, but higher levels of IL-1 are associated with pathology, it is likely that moderate levels of IL-1 or precisely timed bursts of release are needed for a successful response against infection, while low or chronically high levels result in a non-protective response (Fig. 3).

The Erdman study discussed above [58] has implicated autophagy and STING in the recognition of cytosolic Mtb eDNA [58]. It is now known that STING can recognize the second messenger, cyclic GMP-AMP (cGAMP) [102] generated by the recently discovered host DNA-receptor, cGAMP synthase, and then help activate type I IFN pathway via TBK-1 signaling [103]. It might be possible that autophagy eliminates Mtb eDNA preventing it to act as a stimulus for cGAMP generation, besides the function proposed in clearance of *M. tuberculosis*, and thus could suppress pathogenic type I IFN response.

In summary, besides its role in direct elimination of bacteria, autophagy may play just as important role in responses to endogenous (e.g. mitochondrial) and pathogen (mycobacterial) products and irritants to prevent excessive inflammation (increased IL-1 α), activation of non-protective responses (type I IFN), or prolonged duration of strong and thus damaging proinflammatory states (Th17).

Conclusions

Autophagy has been shown to be involved in multiple aspects of *M. tuberculosis* infection. Autophagy can limit mycobacterial survival by autolysosomal killing of bacteria, generation of antimicrobial peptides, and potentiation of antibiotics. Furthermore, autophagy can limit potentially damaging inflammation and can regulate type I IFN responses, which have implications in mycobacterial pathogenesis. Importantly, pharmacological regulation of autophagy has shown beneficial effects in vitro, suggesting potential therapeutic host-targeted options for autophagic control of mycobacterial infection. Future topics for research include the relationship between autophagy and type I IFN responses in infection, and in vivo studies on the interaction between autophagy, host responses, and mycobacterial infection.

References

1. Mizushima N, Yoshimori T, Ohsumi Y. The role of atg proteins in autophagosome formation. Annual review of cell and developmental biology. 2011; 27:107–132.
2. Cemma M, Brumell JH. Interactions of pathogenic bacteria with autophagy systems. Current biology : CB. 2012; 22:R540–545. [PubMed: 22790007]
3. Deretic V, Jiang S, Dupont N. Autophagy intersections with conventional and unconventional secretion in tissue development, remodeling and inflammation. Trends in cell biology. 2012; 22:397–406. [PubMed: 22677446]
4. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. Nature. 2011; 469:323–335. [PubMed: 21248839]
5. 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]
6. 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]
7. Dupont N, Lacas-Gervais S, Bertout J, Paz I, Freche B, Van Nhieu GT, van der Goot FG, Sansonetti PJ, Lafont F. Shigella phagocytic vacuolar membrane remnants participate in the cellular response to pathogen invasion and are regulated by autophagy. Cell Host Microbe. 2009; 6:137–149. [PubMed: 19683680]
8. Mostowy S, Sancho-Shimizu V, Hamon MA, Simeone R, Brosch R, Johansen T, Cossart P. p62 and NDP52 Proteins Target Intracytosolic Shigella and Listeria to Different Autophagy Pathways. The Journal of biological chemistry. 2011; 286:26987–26995. [PubMed: 21646350]
9. Ogawa M, Yoshikawa Y, Kobayashi T, Mimuro H, Fukumatsu M, Kiga K, Piao Z, Ashida H, Yoshida M, Kakuta S, et al. A Tecpr1-dependent selective autophagy pathway targets bacterial pathogens. Cell host & microbe. 2011; 9:376–389. [PubMed: 21575909]
10. Huett A, Heath RJ, Begun J, Sassi SO, Baxt LA, Vyas JM, Goldberg MB, Xavier RJ. The LRR and RING Domain Protein LRSAM1 Is an E3 Ligase Crucial for Ubiquitin-Dependent Autophagy of Intracellular Salmonella Typhimurium. Cell host & microbe. 2012; 12:778–790. [PubMed: 23245322]
- 11**. 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; 482:414–418. [PubMed: 22246324] In this study, galectin 8 was shown to bind damaged vacuoles, including those containing bacteria, and target them for autophagic destruction via NDP52 recruitment.

