Autophagy in Tuberculosis

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Autophagy as an immune mechanism controls inflammation and acts as a cell-autonomous defense against intracellular microbes including *Mycobacterium tuberculosis*. An equally significant role of autophagy is its anti-inflammatory and tissue-sparing function. This combination of antimicrobial and anti-inflammatory actions prevents active disease in animal models. In human populations, genetic links between autophagy, inflammatory bowel disease, and susceptibility to tuberculosis provide further support to these combined roles of autophagy. The autophagic control of *M. tuberculosis* and prevention of progressive disease provide novel insights into physiological and immune control of tuberculosis. It also offers host-based therapeutic opportunities because autophagy can be pharmacologically modulated.

Autophagy in a broader sense is a collection of homeostatic processes (macroautophagy, chaperone mediate autophagy, microautophagy) in the eukaryotic cell that deliver cytoplasmic portions or specific cytosolic targets to lysosomes for degradation or removal (Mizushima et al. 2011). Macroautophagy, commonly referred to as autophagy (and in this text), is a pathway defined in genetic terms as dependent on autophagy-related (Atg) genes (Mizushima et al. 2011) and in morphological terms as the appearance in the cytoplasm of double-membrane organelles termed autophagosomes that capture cytosolic cargo and fuse with lysosomes (Deter and De Duve 1967; Deter et al. 1967). Autophagy affects human health and diseases including aging, neurodegeneration, cancer, and metabolic disorders (Mizushima et al. 2008). The recognition that autophagy plays an antimicrobial role against pathogens (Deretic 2005) when they invade the mammalian cell interior came from two nearly simultaneous reports in 2004 (Gutierrez et al. 2004; Nakagawa et al. 2004). One of these studies reported on the autophagic elimination of virulent *Mycobacterium tuberculosis* and the vaccine strain *Mycobacterium bovis* BCG (Bacillus Calmette–Guérin) in infected murine and human macrophages (Gutierrez et al. 2004). These and additional studies have established that autophagy is a bona fide immunological process (Deretic 2005; Levine et al. 2011). Today we recognize that autophagy is widely integrated with immunity starting from cell-autonomous defense against invading bacteria and viruses to regulation of innate and adaptive immunity in general (Deretic et al. 2013). The initial observations uncovering the role of autophagy against *M. tuberculosis* (Gutierrez et al. 2004; Singh et al. 2006) have been followed by a gradual increase in

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studies supporting and extending these early findings (Alonso et al. 2007; Harris et al. 2007; Xu et al. 2007; Biswas et al. 2008; Jagannath et al. 2009; Yuk et al. 2009; Ghadimi et al. 2010; Kumar et al. 2010; Ponpuak et al. 2010; Shin et al. 2010b; Singh et al. 2010; Fabri et al. 2011a,b; Campbell and Spector 2012; Juarez et al. 2012; Petruccioli et al. 2012; Watson et al. 2012; Zullo and Lee 2012; Anandaiah et al. 2013; Klug-Micu et al. 2013; Manzanillo et al. 2013). In this article we briefly cover autophagy as a pathway and its broad roles in immunity and summarize what has been learned about autophagy in tuberculosis thus far.

AUTOPHAGY PATHWAY

Autophagy is a cytoplasmic quality and quantity control pathway ubiquitous in eukaryotes. The core autophagy pathway is depicted in its minimalistic rendition in Figure 1. The membranes forming autophagic organelles come primarily from the endoplasmic reticulum, but additional compartments provide sources of membrane or lipids for the growing autophagosomal isolation membrane (phagophore) (Fig. 1). Autophagosome formation is dependent on a suite of Atg factors, which are consecutively numbered: for example, Atg1–Atg35 in yeast, with alternative names in mammals such as the Atg1 paralogs in mammals ULK1 and 2 (ULK1 being more studied) and the Atg6 ortholog Beclin 1. Autophagy is often equated with the emergence or modifications of endomembranes decorated with "LC3" (LC3B), which is one of six mammalian Atg8s (LC3A, LC3B, LC3C, GABARAP, GABARAPL1, and GABRAPL2) (Mizushima et al. 2011), with a key event being the carboxy-terminal lipidation of LC3 to generate LC3-II (LC3-phosphatidylethanolamine). Autophagosomes capture cytosolic or cytoplasmic cargo destined for elimination, most commonly through lysosomal degradation (Deter and De Duve 1967; Deter et al. 1967; Itakura et al. 2012), although other modes of cargo disposal have been observed (Jiang et al. 2013). Autophagy can be modulated by metabolic, physiological, immunological (cytokines and innate immunity signaling),

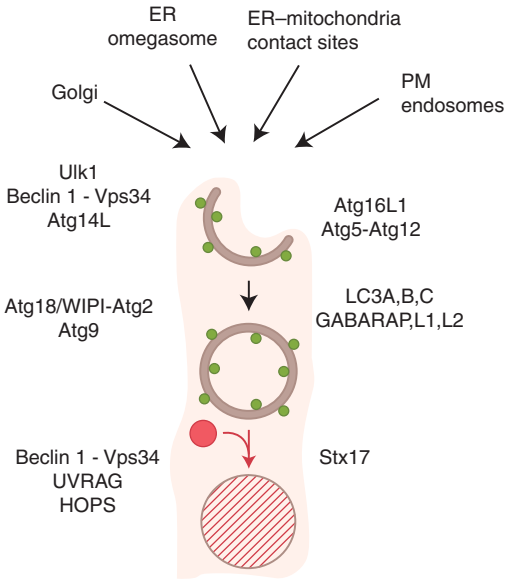

Figure 1. Autophagy pathway. Shown are a simplified macroautophagy pathway, protein factors, and membrane sources for the formation of autophagosomes in mammalian cells. (*Top*) Different sources of membranes contributing to the formation of autophagosomes. ER, endoplasmic reticulum; PM, plasma membrane. Within the cartoon: Crescent, autophagic phagophore or isolation membrane; double-membrane, closed autophagosome; red circle, lysosome; hatched circle delimited by a single membrane, autolysosome; green dots, LC3B (one of six mammalian Atg8s listed on the *right* side; typically used as a marker for autophagosomes). Other key factors are shown using their mammalian nomenclature.

