Autophagy in the fight against tuberculosis

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Abstract

Tuberculosis (TB) is a chronic infectious disease mainly caused by the tubercle bacillus *Mycobacterium tuberculosis* and one of the world's deadliest diseases that has afflicted humanity since ancient times. Although the number of people falling ill with TB each year is declining, its incidence in many developing countries is still a major cause of concern. Upon invading host cells by phagocytosis, *M. tuberculosis* can replicate within infected cells by arresting the maturation of the phagosome, whose function is to target the pathogen for elimination. Host cells have mechanisms of controlling this evasion by inducing autophagy, an elaborate cellular process that targets bacteria for progressive elimination, decreasing bacterial loads within infected cells. In addition, autophagy activation also aids in the control of inflammation, contributing to a more efficient innate immune response against *M. tuberculosis*. Several innovative TB therapies have been envisaged based on autophagy manipulation, with some of them revealing high potential for future clinical trials and eventual implementation in health care systems. Thus, this review highlights the recent advances on the innate immune response regulation by autophagy upon *M. tuberculosis* infection and the promising new autophagy-based therapies for TB.

Mycobacterium tuberculosis: biology and infection

Mycobacterium tuberculosis, the major causative agent of tuberculosis (TB), is estimated to latently infect one third of the world population and continues to claim more lives than any single other bacterial pathogen, despite the availability of drugs since 1944, when streptomycin was first administered to a TB patient. Although only a small percentage of the latently infected individuals will ever develop active disease, this translates into a huge number of 8 million new TB cases and nearly 1.5 million deaths per year.

TB is often regarded as a developing world disease where debilitated health care systems together with the HIV epidemic allow it to remain rampant. In addition, the increasing incidence of multidrug-resistant (MDR) and extensively-drug resistant (XDR) TB, which in some countries accounts for over 20% of new infections, has the potential to exert a heavy toll also in developed countries (WHO Global tuberculosis report 2014).

Mycobacterium tuberculosis and all other species of mycobacteria, many of which are opportunistic intracellular pathogens, owe much of their resilience to a distinctive lipid-rich cell envelope that not only protects the cells against harsh environments, but also contains many molecules that are immune effectors crucial in evading the host immune response (Briken et al., 2004, Court et al., 2010, Ehlers 2010, Ishikawa et al., 2009, Lang 2013, Nobre et al., 2014, Philips and Ernst 2012, Schafer et al., 2009). Besides glycolipids and glucans that constitute most of *M. tuberculosis* cell envelope, several secreted proteins have been identified and shown to also play fundamental roles in *M. tuberculosis* survival and proliferation within the host (Abdallah et al., 2007, Philips 2008, Philips and Ernst 2012).

This extremely successful human pathogen enters our bodies through inhalation of aerosols containing *M. tuberculosis* cells. This organism then employs an array of immune modulators to invade and thrive in the host professional phagocytic cells, such as macrophages, neutrophils, monocytes and dendritic cells (DCs) by arresting phagosome maturation and fusion with lysosomes (Cooper 2009, Ernst 2012). While for many other pathogens the recruitment of phagocytic cells to the infection site halts and eradicates invading organisms, in mycobacterial infections it actually helps the pathogen to proliferate by providing further cells for infection (Ernst 2012, Philips and Ernst 2012).

As recruited cells get infected, the host immune system coordinates the edification of specific protective structures named granulomas, the histological hallmark of this disease (Ernst 2012, Philips and Ernst 2012). Granulomas are traditionally viewed as an attempt by the host to control the infection that is achieved with variable degrees of success (Davis and Ramakrishnan 2009, Lin et al., 2014, Philips and Ernst 2012). The dogma claims that *M. tuberculosis* resides inside phagosomes, however some reports have described that this pathogen can also grow in the cytoplasm environment (van der Wel et al., 2007). The bacteria that replicate inside phagocytes control cell death pathways towards necrosis and recruit more non-infected macrophages, expanding the infection (Lee et al., 2011, Philips and Ernst 2012).

Understanding how this organism evades and exploits our immune defense mechanisms has the potential to change significantly how we tackle this disease, improving the lives and health of millions worldwide.