- 12*. Wild P, Farhan H, McEwan DG, Wagner S, Rogov VV, Brady NR, Richter B, Korac J, Waidmann O, Choudhary C, et al. Phosphorylation of the autophagy receptor optineurin restricts *Salmonella* growth. *Science*. 2011; 333:228–233. [PubMed: 21617041] This study showed involvement of TBK-1 phosphorylation in modulating affinity of an autophagic adaptor for LC3.
13. Yoshikawa Y, Ogawa M, Hain T, Yoshida M, Fukumatsu M, Kim M, Mimuro H, Nakagawa I, Yanagawa T, Ishii T, et al. *Listeria monocytogenes* ActA-mediated escape from autophagic recognition. *Nat Cell Biol*. 2009; 11:1233–1240. [PubMed: 19749745]
14. Nakagawa I, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, Nara A, Funao J, Nakata M, Tsuda K, et al. Autophagy defends cells against invading group A *Streptococcus*. *Science*. 2004; 306:1037–1040. [PubMed: 15528445]
- 15*. Shoji-Kawata S, Sumpter R, Leveno M, Campbell GR, Zou Z, Kinch L, Wilkins AD, Sun Q, Pallauf K, MacDuff D, et al. Identification of a candidate therapeutic autophagy-inducing peptide. *Nature*. 2013; 494:201–206. [PubMed: 23364696] An autophagy-inducing peptide containing a region of beclin-1 fused to tat was shown to inhibit replication of HIV-1 in vitro. In addition, in vivo treatment with this peptide showed efficacy in mice against West Nile Virus or Chikungunya virus infection.
16. Zheng YT, Shahnazari S, Brech A, Lamark T, Johansen T, Brumell JH. The adaptor protein p62/SQSTM1 targets invading bacteria to the autophagy pathway. *J Immunol*. 2009; 183:5909–5916. [PubMed: 19812211]
17. 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; 10:1215–1221. [PubMed: 19820708]
- 18*. Li S, Randow F. A crystal structure of an autophagy receptor with a ligand reveals how *Salmonella* is specifically targeted for degradation. *Science Signaling*. 2013 In press. This study showed precisely how galectins bind to NDP52, a dual specificity autophagic adaptor, recognizing both ubiquitin and galectin tags. Of note, NDP52 is expressed in human cells but not in murine cells, where this gene has been truncated by a C-terminal deletion that removes both of its binding motifs for galectins and ubiquitin.
19. Deretic V, Levine B. Autophagy, immunity, and microbial adaptations. *Cell Host Microbe*. 2009; 5:527–549. [PubMed: 19527881]
20. 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]
21. 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]
22. Ku B, Woo JS, Liang C, Lee KH, Hong HS, E X, Kim KS, Jung JU, Oh BH. Structural and biochemical bases for the inhibition of autophagy and apoptosis by viral BCL-2 of murine gamma-herpesvirus 68. *PLoS Pathog*. 2008; 4:e25. [PubMed: 18248095]
23. Lee JS, Li Q, Lee JY, Lee SH, Jeong JH, Lee HR, Chang H, Zhou FC, Gao SJ, Liang C, et al. FLIP-mediated autophagy regulation in cell death control. *Nat Cell Biol*. 2009; 11:1355–1362. [PubMed: 19838173]
24. Gannage M, Dormann D, Albrecht R, Dengjel J, Torossi T, Ramer PC, Lee M, Strowig T, Arrey F, Conenello G, et al. Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. *Cell Host Microbe*. 2009; 6:367–380. [PubMed: 19837376]
25. Kyei GB, Dinkins C, Davis AS, Roberts E, Singh SB, Dong C, Wu L, Kominami E, Ueno T, Yamamoto A, et al. Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J Cell Biol*. 2009; 186:255–268. [PubMed: 19635843]
26. Seto S, Tsujimura K, Koide Y. Coronin-1a inhibits autophagosome formation around *Mycobacterium tuberculosis*-containing phagosomes and assists mycobacterial survival in macrophages. *Cellular microbiology*. 2012; 14:710–727. [PubMed: 22256790]
27. Dortet L, Mostowy S, Samba-Louaka A, Gouin E, Nahori MA, Wiemer EA, Dussurget O, Cossart P. Recruitment of the major vault protein by InlK: a *Listeria monocytogenes* strategy to avoid autophagy. *PLoS pathogens*. 2011; 7:e1002168. [PubMed: 21829365]