and pharmacological (e.g., rapamycin) agonists and antagonists. There are two forms of autophagy—nonselective (bulk or generalized) autophagic degradation of the cytoplasm, usually as a response to starvation, and selective autophagy, whereby specific targets in the cytosol are recognized by autophagic receptors and captured by autophagosomes (Birgisdottir et al. 2013).

INDUCTION OF BULK AUTOPHAGY BY NUTRITIONAL SIGNALS

The classical signal for generalized autophagy is starvation, which can be caused by low levels of amino acids, low energy, or absence of growth

factors. Starvation can induce autophagy to kill virulent *M. tuberculosis* in macrophages (Fig. 2A) (Gutierrez et al. 2004). During starvation, portions of the cytosol are corralled into autophagosomes to generate amino acids (Lum et al. 2005) and energy sources, for example, via lipolysis (Settembre et al. 2013). These upstream metabolic events leading to autophagy activation are controlled via mTOR (mammalian target of rapamycin) and AMPK (AMP-activated Ser/Thr protein kinase). The mTOR Ser/Thr protein kinase negatively controls the most upstream of the Atg factors, ULK1, by phosphorylating it at inactivating sites (e.g., Ser 757), whereas AMPK positively regulates ULK1 by phosphorylating ULK1 at activating sites (e.g.,

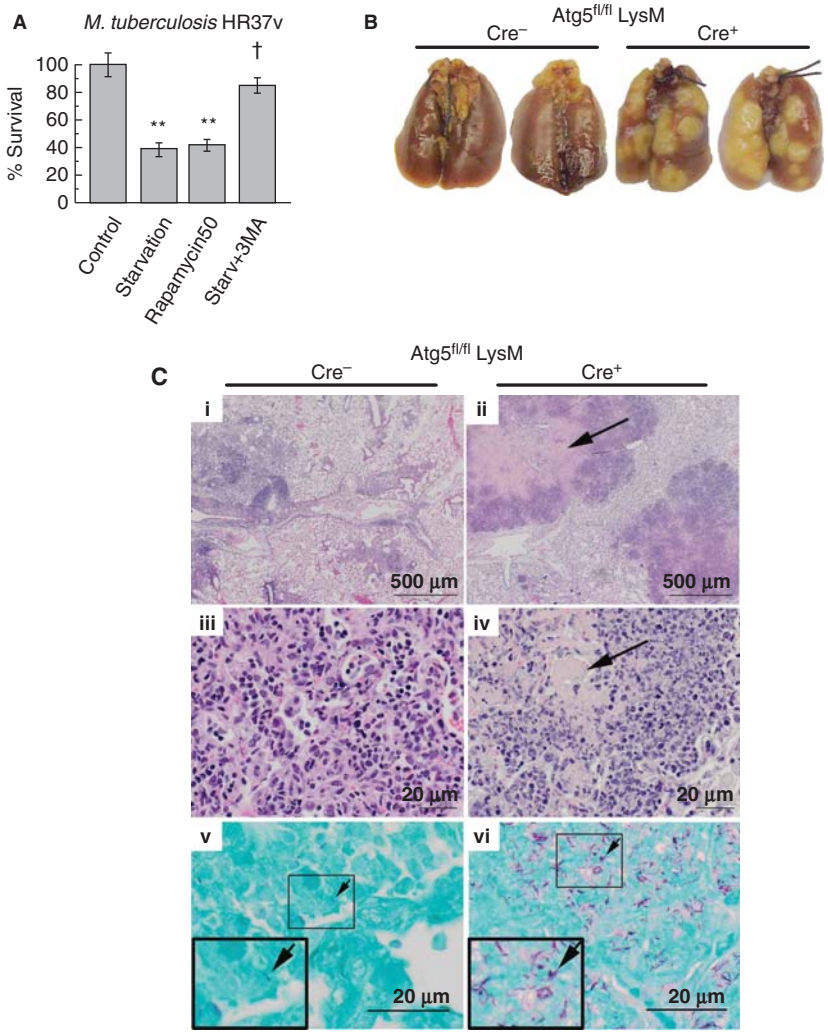

Figure 2. Autophagy protects against *M. tuberculosis* infection and pathogenesis. (*A*) Autophagy induction by starvation or rapamycin kills virulent *M. tuberculosis* in macrophages. (*B*) Increased gross lung pathology in mice defective for autophagy in the myeloid lineage (Cre^+) relative to autophagy-competent (Cre^-) mice infected with *M. tuberculosis* H37Rv. (*C*) Increased lung tissue necrosis and bacillary load in the lungs of mice defective for autophagy in the myeloid lineage (Cre^+) relative to their autophagy-competent (Cre^-) littermates. (Bottom panels) Acid-fast stain. Arrows, *M. tuberculosis* H37Rv bacilli. (*A*, Reprinted, with permission, from Gutierrez et al. 2004; *B*,*C*, reprinted, with permission, from Castillo et al. 2012.)