Autophagy: a general antibacterial host mechanism

Macroautophagy (hereafter referred as autophagy) is a mechanism that relies on the formation of a double-membrane vesicle, the autophagosome, which engulfs components of the cytoplasm and delivers them to degradation in the lysosome. Autophagy is crucial for the maintenance of cellular homeostasis by continuously degrading damaged organelles, long-lived proteins, protein aggregates, and intracellular pathogenic microorganisms (Mizushima 2011). It also provides a way of recycling nutrients, which then participate in *de novo* protein synthesis and energy production. Autophagy can be induced by a variety of stimuli and/or environmental stresses, as for example nutrient starvation, low oxygen levels, oxidative stress, pathogen infection and certain drugs treatments (Bento et al., 2013, Gomes and Dikic 2014).

Autophagy-related (ATG) proteins are the key players in the regulation of autophagy, being hierarchically organized in functional complexes that control all the autophagy steps, from the initiation signalling point to the autophagosome fusion with the lysosome. The formation of new autophagosomes is triggered and assisted by a core of ATG proteins that can be subdivided in some

groups: (1) the unc-51-like kinase (ULK) complex, composed by the mammalian Atg1 orthologues ULK1 and ULK2, ATG13 and the focal adhesion kinase-family interacting protein of 200 kDa (FIP200), that is controlled by AMP-activated protein kinase (AMPK) and mechanistic target of rapamycin (mTOR), both being responsive to signals such as amino acids and glucose availability, growth factors stimulation, stress conditions and AMP/ATP energetic status of the cell; (2) class III phosphatidylinositol 3-kinase (PI3K) complex (controlled via phosphorylation by the ULK complex), composed by VPS34, Atg6 (also known as Beclin 1), Atg14 (also known as Barkor) and VPS15 (also known as p150), that is involved in the synthesis of phosphatidylinositol 3-phosphate PI3P, whose function in autophagy is not very clear but seems to favour the recruitment of WD-repeat-domain-phosphoinositide-interacting proteins (WIPIs in mammals and Atg18 in yeast) to the phagophore membrane, marking membranes for autophagosome nucleation (3) ATG9, which is involved in the supply of lipid bilayers to the formation of autophagosomes; (4) ATG12-ATG5-ATG16L1 complex, formed by an ubiquitination-like reaction where ATG12 is conjugated to ATG5 (by a mechanism dependent on ATG7 and ATG10, which act similar to an E1-ubiquitin activating enzyme and an E2-ubiquitin conjugating enzyme, respectively), which then is associated to ATG16L1 and, subsequently to the nascent phagophore; and (5) ubiquitin-like microtubule-associated protein 1-light chain 3 (LC3 in mammals or Atg8 in yeast) family system, where pro-LC3 is cleaved by ATG4B, resulting in LC3-I that is then conjugated to phosphatidylethanolamine (PE) by ATG7 and ATG3 to form LC3-II, the autophagosome-associated form of LC3. The ATG12-ATG5-ATG16L1 complex enhances the recruitment of LC3 to the site of lipidation and conjugation of LC3 to PE (Figure 1). LC3-II is thought to be involved in the elongation and closure of the autophagosome and it is also important in the recruitment of cargo, by a mechanism dependent on proteins similar to P62 (also known as sequestosome 1 or SQSTM1) and neighbor of BRCA gene 1 (NBR1), containing LC3-interacting (LIR) and ubiquitin-associated (UBA) domains. This type of proteins recognizes ubiquitin-tagged substrates through their UBA domains and interacts with LC3-II via the LIR domain, acting as cargo adapters for ubiquitinated proteins that can be degraded by autophagy (Bento et al., 2013, Gomes and Dikic 2014).