28. Mesquita FS, Thomas M, Sachse M, Santos AJ, Figueira R, Holden DW. The Salmonella deubiquitinase SseL inhibits selective autophagy of cytosolic aggregates. *PLoS pathogens*. 2012; 8:e1002743. [PubMed: 22719249]
29. Choy A, Dancourt J, Mugo B, O'Connor TJ, Isberg RR, Melia TJ, Roy CR. The Legionella effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation. *Science*. 2012; 338:1072–1076. [PubMed: 23112293]
30. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature*. 2012; 491:119–124. [PubMed: 23128233]
31. Consortium: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature*. 2007; 447:661–678. [PubMed: 17554300]
32. Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science*. 2006; 313:1438–1441. [PubMed: 16888103]
33. Singh SB, Ornatowski W, Vergne I, Naylor J, Delgado M, Roberts E, Ponpuak M, Master S, Pilli M, White E, et al. Human IRGM regulates autophagy and cell-autonomous immunity functions through mitochondria. *Nat Cell Biol*. 2010; 12:1154–1165. [PubMed: 21102437]
34. Intemann CD, Thye T, Niemann S, Browne EN, Amanua Chinbuah M, Enimil A, Gyapong J, Osei I, Owusu-Dabo E, Helm S, et al. Autophagy gene variant IRGM -261T contributes to protection from tuberculosis caused by *Mycobacterium tuberculosis* but not by *M. africanum* strains. *PLoS Pathog*. 2009; 5:e1000577. [PubMed: 19750224]
35. Che N, Li S, Gao T, Zhang Z, Han Y, Zhang X, Sun Y, Liu Y, Sun Z, Zhang J, et al. Identification of a novel IRGM promoter single nucleotide polymorphism associated with tuberculosis. *Clin Chim Acta*. 2010; 411:1645–1649. [PubMed: 20547146]
- 36*. King KY, Lew JD, Ha NP, Lin JS, Ma X, Graviss EA, Goodell MA. Polymorphic allele of human IRGM1 is associated with susceptibility to tuberculosis in African Americans. *PloS one*. 2011; 6:e16317. [PubMed: 21283700] IRGM, an autophagy-associated gene with a known correlation with Crohn's Disease, was found to be associated with tuberculosis infection in certain patient cohorts.
37. Bahari G, Hashemi M, Taheri M, Naderi M, Eskandari-Nasab E, Atabaki M. Association of IRGM Polymorphisms and Susceptibility to Pulmonary Tuberculosis in Zahedan, Southeast Iran. *The Scientific World Journal*. 2012; 2012:950801.
38. MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. *Science*. 2003; 302:654–659. [PubMed: 14576437]
39. Wang C, Mendonsa GR, Symington JW, Zhang Q, Cadwell K, Virgin HW, Mysorekar IU. Atg16L1 deficiency confers protection from uropathogenic *Escherichia coli* infection in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:11008–11013. [PubMed: 22715292]
40. Wilkinson RJ, Llewelyn M, Toossi Z, Patel P, Pasvol G, Lalvani A, Wright D, Latif M, Davidson RN. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *Lancet*. 2000; 355:618–621. [PubMed: 10696983]
41. Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, Lee ZW, Lee SH, Kim JM, Jo EK. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. *Cell host & microbe*. 2009; 6:231–243. [PubMed: 19748465]
42. Shin DM, Yuk JM, Lee HM, Lee SH, Son JW, Harding CV, Kim JM, Modlin RL, Jo EK. Mycobacterial Lipoprotein Activates Autophagy via TLR2/1/CD14 and a Functional Vitamin D Receptor Signaling. *Cell Microbiol*. 2010
- 43*. Campbell GR, Spector SA. Vitamin D Inhibits Human Immunodeficiency Virus Type 1 and *Mycobacterium tuberculosis* Infection in Macrophages through the Induction of Autophagy. *PLoS pathogens*. 2012; 8:e1002689. [PubMed: 22589721] This study showed that vitamin D induces initiation and maturation of autophagy that results in inhibition of HIV and *M. tuberculosis* replication in a cathelicidin microbial peptide-dependent manner.
44. Anandaiah A, Sinha S, Bole M, Sharma SK, Kumar N, Luthra K, Li X, Zhou X, Nelson B, Han X, 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. *Infection and immunity*. 2013; 81:2–10. [PubMed: 23071135]