Ser 317 and Ser 777) (Egan et al. 2011; Kim et al. 2011). ULK1 phosphorylates a number of key substrates, most pertinently Beclin 1 (mammalian Atg6) at Ser 15 (Russell et al. 2013). Beclin 1 works with other subunits of class III phosphatidylinositol 3 kinase hVPS34 to generate phosphatidylinositol 3-phosphate (PI3P) on donor (e.g., ER [Axe et al. 2008]) membranes. PI3P marks the spot for initiation of autophagic phagophore formation. A phagophore expands into a full autophagosome. This is concomitant with or preceding the acquisition of SNARE Syntaxin 17 (Itakura et al. 2012; Hamasaki et al. 2013), which promotes fusion with lysosomes whereby a terminal degradative organelle, autolysosome, is formed where the captured cargo is degraded.

SELECTIVE AUTOPHAGY

Selective autophagy is driven by the recognition of cargos for selective autophagy via autophagic receptors (Fig. 3). The spectrum of selective autophagy targets includes defunct organelles such as depolarized mitochondria, protein aggregates, and intracellular microbes in contact with the cytosol. A subset of autophagic receptors involved in antimicrobial defense is termed

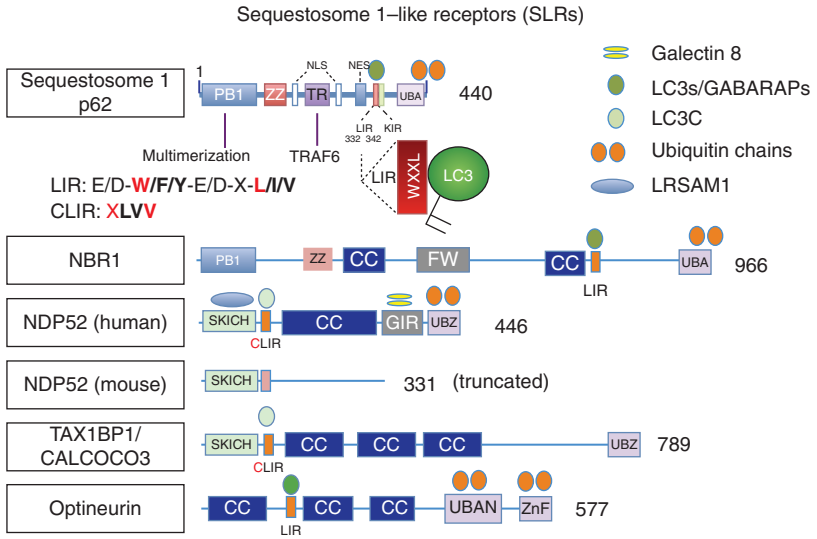

Figure 3. Sequestosome 1/p62–like receptors (SLRs), a new class of pattern-recognition receptors involved in autophagic elimination of intracellular microbes. LIR, LC3-interacting region (consensus sequence shown and key positions in red); aromatic pocket-filling W (or F/Y) and aliphatic pocket-filling L (or I/V) form an intermolecular parallel β sheet with LC3s or GABARAPs, at the interface between the amino-terminal α-helical domain and ubiquitin-like fold of LC3s/GABARAPs. CLIR, a LIR specific for LC3C, whereby aromatic residue is not present (X) to fill in the aromatic pocket and instead the interactions are stabilized by compensatory hydrophobic contacts provided by additional aliphatic residues located between the W and L position anchors. KIR, KAEP1-interacting region; NES, nuclear export signal; NLS, nuclear localization signal; PB1, protein-binding domain (homopolymerization of p62 hetero-oligomerization between p62 and NBR1, or interactions with other partners); TR, TRAF6-interacting region (also a multipartner binding region); ZZ, ZZ-type zinc finger (ZnF) domain; FW, four-Trp domain, also known as the NBR1 box; UBAN, a parallel coiled-coiled dimer UBD with specificity for linear ubiquitin chains; CC, coiled coil; UBA, a three-helix bundle UBD (ubiquitin-binding domain) with affinity for monoubiquitin and the more open conformation of K63 ubiquitin linkages; UBZ, a Zn finger ββα fold UBD binding mono- and polyubiquitin; SKICH, skeletal muscle and kidney enriched inositol phosphatase carboxyl homology domain; GIR, galectin-interacting region. Sequestosome 1/p62 has been shown to affect *M. tuberculosis* clearance (Ponpuak et al. 2010). NDP52 has been implicated in elimination of *M. tuberculosis* in murine macrophages (Watson et al. 2012); note, however, that NDP52 is severely truncated in this species. (Image modified from Deretic et al. 2013.)

SLRs, for Sequestosome 1/p62-like receptors (Fig. 3). The cargo, such as a cytosolic bacterium or a depolarized mitochondrion (bacteria and mitochondria share recognition machinery [Deretic 2010]), is often tagged by ubiquitin (Bjorkoy et al. 2005; Kirkin et al. 2009), galectins (Thurston et al. 2012; Li et al. 2013), and possibly other modifications (Deretic et al. 2013). Ubiquitin is placed on or around bacteria via specific E3 ligases such as LRSAM1, which possesses a leucine-rich repeat characteristic of pattern recognition receptors and a RING domain conferring its E3 ubiquitin ligase activity (Huett et al. 2012), or another E3 ligase, Parkin (Manzanillo et al. 2013). Parkin is well known for ubiquitination of depolarized or damaged mitochondria en route for autophagic elimination via mitophagy (Youle and Narendra 2011). Ubiquitin and other earmarks are recognized by autophagic receptors that bind the tags at one end and interact at the other end with mammalian Atg8s via an LC3-interacting region (LIR) (Birgisdottir et al. 2013). Whether and how cargo capture triggers signaling and execution of membrane growth during selective autophagy are not well understood, but many of the factors participating in generalized/nonselective autophagy are engaged.