The formation of autophagosomes can be regulated by a wide variety of signals that are usually categorized into mTOR-dependent and mTOR-independent (Sarkar 2013). mTOR is a classic negative regulator of autophagy and its activity is canonically inhibited by starvation or rapamycin treatment, which leads to activation of Atg13-ULK1/ULK2-FIP200 complex, thereby inducing autophagy. ULK1 phosphorylation/activation by AMPK and upregulation of phosphatase and tensin homologue (PTEN) via inhibition of AKT kinase, both induced by p53, also inhibit mTOR. AMPK can also phosphorylate tuberous sclerosis complex 2 (TSC2), which impacts on the activity of the TSC-Ras homology enriched in brain (RHEB) axis, ultimately leading to mTOR inactivation and autophagy induction. Among the mTOR-independent mechanisms, inhibition of inositol monophosphatase (IMPase), which reduces the levels of free inositol and inositol (1,4,5)-triphosphate, and activation of AMPK via Ca^{2+}-transfer from the endoplasmic reticulum to the mitochondria, are some of the most well characterized mechanisms regulating autophagy (Bento et al., 2013, Ravikumar et al., 2009).

With particular interest for the context of this review, autophagy constitutes a cell-autonomous defence mechanism against a wide-range of intracellular pathogens from bacteria (i.e. *Mycobacterium tuberculosis*, *Streptococcus pyogenes*, *Shigella flexneri, Salmonella enterica*) to protozoa and viruses (Gomes and Dikic 2014).

In the specific case of bacteria, after invading host cells, they reside within vacuoles or phagosomes, whose maturation tends to be blocked. Eventually, some bacteria damage the membrane of the phagosome and escape into the cytosol. Bacteria in damaged phagosomes or in the cytosol can then be targeted to autophagy and degradation in the lysosome, by a mechanism that relies on the binding of ubiquitin or galectin to bacteria and/or to the membrane of phagosome-containing bacteria, which are recognized by the autophagic adaptors P62, NBR1, optineurin or calcium binding and coiled-coil domain 2 (CALCOCO2 or NDP52). However, some bacteria have the ability to manipulate autophagy for survival by secreting effectors that inhibit the pathway (Gomes and Dikic 2014, Huang and Brumell 2014). In the next sections, we will revise the main findings that have implicated autophagy in the clearance of mycobacteria, as well as some of the mycobacterial defence mechanisms against

elimination by host cells and potential tuberculosis therapeutics based on autophagy antimicrobial responses.

Autophagy: a host defence mechanism against *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is an intracellular pathogen that can replicate within infected macrophages, by arresting the maturation of the phagosome where the bacteria reside. This is at least in part attributed to the failure of phagosomes to undergo fusion with lysosomes by selective exclusion of Rab7 GTPase and lysosomal-associated membrane protein 1 or LAMP-1 (markers of late endosome and lysosome), coupled with the retention of Rab5 (an early endosome marker) on the phagosome, which allows *M. tuberculosis* to avert the usual physiological destination of phagocytosed material (Via et al., 1997). In addition, *M. tuberculosis* also interferes with the delivery of V-ATPase subunits and lysosomal hydrolases from the *trans*-Golgi network (TGN) to the phagocytic compartment, which impacts on its biogenesis and function. This is connected to the fact that *M. tuberculosis* produces an array of lipids and lipoglycans (i.e. lipoarabinomannan) that mimic certain mammalian phosphatidylinositols important for the synthesis of PI3P via VPS34 which not only inhibits autophagy but also blocks PI3P-dependent trafficking pathways, such as the one between the TGN and the phagosome (Fratti et al., 2003, Shui et al., 2011, Vergne et al., 2004). *M. tuberculosis* also secrets a tyrosine phosphatase (MptpA) that further reduces the phagosomal levels of PI3P and blocks phagosome-lysosome fusion by interacting with vacuolar protein sorting 33b (Vps33b), a host protein typically associated with vesicle trafficking steps in the endosome/lysosome pathway (Bach et al., 2008, Vergne et al., 2005) (Figure 2).