45. Klug-Micu GM, Stenger S, Sommer A, Liu PT, Krutzik SR, Modlin RL, Fabri M. CD40L and IFN-gamma induce an antimicrobial response against *M. tuberculosis* in human monocytes. *Immunology*. 2013
 - 46**. Fabri M, Stenger S, Shin DM, Yuk JM, Liu PT, Realegeno S, Lee HM, Krutzik SR, Schenk M, Sieling PA, et al. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. *Science translational medicine*. 2011; 3:104ra102. In this study, T cell-produced IFN- γ was shown to induce autophagy, antimicrobial peptide production, and antimicrobial activity in *M. tuberculosis*-infected macrophages in a vitamin D-dependent manner.
 47. Fabri M, Realegeno SE, Jo EK, Modlin RL. Role of autophagy in the host response to microbial infection and potential for therapy. *Current opinion in immunology*. 2011; 23:65–70. [PubMed: 21071195]
 48. Iijima H, Shinzaki S, Takehara T. The importance of vitamins D and K for the bone health and immune function in inflammatory bowel disease. *Curr Opin Clin Nutr Metab Care*. 2012; 15:635–640. [PubMed: 22914505]
 49. 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]
 50. 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]
 51. Ponpuak M, Davis AS, Roberts EA, Delgado MA, Dinkins C, Zhao Z, Virgin HW, Kyei GB, Johansen T, Vergne I, et al. Delivery of cytosolic components by autophagic adaptor protein p62 endows autophagosomes with unique antimicrobial properties. *Immunity*. 2010; 32:329–341. [PubMed: 20206555]
 52. 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]
 53. Ghadimi D, de Vrese M, Heller KJ, Schrezenmeir J. Lactic acid bacteria enhance autophagic ability of mononuclear phagocytes by increasing Th1 autophagy-promoting cytokine (IFN-gamma) and nitric oxide (NO) levels and reducing Th2 autophagy-restraining cytokines (IL-4 and IL-13) in response to *Mycobacterium tuberculosis* antigen. *Int Immunopharmacol*. 2010; 10:694–706. [PubMed: 20381647]
 54. Kumar D, Nath L, Kamal MA, Varshney A, Jain A, Singh S, Rao KV. Genome-wide analysis of the host intracellular network that regulates survival of *Mycobacterium tuberculosis*. *Cell*. 2010; 140:731–743. [PubMed: 20211141]
 55. Petruccioli E, Romagnoli A, Corazzari M, Coccia EM, Butera O, Delogu G, Piacentini M, Girardi E, Fimia GM, Goletti D. Specific T cells restore the autophagic flux inhibited by *Mycobacterium tuberculosis* in human primary macrophages. *The Journal of infectious diseases*. 2012; 205:1425–1435. [PubMed: 22457295]
 56. Juarez E, Carranza C, Hernandez-Sanchez F, Leon-Contreras JC, Hernandez-Pando R, Escobedo D, Torres M, Sada E. NOD2 enhances the innate response of alveolar macrophages to *Mycobacterium tuberculosis* in humans. *European journal of immunology*. 2012; 42:880–889. [PubMed: 22531915]
 57. Zullo AJ, Lee S. *Mycobacterial* induction of autophagy varies by species and occurs independently of mammalian target of rapamycin inhibition. *The Journal of biological chemistry*. 2012; 287:12668–12678. [PubMed: 22275355]
 - 58**. Watson RO, Manzanillo PS, Cox JS. Extracellular *M. tuberculosis* DNA Targets Bacteria for Autophagy by Activating the Host DNA-Sensing Pathway. *Cell*. 2012; 150:803–815. [PubMed: 22901810] ESX-1-mediated permeabilization resulted in recruitment of autophagy machinery to *M. tuberculosis*-containing phagosomes in a p62- and NDP52-dependent manner in murine macrophages. Mice lacking autophagy in macrophages were much more susceptible to infection.
 - 59*. Kim JJ, Lee HM, Shin DM, Kim W, Yuk JM, Jin HS, Lee SH, Cha GH, Kim JM, Lee ZW, et al. Host cell autophagy activated by antibiotics is required for their effective antimycobacterial drug