LC3-ASSOCIATED PHAGOCYTOSIS

An example of a known exception to using all parts of the autophagy pathway is a process called LC3-associated autophagy (LAP), which is a merger between conventional phagocytosis and autophagy (Cemma and Brumell, 2012). LAP appears to be a modification of the conventional phagocytic pathway distinguished by the recruitment of LC3, which enhances phagosomal fusogenicity with lysosomes (Fig. 4). LAP does not require ULK1 but needs Beclin 1-hVPS34 and LC3-conjugation systems (Henault et al. 2012). LAP occurs following uptake of various extracellular targets: bacteria or particles coated with Toll-like receptor (TLR) agonists (Sanjuan et al. 2007; Xu et al. 2007; Lee et al. 2010), phagocytosed dead cells (Martinez et al. 2011), live epithelial cells engulfed via entotisis by neighboring cells (Florey et al. 2011),

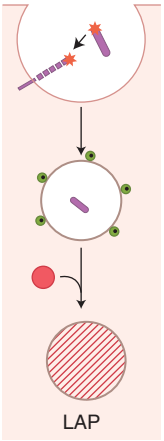

Figure 4. LC3-associated phagocytosis (LAP) as an intersection between autophagy and phagocytosis. Autophagy is frequently morphologically described as formation of double-membrane autophagosomes in the cytoplasm. This is requisite when an internal phagosome is derived from intracellular membranes such as the endoplasmic reticulum (ER). One important exception in the context of the role of autophagy during infection is the formation of conventional phagosomes that are also decorated with LC3 (green dots). Note that LC3 is only on the cytofacial side of the phagosomal membrane, and that this is a single membrane as in the case of conventional phagosomes. Depicted also is a Toll-like receptor molecule recognizing pathogen-associated molecular patterns (asterisks), involved in the induction of LAP and capable of concomitantly inducing conventional autophagy in the cell. The presence of LC3 on these phagosomes promotes maturation of the standard phagosome into autolysosomes. LAP depends on Beclin 1-hVPS34, LC3-conjugation systems, and other parts of autophagy pathway but is independent of ULK1, which is needed to generate double-membrane autophagosomes during starvation from internal ER membranes. The role of LC3 may be a manifestation of the tethering and fusogenic properties of LC3; furthermore, concomitantly generated autolysosomes are enriched in their bactericidal properties, whereas other immunologically active compartments such as those involved in antigen presentation and TLR signaling (e.g., TLR9) can enhance or exacerbate a variety of immune responses. (Image modified from Deretic et al. 2013.)

rod outer segments phagocytosed by retinal pigment epithelial cells (Kim et al. 2013), and FcγR-dependent uptake of immune complexes (Henault et al. 2012). *M. tuberculosis* and

M. bovis BCG bacilli that reside inside the phagosome can be subjected to LAP on induction of autophagy by physiological, immunological, or pharmacological means (Gutierrez et al. 2004; Harris et al. 2007; Xu et al. 2007; Jagannath et al. 2009; Pilli et al. 2012). LAP enhances conventional phagosomes by conferring improved lytic properties, enhanced bactericidal functions (Gutierrez et al. 2004; Harris et al. 2007; Sanjuan et al. 2007; Cemma and Brumell 2012; Pilli et al. 2012), better antigen presentation (Jagannath et al. 2009; Lee et al. 2010), and enhanced intracellular trafficking of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) complexed with pattern-recognition receptors (PRRs) (Chaturvedi et al. 2008; Lee et al. 2010; Henault et al. 2012). The recapitulation of phagosomal/autophagosomal maturation steps in LAP is linked to PI3P generation, long known to be key for phagosomal maturation (Vergne et al. 2004), whereas the role of LC3 may be a manifestation of the tethering and fusogenic properties of LC3 (Nakatogawa et al. 2007; Weidberg et al. 2011).