Nutrient starvation, a conventional inducer of the VPS34 kinase complex, has been shown to act as an effective promoter of biogenesis, acidification and maturation of mycobacterial phagosomes, by increasing the delivery of late endosome/ lysosome markers (e.g. vacuolar-type H^+-ATPase or V-ATPase, LAMP1 and cathepsin D) and the recruitment of membrane-associated LC3 to the phagosome, which directly impacts on the survival of mycobacteria in infected cells (Gutierrez et al., 2004). Interferon-γ (IFN- γ), a cytokine associated with protective immunity against *M. tuberculosis*,

and immunity-related GTPase family, M (IRGM or LRG-47), a downstream effector of IFN-γ, show similar effects to starvation, while the PI3 kinases inhibitors 3-methyladenine (3-MA) and wortmannin abrogate this response (Gutierrez et al., 2004). Depletion of Beclin-1 and Atg7, critical autophagy regulators, also inhibit IFN-γ- or Irgm1-induced phagosomal maturation (Singh et al., 2006). Therefore, immunological or pharmacological VPS34- or autophagy-targeted manipulation can render infected cells more resistant to mycobacterial infection.

Besides from inhibiting VPS34, mycobacteria have other ways of protecting themselves against autophagy-mediated clearance. For instance, the "enhanced intracellular survival" (Eis) gene enhances the survival of *M. tuberculosis* and other mycobacteria inside of cells by diminishing autophagy and pro-inflammation (Shin et al., 2010a, Wei et al., 2000). Infection of macrophages with an *eis*-deleted *M. tuberculosis* strain (Mtb-Δ*eis*) was shown indeed to augment the formation of LC3-positive and double membrane vesicles (autophagosomes) as compared to the Mtb-WT strain. These vesicles enclose the bacilli and subsequently fuse with multivesicular structures, leading to the formation of late or degradative autophagic vacuoles, which correspond to autolysosomes. In addition, Mtb-Δ*eis* also upregulates the production of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and the generation of ROS by a c-Jun N-terminal kinase (JNK)-dependent mechanism in host macrophages, being the last event the one triggering autophagy and pro-inflammation (Shin et al., 2010a). Interestingly, Eis protein secreted by *M. tuberculosis* appears to enhance survival of other mycobacteria, namely *M. smegmatis*, in macrophages. *M. tuberculosis*-Eis is an efficient N^{ε}-acetyltransferase, rapidly acetylating Lys55 of dual-specificity protein phosphatase 16 (DUSP16; also known as MKP-7), a JNK-specific phosphatase, whereas *M. smegmatis*-Eis is more efficient as a N^{α}-acetyltransferase and preferentially acetylates the terminal amino group of peptides (Kim et al., 2012b). This difference between both proteins is likely to be explained by a structural dissimilarity in the peptide recognition pocket of the enzymes; *M. tuberculosis*-Eis is characterized by the presence of a narrow channel, while *M. smegmatis*-Eis has a deep, round-shaped substrate-binding pocket, which seems more suitable for accommodating the terminal amino group of peptides than specific sequences within proteins (Kim et al., 2012b). This structural difference seems indeed to have a physiological impact on the survival of both species.

While *M. tuberculosis*-Eis significantly down-regulates lipopolysaccharides-induced JNK phosphorylation, *M. smegmatis*-Eis does not reveal this function. Therefore, acetylation of DUSP16 by *M. tuberculosis*-Eis seems to be the key initial event in the JNK-dependent inhibition of autophagy, phagosome maturation and ROS generation, which ultimately contributes to enhanced survival of *M. tuberculosis* within the macrophage cells (Kim et al., 2012b). This also suggests that *M. tuberculosis* developed adaptive evolutionary strategies to potentiate the suppression of the host innate immune system.

M. tuberculosis has also been suggested to pervert the function of some intrinsic host mechanisms favouring the survival of the bacteria in macrophages. This is the case of coronin 1a (Coro1a), a host F-actin-binding protein, which inhibits autophagosome formation around *M. tuberculosis*-containing phagosomes, most likely by inhibiting the activation of p38 mitogen-activated protein kinase (p38 MAPK) necessary for autophagy induction via Toll-like receptor (TLR) signalling pathways in innate immunity (Seto et al., 2012).