- action. *Cell host & microbe*. 2012; 11:457–468. [PubMed: 22607799] This study showed that two anti-*M. tuberculosis* antibiotics induced autophagy and required autophagy for efficacy.
60. 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]
 61. Huang J, Canadien V, Lam GY, Steinberg BE, Dinauer MC, Magalhaes MA, Glogauer M, Grinstein S, Brumell JH. Activation of antibacterial autophagy by NADPH oxidases. *Proc Natl Acad Sci U S A*. 2009
 62. Deretic V. Autophagy: an emerging immunological paradigm. *Journal of immunology*. 2012; 189:15–20.
 - 63**. Castillo EF, Dekonenko A, Arko-Mensah J, Mandell MA, Dupont N, Jiang S, Delgado-Vargas M, Timmins GS, Bhattacharya D, Yang H, et al. Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:E3168–3176. [PubMed: 23093667] Mice lacking autophagy in myeloid cells were found to be more susceptible to *M. tuberculosis* infection. This susceptibility correlated with increased IL-1 alpha and IL-17 production.
 64. 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]
 65. Lam KK, Zheng X, Forestieri R, Balgi AD, Nodwell M, Vollett S, Anderson HJ, Andersen RJ, Av-Gay Y, Roberge M. Nitazoxanide Stimulates Autophagy and Inhibits mTORC1 Signaling and Intracellular Proliferation of Mycobacterium tuberculosis. *PLoS pathogens*. 2012; 8:e1002691. [PubMed: 22589723]
 66. Sundaramurthy V, Barsacchi R, Samusik N, Marsico G, Gilleron J, Kalaidzidis I, Meyenhofer F, Bickle M, Kalaidzidis Y, Zerial M. Integration of chemical and RNAi multiparametric profiles identifies triggers of intracellular mycobacterial killing. *Cell host & microbe*. 2013; 13:129–142. [PubMed: 23414754]
 67. Shui W, Petzold CJ, Redding A, Liu J, Pitcher A, Sheu L, Hsieh TY, Keasling JD, Bertozzi CR. Organelle membrane proteomics reveals differential influence of mycobacterial lipoglycans on macrophage phagosome maturation and autophagosome accumulation. *J Proteome Res*. 2011; 10:339–348. [PubMed: 21105745]
 68. Kim KH, An DR, Song J, Yoon JY, Kim HS, Yoon HJ, Im HN, Kim J, Kim do J, Lee SJ, et al. Mycobacterium tuberculosis Eis protein initiates suppression of host immune responses by acetylation of DUSP16/MKP-7. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:7729–7734. [PubMed: 22547814]
 69. Shin DM, Jeon BY, Lee HM, Jin HS, Yuk JM, Song CH, Lee SH, Lee ZW, Cho SN, Kim JM, et al. Mycobacterium tuberculosis eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. *PLoS pathogens*. 2010; 6:e1001230. [PubMed: 21187903]
 70. Ganaie AA, Lella RK, Solanki R, Sharma C. Thermostable hexameric form of Eis (Rv2416c) protein of M. tuberculosis plays an important role for enhanced intracellular survival within macrophages. *PloS one*. 2011; 6:e27590. [PubMed: 22110681]
 71. Romagnoli A, Etna MP, Giacomini E, Pardini M, Remoli ME, Corazzari M, Falasca L, Goletti D, Gafa V, Simeone R, et al. ESX-1 dependent impairment of autophagic flux by Mycobacterium tuberculosis in human dendritic cells. *Autophagy*. 2012; 8:1357–1370. [PubMed: 22885411]
 72. Zhang L, Zhang H, Zhao Y, Mao F, Wu J, Bai B, Xu Z, Jiang Y, Shi C. Effects of Mycobacterium tuberculosis ESAT-6/CFP-10 fusion protein on the autophagy function of mouse macrophages. *DNA and cell biology*. 2012; 31:171–179. [PubMed: 21740189]
 73. Russell DG, Cardona PJ, Kim MJ, Allain S, Altare F. Foamy macrophages and the progression of the human tuberculosis granuloma. *Nat Immunol*. 2009; 10:943–948. [PubMed: 19692995]
 74. 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; 12:669–681. [PubMed: 23159056]