IMMUNE SIGNALING AND AUTOPHAGY AS AN ANTI-*M. tuberculosis* EFFECTOR

In addition to being controlled globally by starvation, autophagy responds to innate immunity signals and cytokine stimulation during immune responses (Deretic et al. 2013). Autophagy can be induced via PRRs. TLR ligands and TLRs activate autophagy (Xu et al. 2007; Delgado et al. 2008) via TRAF6 (Shi and Kehrl 2010), which activates or stabilizes Beclin 1 (Shi and Kehrl 2010) and ULK1 (Nazio et al. 2013). NOD2 can activate autophagy via RIPK2 and ULK1 (Lupfer et al. 2013). Second messenger 2′-5′ cyclic GMP-AMP (cGAMP) generated by mammalian cGAMP synthase in response to the presence of cyctosolic DNA (viral, mitochondrial, or bacterial, including mycobacterial) or secreted bacterial 3′-5′ cyclic-di-GMP or cyclic-di-AMP can stimulate autophagy and ULK1 (Watson et al. 2012; Konno et al. 2013). IL-1β stimulates autophagy (Pilli et al. 2012), which is of high relevance for *M. tuberculosis* control. MyD88 signaling is key to early protection against *M. tuberculosis,* although TLRs, which act via MyD88, appear not to be essential for protection (Fremond et al. 2004, 2007; Scanga et al. 2004; Holscher et al. 2008). In contrast, IL-1 receptor signaling, which also relies on MyD88 as an adapter, is key to early control of *M. tuberculosis* (Mayer-Barber et al. 2010, 2011; Guler et al. 2011). IL-1β eliminates *M. tuberculosis* through autophagy (Pilli et al. 2012). Th1 cytokines activate autophagy to kill intracellular *M. tuberculosis* (Harris et al. 2007). Stimulation of autophagy mediated through P2X purinergic receptors can eliminate intracellular *M. tuberculosis* (Biswas et al. 2008). Activation of anti-*M. tuberculosis* autophagy has also been reported via direct cell–cell contacts between specific T cells and *M. tuberculosis*–infected macrophages (Petruccioli et al. 2012).

MECHANISMS OF INTRACELLULAR KILLING OF *M. tuberculosis* BY AUTOPHAGY

Autophagy eliminates intracellular *M. tuberculosis* through lytic and antimicrobial properties unique to autolysosomes, which are much more robust antimicrobial compartments than conventional phagosomes (Ponpuak et al. 2010). A step-by-step analysis of autophagy factors, starting with an SLR, Sequestosome 1/p62, has established that the entire pathway is important for anti–*M. tuberculosis* action of autophagy (Ponpuak et al. 2010). Another SLR, NDP52, has been reported as being necessary for elimination of *M. tuberculosis* in mouse macrophages (Watson et al. 2012), but this cannot be the case, at least in murine cells, because *Mus musculus* lacks a functional NDP52 gene (Fig. 3) (Deretic et al. 2013). Induction of autophagy promotes maturation and acidification of *M. tuberculosis* phagosomes and their conversion into mycobactericidal organelles (Gutierrez et al. 2004; Harris et al. 2007; Fabri et al. 2011b) via processes akin to LAP (Cemma and Brumell 2012). Thus, induction of autophagy overcomes the classical *M. tuberculosis* virulence determinant known as inhibition of phagosome-lysosome (Hart et al. 1987; Vergne et al. 2004). Among the constituents delivered via these enhanced traffick-

ing pathways are conventional anti–*M. tuberculosis* antimicrobial peptides including cathelicidin potentially through autophagy-stimulated fusion with lysosomes where cathelicidin is stored (Yuk et al. 2009; Fabri et al. 2011b). Moreover, autophagy captures cytosolic proteins and partially digests them. These autophagosomal contents, which have enhanced antimycobacterial properties (Ponpuak et al. 2010), are delivered to the *M. tuberculosis* phagosome in cells induced for autophagy. A specific manifestation of this is that induction of autophagy generates and delivers to *M. tuberculosis* a mixture of neo-antimicrobial peptides (known as cryptides [Ponpuak and Deretic 2011]) produced through autophagic proteolysis of otherwise innocuous cytosolic proteins such as ribosomal proteins (Ponpuak et al. 2010) and ubiquitin (Alonso et al. 2007). A certain fraction of intracellular *M. tuberculosis* bacilli escape from phagosomes into the cytoplasm or are in contact with the cytosol (van der Wel et al. 2007). The bacilli that are in contact with the cytosol represent a minor proportion of the total intracellular *M. tuberculosis*, but are nevertheless subject to a cleanup through selective autophagy (Watson et al. 2012).

An unexpected link between action of the frontline antituberculosis chemotherapeutics and autophagy has been reported (Kim et al. 2012a). The key antituberculosis drugs, isoniazid and pyrazinamide, express their full potency in cooperation with a functional autophagy (Kim et al. 2012a). During treatment of *M. tuberculosis* with isoniazid or pyrazinamide, the release of bacterial products induces autophagy in infected macrophages in association with a generation of reactive oxygen species (ROS) from mitochondrial and NADPH oxidase sources (Kim et al. 2012a). This is in keeping with induction of autophagy by ROS released by mitochondria and NADPH oxidase (Scherz-Shouval et al. 2007; Huang et al. 2009). The enhanced effectiveness of these antibiotics in synergy with autophagy may explain the sterilizing properties of pyrazinamide. Furthermore, several compounds that can induce autophagy have been shown to inhibit *M. tuberculosis* (Floto et al. 2007; Lam et al. 2012; Sundaramurthy et al. 2013), raising the possibility of host-based therapies based on autophagy-inducing drugs.

M. tuberculosis POSSESSES COUNTERMEASURES AGAINST AUTOPHAGY

Mycobacterial infections show evidence of mTOR activation (mTOR inhibits autophagy) or autophagy induction, correlating with the species or strain virulence (Zullo and Lee 2012). Furthermore, metabolic reprogramming of host macrophages by *M. tuberculosis* (Russell et al. 2009; Singh et al. 2012) leads to an increase in lipid droplets, the cytoplasmic stores of neutral lipids such as triglycerides and cholesterol esters. Lipid droplets are dynamic organelles (Fujimoto and Parton 2011) that are known to intersect with the autophagic pathway (Singh et al. 2009; Velikkakath et al. 2012). The host cell reprogramming by *M. tuberculosis* reduces autophagic capacity of the cell and protects intracellular *M. tuberculosis* from autophagic elimination (Singh et al. 2012).