However, host adaptive responses have also evolved in order to take advantage of some intrinsic mycobacteria mechanisms, as for example the type VII secretion system ESX-1 (Watson et al., 2012). Recruitment of LC3 to *M. bovis* bacilli Calmette Guerin (BCG)-containing vacuoles depends on exogenous stimulation of autophagy (Gutierrez et al., 2004, Singh et al., 2006, Watson et al., 2012). On the contrary, targeting of LC3 to *M. tuberculosis*-containing vacuoles seems to be triggered without any extrinsic stimulatory signal. One of the major differences between BCG and virulent mycobacteria is the lack of ESX-1 from the BCG strain, which may explain their different effectiveness in replicating within macrophages and in activating innate immune responses by the host (Harboe et al., 1996).

Mycobacterium marinum has been used in many studies as a surrogate model of *M. tuberculosis* due to its relative safety for humans (fish and amphibians are the preferred hosts) and its shared mechanisms of pathogenesis (Tobin and Ramakrishnan 2008). Although ESX-1 is present in both pathogenic species, it seems to play different roles in targeting bacteria to degradation. For instance, ESX-1 is required for total vacuolar escape of *M. marinum* (contributing to an intracellular phase

where it resides in the cytosol without being enclosed by any membrane organelle), which becomes ubiquitinated and sequestered by LAMP-1-positive host vesicles by a mechanism that seems to be Atg5-independent and to occur in the absence of LC3-membrane association (Collins et al., 2009). In contrast, ESX-1 has been suggested to induce *M. tuberculosis*-containing phagosomes permeabilization/perforation, which allows cytosolic components of the ubiquitin-mediated autophagy pathway access the enclosed bacillus. Stimulator of interferon gene (STING), which works both as a direct cytosolic DNA sensor and an adaptor protein that functions upstream of TANK-binding kinase 1 (TBK1) in type 1 interferon signalling, recognizes extracellular bacterial DNA (which has been shown to be exposed during macrophage infection), promotes ubiquitin tagging of the bacilli (mostly through K63-linkage) and subsequent LC3 recruitment to the phagosome by a mechanism dependent on the ubiquitin-autophagy adaptors p62 and NDP52 (Watson et al., 2012). This leads to the formation of bacilli-containing autophagosomes that mature via fusion with lysosomes to create autolysosomes (Figure 2). In opposition to *M. marinum*, this mechanism was shown to require the activity of Atg5, as macrophages with Atg5 genomic deletion were unable to recruit LC3 to phagosomes containing *M. tuberculosis* and induce their maturation into autolysosomes, as assessed by strong decrease of LAMP1 co-localization (Collins et al., 2009, Watson et al., 2012). This whole mechanism is clearly dependent on ESX-1 since an *M. tuberculosis* mutant defective in early secreted Ag of 6 kDa or ESAT-6 (the major ESX-1-secreted substrate with membrane damaging activity) fails to recruit LC3 to the phagosome. Therefore, *M. tuberculosis* clearance by the autophagy-lysosomal pathway provides a way of cell-autonomous control of bacterial replication within macrophages and appears to be fundamental for the host survival upon *M. tuberculosis* infection. Indeed, $Atg5^{-/-}$ deletion render mice extremely sensitive to *M. tuberculosis*, as all mice succumb to infection by 4 weeks post-infection and a 1,000-fold increase in bacilli replication is observed within the lungs, as compared to $Atg5^{+/+}$ infected-mice (Watson et al., 2012). However, it is interesting to note that only one-third of intracellular bacteria are targeted by the ubiquitin-autophagy pathway, which suggests that infection control may be potentiated by strategies that augment autophagy activity in the host cells and that avoid autophagy evasion by the remaining bacilli.

TBK-1 also plays a critical role in regulating mycobacteria clearance by autophagy and cell defence against mycobacteria-triggered infection (Pilli et al., 2012). TBK-1 was previously shown to control type I interferon response elicited by intracellular DNA and, more recently, to orchestrate autophagy clearance of mycobacteria by a mechanism dependent on Rab8b, optineurin and P62. TBK-1 depletion does not suppress formation of autophagosomes but suppresses their maturation into autolysosomes due to inhibited delivery of lysosomal hydrolases to the autophagosomal compartment. In addition, TBK-1 also triggers phosphorylation of serine 403 in the ubiquitin-associated (UBA) domain of p62, strongly increasing its affinity for ubiquitin-tagged substrates, such as mycobacteria tagged with K63-linked ubiquitin chains, which induces their elimination by autophagy (Figure 2). Of interest is also the fact that TBK-1 is required for IL-1β-induced clearance of *M. tuberculosis* by autophagy, since the TBK-1 inhibitor BX795 or TBK-1 depletion reduces mycobacterial killing when autophagy is induced by IL-1β (Pilli et al., 2012).