75. Fujimoto T, Parton RG. Not just fat: the structure and function of the lipid droplet. *Cold Spring Harbor perspectives in biology*. 2011;3.
76. Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ. Autophagy regulates lipid metabolism. *Nature*. 2009; 458:1131–1135. [PubMed: 19339967]
77. Velikkakath AK, Nishimura T, Oita E, Ishihara N, Mizushima N. Mammalian Atg2 proteins are essential for autophagosome formation and important for regulation of size and distribution of lipid droplets. *Molecular biology of the cell*. 2012; 23:896–909. [PubMed: 22219374]
78. Koga H, Kaushik S, Cuervo AM. Inhibitory effect of intracellular lipid load on macroautophagy. *Autophagy*. 2010; 6:825–827. [PubMed: 20647740]
- 79*. Manzanillo PS, Shiloh MU, Portnoy DA, Cox JS. Mycobacterium Tuberculosis Activates the DNA-Dependent Cytosolic Surveillance Pathway within Macrophages. *Cell host & microbe*. 2012; 11:469–480. [PubMed: 22607800] This study demonstrated that mycobacterial DNA released from virulent ESX-1-competent *M. tuberculosis* induces type I interferon responses that are pathogenic in mice during infection.
80. Manca C, Tsenova L, Freeman S, Barczak AK, Tovey M, Murray PJ, Barry C, Kaplan G. Hypervirulent *M. tuberculosis* W/Beijing strains upregulate type I IFNs and increase expression of negative regulators of the Jak-Stat pathway. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research*. 2005; 25:694–701. [PubMed: 16318583]
81. Fremond CM, Yermeev V, Nicolle DM, Jacobs M, Quesniaux VF, Ryffel B. Fatal Mycobacterium tuberculosis infection despite adaptive immune response in the absence of MyD88. *The Journal of clinical investigation*. 2004; 114:1790–1799. [PubMed: 15599404]
82. Scanga CA, Bafica A, Feng CG, Cheever AW, Hieny S, Sher A. MyD88-deficient mice display a profound loss in resistance to Mycobacterium tuberculosis associated with partially impaired Th1 cytokine and nitric oxide synthase 2 expression. *Infection and immunity*. 2004; 72:2400–2404. [PubMed: 15039368]
83. Holscher C, Reiling N, Schaible UE, Holscher A, Bathmann C, Korbel D, Lenz I, Sonntag T, Kroger S, Akira S, et al. Containment of aerogenic Mycobacterium tuberculosis infection in mice does not require MyD88 adaptor function for TLR2, -4 and -9. *Eur J Immunol*. 2008; 38:680–694. [PubMed: 18266299]
84. Fremond CM, Togbe D, Doz E, Rose S, Vasseur V, Maillet I, Jacobs M, Ryffel B, Quesniaux VF. IL-1 receptormediated signal is an essential component of MyD88-dependent innate response to Mycobacterium tuberculosis infection. *J Immunol*. 2007; 179:1178–1189. [PubMed: 17617611]
85. Xiong Y, Song C, Snyder GA, Sundberg EJ, Medvedev AE. R753Q polymorphism inhibits Toll-like receptor (TLR) 2 tyrosine phosphorylation, dimerization with TLR6, and recruitment of myeloid differentiation primary response protein 88. *J Biol Chem*. 2012; 287:38327–38337. [PubMed: 22992740]
86. Mayer-Barber KD, Barber DL, Shenderov K, White SD, Wilson MS, Cheever A, Kugler D, Hieny S, Caspar P, Nunez G, et al. Caspase-1 independent IL-1beta production is critical for host resistance to mycobacterium tuberculosis and does not require TLR signaling in vivo. *J Immunol*. 2010; 184:3326–3330. [PubMed: 20200276]
- 87*. Mayer-Barber KD, Andrade BB, Barber DL, Hieny S, Feng CG, Caspar P, Oland S, Gordon S, Sher A. Innate and Adaptive Interferons Suppress IL-1alpha and IL-1beta Production by Distinct Pulmonary Myeloid Subsets during Mycobacterium tuberculosis Infection. *Immunity*. 2011; 35:1023–1034. [PubMed: 22195750] Production of IL-1 in inflammatory macrophage and dendritic cell populations were shown to be regulated by type I IFN production during *M. tuberculosis* infection in mice. IL-1 production was required for protection in vivo.
88. Guler R, Parihar SP, Spohn G, Johansen P, Brombacher F, Bachmann MF. Blocking IL-1alpha but not IL-1beta increases susceptibility to chronic Mycobacterium tuberculosis infection in mice. *Vaccine*. 2011; 29:1339–1346. [PubMed: 21093494]
89. Sun H, Wang Y, Ma X, Pei F, Sun H, Zhang Y, Yu B. A method of oligochip for single nucleotide polymorphism genotyping in the promoter region of the interleukin-1 beta gene and its clinical application. *Oligonucleotides*. 2007; 17:336–344. [PubMed: 17854273]
- 90*. Pilli M, Arko-Mensah J, Ponpuak M, Roberts E, Master S, Mandell MA, Dupont N, Ornatowski W, Jiang S, Bradfute SB, et al. TBK-1 Promotes Autophagy-Mediated Antimicrobial Defense by