A number of candidate antiautophagy factors encoded by *M. tuberculosis* have been reported. An *M. tuberculosis* protein termed Eis (enhanced intracellular survival) (Shin et al. 2010a; Ganaie et al. 2011; Kim et al. 2012b) may interact with specific autophagy factors or affect upstream signaling regulators. Eis is an ε-aminoacetyltransferase that acetylates Lys-55 in a JNK-specific phosphatase (Kim et al. 2012b). JNK action is important for activation of the key autophagy regulator Beclin 1, and thus Eis may modulate autophagy (Shin et al. 2010a; Ganaie et al. 2011; Kim et al. 2012b). A mycobacterial glycolipid lipoarabinomannan has also been reported as inhibiting autophagy (Shui et al. 2011). ESX-1, a type VII secretion system of *M. tuberculosis*, releases a 6-kDa protein ESAT-6 to block *M. tuberculosis* phagosomal maturation into degradative autolysosomal organelles (Romagnoli et al. 2012; Zhang et al. 2012). Pharmacological agonists of autophagy can overcome the ESAT-6-based block (Romagnoli et al. 2012), in keeping with the observations that physiological or immunological

stimulation of autophagy (e.g., by starvation or IFN-γ) (Gutierrez et al. 2004; Harris et al. 2007) can kill *M. tuberculosis* overpowering antiautophagy mechanisms of *M. tuberculosis.*

IFN-γ, TH1 VERSUS TH2 CYTOKINES, AND VITAMIN D IN ANTI-*M. tuberculosis* AUTOPHAGY

Response of autophagy, as an antimicrobial effector, to Th1 and Th2 cytokines correlates with the general association of Th1 versus Th2 cytokines with their restriction versus permissiveness to intracellular pathogens. IFN-γ, a key Th1 cytokine, can turn on autophagy (Gutierrez et al. 2004; Harris et al. 2007). Th2 cytokines IL-4 and IL-13 inhibit IFN-γ-dependent autophagy in a dominant fashion; that is, they can trump IFN-γ effects during simultaneous exposure (Harris et al. 2007; Ghadimi et al. 2010). Th2 cytokines can also override other physiological inducers of autophagy, although the inhibitory signaling pathways differ in terms of interference with IFN-γ or, for example, starvation as inducers of autophagy (Harris et al. 2007). IL-4 and IL-13 inhibit IFN-γ-induced autophagy via a pathway dependent on STAT6, whereas they inhibit starvation-induced autophagy via Akt signaling. IL-10 can also be inhibitory to autophagy (Van Grol et al. 2010; Park et al. 2011).

There are important hormonal agonists of IFN-γ activation of autophagy, including sterol-derived vitamin D metabolite calcitriol, also known by various names as 1,25-dihydroxyvitamin D3, 1,25-dihydroxycholecalciferol, or 1,25(OH)D3. Calcitriol circulates at very low concentrations in the serum and is generated in the kidney. However, calcitriol can additionally be generated in human macrophages from its precursor calcidiol (25-hydroxy vitamin D3; present at much higher concentrations in the serum) by a specific 1-α hydroxylase induced in macrophages in response to TLR activation (Liu et al. 2006), stimulation with IFN-γ (Reichel et al. 1987), or exposure to other stimuli (Evans et al. 2006). Low levels of calcitriol and its precursor calcidiol in the serum have been associated with susceptibility to tuberculosis (Nnoaham and Clarke 2008). Of note, lower calcidiol and calcitriol levels also factor in HIV–*M. tuberculosis* coinfections (Campbell and Spector 2012). Recent studies have indicated a strong synergistic role between calcitriol with IFN-γ during induction of autophagy in human macrophages as a contributing mechanism to *M. tuberculosis* killing (Yuk et al. 2009; Shin et al. 2010b; Fabri et al. 2011a,b; Campbell and Spector 2012; Anandaiah et al. 2013; Klug-Micu et al. 2013). Calcitriol induces autophagy via Ca^{2+} and Ca^{2+}/calmodulin-dependent kinase kinase-β (CaMKKβ) (Hoyer-Hansen et al. 2007), which in turn activates AMPK (AMP-activated protein kinase) (Mihaylova and Shaw 2011). Studies outside of the tuberculosis field have shown that CaMKKβ, which responds to calcitriol via Ca^{2+}, promotes induction of autophagy in a manner sensitive to membrane-permeant Ca^{2+} chelators (Hoyer-Hansen et al. 2007) by activating a phosphorylation cascade involving ULK1 and then Beclin 1, as discussed above in the section Autophagy Pathway (Egan et al. 2011; Kim et al. 2011; Russell et al. 2013). The phosphorylation of AMPK by CaMKKβ occurs on the same critical Thr 172 residue as with other key kinases that control AMPK activation (Mihaylova and Shaw 2011). The aforementioned cathelicidin has also been reported to play a role in induction of autophagy by calcitriol (Yuk et al. 2009). However, the proposed activation of autophagy by cathelicidin has no defined mechanism in contrast to its documented effector functions of an antimicrobial peptide with enhanced delivery to intracellular *M. tuberculosis* through autophagy (Fabri et al. 2011b). Whereas in human macrophages calcitriol is required for IFN-γ-induced autophagy (Fabri et al. 2011b), it is not necessary in human macrophages when autophagy is induced by nutritional (starvation) or pharmacological (rapamycin) means (Fabri et al. 2011b). Calcidiol and calcitriol levels appear not to be a determinant of autophagy induction by IFN-γ in murine macrophages (Gutierrez et al. 2004; Singh et al. 2006; Harris et al. 2007). This is in keeping with the notion that expression of antimicrobial peptides (e.g., cathelcidin) in murine cells is not controlled

by vitamin D response elements (Fabri et al. 2011b).

GENETIC PREDISPOSITIONS TO TUBERCULOSIS AND AUTOPHAGY

In keeping with the above role of calcitriol in autophagy, human genetic polymorphisms in the gene encoding vitamin D receptor are associated with susceptibility to tuberculosis when combined with low serum levels of calcidiol, a precursor to calcitriol (Wilkinson et al. 2000). There are further genetic links between autophagy and risks for active tuberculosis. A recent genome-wide association study (GWAS) shows a widespread overlap between genetic risks for inflammatory bowel disease (IBD) and tuberculosis (Jostins et al. 2012). As a subset of these overlaps, genetic polymorphisms in autophagy genes affect both IBD and tuberculosis. For example, polymorphisms in *ATG16L1* and *IRGM*, have been initially identified through GWASs as autophagy risk loci for Crohn's disease (Wellcome Trust Case Control Consortium 2007), a common form of IBD. Subsequent human population studies have shown that *IRGM*, encoding an autophagy-modulating factor (Singh et al. 2006, 2010), is also a risk factor for tuberculosis (Intemann et al. 2009; Che et al. 2010; King et al. 2011; Bahari et al. 2012).

AUTOPHAGY PROTECTS AGAINST *M. tuberculosis* INFECTION IN VIVO

Autophagy protects in vivo against bacillary burden, inflammation, lung pathology (Fig. 2B,C), and death in transgenic mouse models of *M. tuberculosis* infection (Castillo et al. 2012; Watson et al. 2012). This has been shown with virulent *M. tuberculosis* strains, H37Rv (Castillo et al. 2012) and Erdman (Watson et al. 2012), using $Atg5^{fl/fl}$ LysM-Cre transgenic mice with autophagy defective in myeloid cells. $Atg7^{fl/fl}$ LysM-Cre mice with a similar autophagy defect in myeloid cells show increased pathology when challenged with *M. bovis* BCG, whereas higher numbers of bacilli are taken up by $Atg7^{fl/fl}$ LysM-Cre^{+} macrophages ex vivo ascribed in part to changes in phagocytic receptors (Bonilla et al. 2013). Although the details of respiratory infection and strains used varied, all studies observed higher bacillary load, exacerbated lung involvement, and increased lung pathology relative to autophagy-competent mice (Castillo et al. 2012; Watson et al. 2012; Bonilla et al. 2013). The two studies with virulent *M. tuberculosis* strains showed increased mortality of $Atg5^{fl/fl}$ LysM-Cre^{+} mice (Castillo et al. 2012; Watson et al. 2012).

AUTOPHAGY PROTECTS AGAINST EXCESSIVE INFLAMMATION AND TUBERCULOSIS PATHOGENESIS

Most prominently, IL-1 has been found elevated in the infected $Atg5^{fl/fl}$ LysM-Cre^{+} mice relative to their $Atg5^{fl/fl}$ LysM-Cre^{-} littermates (Castillo et al. 2012; Watson et al. 2012). In contrast, IFN-γ or IL-4 responses are equal in autophagy-proficient and autophagy-deficient mice (Castillo et al. 2012). Of IL-1s, IL-1α has turned out to be the dominant cytokine in the infected $Atg5^{fl/fl}$ LysM-Cre^{+} lung. IL-1β, albeit increased in autophagy-deficient mice, is present at low absolute levels in the lung challenged with disparate pathogens (Castillo et al. 2012; Lupfer et al. 2013). Although inflammasome is hyperactivated in autophagy-defective mice (Saitoh et al. 2008; Nakahira et al. 2011; Zhou et al. 2011), the lung environment may suppress excess IL-1β (Castillo et al. 2012; Lupfer et al. 2013). Nevertheless, the lung immune cells from mice with autophagic defect in myeloid cells show propensity towards extended Th17 polarization (Castillo et al. 2012). This is attributable to a cell-autonomous defect in autophagy-deficient macrophages, which secrete excess IL-1α (Castillo et al. 2012). IL-1α, just like IL-1β, promotes Th17 polarization leading to extended IL-17 response during *M. tuberculosis* infection of $Atg5^{fl/fl}$ LysM-Cre^{+} mice (Castillo et al. 2012). This scenario makes it impossible for IFN-γ, one of the functions of which is a suppression of excessive and pathology-inducing IL-1β responses by inhibiting inflammasome activation (Mishra et al. 2013), to overcome the alternative IL-1 responses via IL-1α. Unlike IL-1β, which depends on inflammasome

activation, IL-1α can be activated independently of inflammasome via calpain (Castillo et al. 2012; Gross et al. 2012).