Controlling Autophagosome Maturation. *Immunity*. 2012; 37:223–234. [PubMed: 22921120]
This study showed that IL-1 β induces autophagy. It also demonstrated that TBK-1 controls autophagosomal maturation.

91. Mishra BB, Rathinam VA, Martens GW, Martinot AJ, Kornfeld H, Fitzgerald KA, Sasseti CM. Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1 β . *Nat Immunol*. 2013; 14:52–60. [PubMed: 23160153]
92. Master SS, Rampini SK, Davis AS, Keller C, Ehlers S, Springer B, Timmins GS, Sander P, Deretic V. *Mycobacterium tuberculosis* prevents inflammasome activation. *Cell Host Microbe*. 2008; 3:224–232. [PubMed: 18407066]
93. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature*. 2011; 469:221–225. [PubMed: 21124315]
94. Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, Kim HP, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nature immunology*. 2011; 12:222–230. [PubMed: 21151103]
95. Berry MP, Graham CM, McNab FW, Xu Z, Bloch SA, Oni T, Wilkinson KA, Banchereau R, Skinner J, Wilkinson RJ, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature*. 2010; 466:973–977. [PubMed: 20725040]
96. Ottenhoff TH, Dass RH, Yang N, Zhang MM, Wong HE, Sahiratmadja E, Khor CC, Alisjahbana B, van Crevel R, Marzuki S, et al. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. *PloS one*. 2012; 7:e45839. [PubMed: 23029268]
97. Antonelli LR, Gigliotti Rothfuchs A, Goncalves R, Roffe E, Cheever AW, Bafica A, Salazar AM, Feng CG, Sher A. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. *The Journal of clinical investigation*. 2010; 120:1674–1682. [PubMed: 20389020]
98. Novikov A, Cardone M, Thompson R, Shenderov K, Kirschman KD, Mayer-Barber KD, Myers TG, Rabin RL, Trinchieri G, Sher A, et al. *Mycobacterium tuberculosis* triggers host type I IFN signaling to regulate IL-1 β production in human macrophages. *Journal of immunology*. 2011; 187:2540–2547.
99. Stanley SA, Johndrow JE, Manzanillo P, Cox JS. The Type I IFN response to infection with *Mycobacterium tuberculosis* requires ESX-1-mediated secretion and contributes to pathogenesis. *Journal of immunology*. 2007; 178:3143–3152.
100. Pandey AK, Yang Y, Jiang Z, Fortune SM, Coulombe F, Behr MA, Fitzgerald KA, Sasseti CM, Kelliher MA. NOD2, RIP2 and IRF5 play a critical role in the type I interferon response to *Mycobacterium tuberculosis*. *PLoS pathogens*. 2009; 5:e1000500. [PubMed: 19578435]
101. Ludigs K, Parfenov V, Du Pasquier RA, Guarda G. Type I IFN-mediated regulation of IL-1 production in inflammatory disorders. *Cellular and molecular life sciences: CMLS*. 2012; 69:3395–3418. [PubMed: 22527721]
102. Wu J, Sun L, Chen X, Du F, Shi H, Chen C, Chen ZJ. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science*. 2013; 339:826–830. [PubMed: 23258412]
103. 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; 339:786–791. [PubMed: 23258413]