The above anti-inflammatory action of autophagy may not stop at direct control of intracellular *M. tuberculosis* and tempering of the IL-1 response (see Fig. 5 for an overall model). We propose a model that extends to potential effects on type I IFN responses. Type I interferon (IFN) is a key biosignature of active tuberculosis (Berry et al. 2010; Maertzdorf et al. 2012; Ottenhoff et al. 2012; Teles et al. 2013). *M. tuberculosis*–mediated induction of type I IFN requires the ESX-1 system (Stanley et al. 2007; Pandey et al. 2009; Novikov et al. 2011; Manzanillo et al. 2012), and this signaling has been shown to be induced by *M. tuberculosis* extracellular DNA through IRF3 (Manzanillo et al. 2012). Mice lacking IRF3 (or lacking IFNAR) survive *M. tuberculosis* infection longer than wild-type mice (Manca et al. 2005; Manzanillo et al. 2012). Because autophagy clears *M. tuber-*

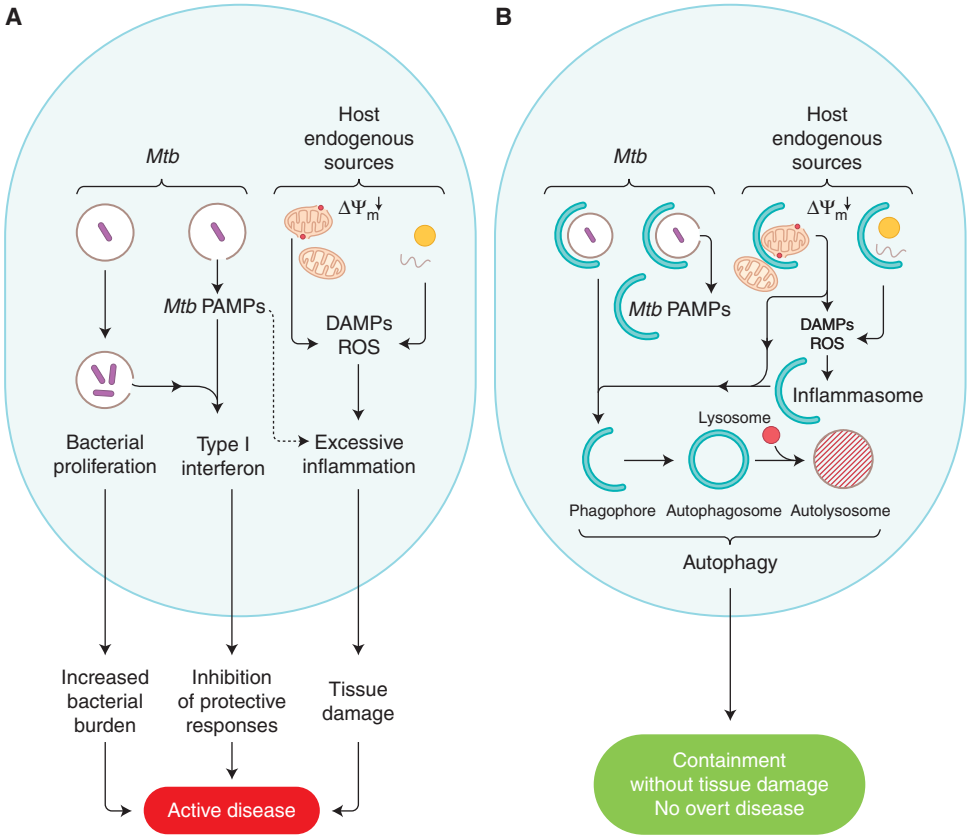

Figure 5. Proposed model of how autophagy interferes with progression to active disease during *M. tuberculosis* infection. (*A*) Proposed processes contributing to progression into active disease pertinent to the role of autophagy: uncontrolled *M. tuberculosis* growth; endogenous sources of excessive inflammation (e.g., damaged organelles such as depolarized mitochondria, which are the source of reactive oxygen species [ROS] and mitochondrial DNA released into the cytosol) acting as damage-associated molecular patterns (DAMPs) that amplify inflammatory responses to the point of causing excessive tissue damage; *M. tuberculosis* pathogen-associated molecular patterns (PAMPs; e.g., mycobacterial *N*-glycolyl muramyl dipeptide or bacterial DNA released from or associated with the bacilli) inducing type I IFN, which is a biomarker of active disease and suppresses measured host responses that inhibit *M. tuberculosis* proliferation and thus curtails their protective effectiveness. (*B*) Autophagy (represented by crescents) eliminates the above promoters of active disease and thus acts as an antibacterial and tissue-sparing process.

culosis along with its extracellular DNA (Watson et al. 2012), it is possible that removal of this PAMP (bacterial extracellular DNA) prevents activation of now well-delineated pathways of induction of type I IFN via dicyclic nucleotide second messengers and STING (Ablasser et al. 2013; Civril et al. 2013; Wu et al. 2013). Specific molecular intersections between autophagy (e.g., ULK1) and these systems (e.g., STING) have been recently uncovered (Konno et al. 2013). It is possible that autophagic removal of other type I IFN-inducing products, such as the *M. tuberculosis* peptidoglycan *N*-glycolyl MDP (Pandey et al. 2009), may also act to prevent excess type I IFN production.

CONCLUSIONS AND OUTLOOK

Autophagy is a cell-autonomous innate immunity defense that enables host cells to capture intracellular pathogens and kill them in lysosomal organelles that during autophagy acquire enhanced bactericidal properties. Equally or perhaps more importantly, autophagy can suppress excessive inflammatory responses that contribute to progression to active disease. This occurs via autophagic elimination of endogenous cellular agonists of inflammasome or calpain activation, thus preventing excess IL-1 response, and may also remove mycobacterial products eliciting other pathogenesis-associated responses such as type I IFN linked to active disease. When balanced, both IL-1 and type I IFN may have their role in protection but without autophagy to remove the contributing agonists, these important cytokine responses may be out of alignment and cause pathology and active disease. Consequently, pharmacological activation of autophagy may offer therapeutic host-targeted options for better control of *M. tuberculosis* infection.

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