Highlights

- Autophagy is involved in control of *M. tuberculosis*
- Autophagy can directly kill *M. tuberculosis* by a variety of mechanisms
- Autophagy can also modulate systemic immune responses in vivo during *M. tuberculosis* infection
- There are genetic links between autophagy and *M. tuberculosis* susceptibility in humans

Figure 1.

Autophagy in defense against intracellular bacteria. Induction of autophagy can lead to 1) xenophagy, whereby bacteria are engulfed by autophagosomes and degraded after fusion with lysosomes (Lys) to form autolysosomes (ALys); 2) LC-3-associated phagocytosis (LAP) and subsequent fusion with lysosomes; 3) formation of antimicrobial peptides that can kill microbes. Antimicrobial peptides can be generated by degradation of ubiquitinated proteins in the lysosome. Abbreviations: mTOR, molecular target of rapamycin; AMPK, 5' AMP-activated protein kinase; Ulk 1, unc-51 (*C. elegans*)-like kinase; adaptor, molecules recognizing tags such as ubiquitin on autophagic targets while also binding to LC3; Mtb, *Mycobacterium tuberculosis*; LC3-II, C-terminally lipidated form of microtubule-associated protein 1 light chain 3.

Figure 2.

Immune-mediated induction of autophagy in *M. tuberculosis* infection. Induction of autophagy by a variety of immunological factors (including T cell/macrophage contact, IFN-γ/Vitamin D, IL-1β, and ATP/P2X7) can lead to induction or maturation of autophagy in *M. tuberculosis* infection, resulting in direct mycobacterial killing. Certain anti-myobacterial antibiotics (such as isoniazid (INH) and pyrazinamide (PZA) work through autophagy, improving efficacy.

Abbreviations: DAPK1, death-associated protein kinase 1; PAMPs, pathogen-associated molecular patterns; TBK1, TANK-binding kinase 1; ROS, reactive oxygen species; P2X7, P2X purinoceptor 7; 1,25D3, 1,25-dihydroxycholecalciferol; cathelicidin (present in lysosomes, delivered in the lumen through a conventional pathway during its biogenesis), cryptide (generated by autophagic digestion from cytosolic proteins).

Figure 3.

Autophagy and systemic immune responses to *M. tuberculosis*. Autophagy has a complex role in modulating systemic in vivo immune responses to *M. tuberculosis* infection. IL-1 α (and possibly Th17) and type I IFN are regulated by (and themselves regulate) autophagy and are involved with protection against *M. tuberculosis* infection. Abbreviations: eDNA (extracellular bacterial DNA). Green boxes signify protective responses; red boxes signify non-protective responses.