Elimination of *M. tuberculosis* through autophagy has been clearly elucidated as an ubiquitin-dependent mechanism where phagosomes-enclosing bacteria are tagged with ubiquitin chains, which are subsequently recognized by the autophagy adaptors p62 and NDP52 that recruit all the autophagy machinery necessary for their degradation (Gomes and Dikic 2014, Huang and Brumell 2014). However, the E3 ligase (Parkin) that triggers K63-ubiquitination of mycobacteria was only recently identified. Parkin (also known as PARK2) mutations and polymorphisms, apart from being well-known Parkinson's disease risk factors, are also associated to increased susceptibility to *Mycobacterium leprae* infection (Mira et al., 2004). In fact, parkin is important for the host defence against *M. tuberculosis* by promoting xenophagy, by a mechanism similar to the one that implicates the protein in mitophagy induction (Geisler et al., 2010, Youle and Narendra 2011). This resemblance is likely to be explained by the fact that endosymbiotic bacteria are the most probable evolutionary origin of mitochondria, which suggests an evolutionarily conserved role for parkin (Manzanillo et al., 2013).

The major evidences implicating parkin in *M. tuberculosis* xenophagy showed that infected-Park2$^{-/-}$ macrophages present a significant reduction in ubiquitin-positive mycobacteria, as compared to

normal cells (Manzanillo et al., 2013). While expression of wild-type Park2 in Park2$^{-/-}$ macrophages restored ubiquitin localization around mycobacterial cells, two parkin pathogenic RING domain mutants with no E3 ligase activity (T240R and P437L) failed to do so (Manzanillo et al., 2013). Infected-Park2$^{-/-}$ macrophages also revealed decreased recruitment of the ubiquitin adaptors P62, NDP52 and NBR1 and the autophagy proteins LC3 and Atg12 to mycobacterial cells, compromising their efficient elimination. Parkin deficiency increased indeed bacterial viability and replication within infected macrophages, being Park2$^{-/-}$ mice extremely susceptible to *M. tuberculosis*; all infected Park2 deficient mice succumbed to *M. tuberculosis* infection by 85 days post-infection, whereas all infected wild-type mice remain alive and with no overt signs of stress (Manzanillo et al., 2013).

Crosstalk between innate immunity and autophagy in tuberculosis

Induction of cytokines expression is a key host defence mechanism against *M. tuberculosis* infection and can be triggered by activation of TLR- and non TLR-dependent signalling cascades (Jo 2013). Apart from regulating other defence responses that are beyond the scope of this review, IFN-γ was shown to be an important cytokine in the regulation of mycobacteria clearance by autophagy, while the cytokines IL-4 and IL-13 seem to inhibit this effect (Harris et al., 2007). On the other hand, TNF-α appears to synergize the antimicrobial and autophagic responses triggered by IFN-γ (Harris et al., 2008), while many other cytokines positively aid in the autophagic response against mycobacteria (i. e. TNF-β, IL-2, IL-6, CCL2) (Harris 2011). Interestingly, maturation of *M. tuberculosis*-containing phagosomes induced by IFN-γ was shown to be abrogated by the TNF blockers adalimumab, etanercept and infliximab (Harris et al., 2008). Overall, T helper-1 (Th1) cytokines appear to induce autophagy, whereas the Th2 cytokines IL-4 and IL-13 seem to inhibit it. Apart from inhibiting IFN-γ-induced autophagy, IL-4 and IL-13 also inhibit starvation-induced autophagy in a way dependent on protein kinase B (AKT) (Harris et al., 2007). Therefore, a precise balance of different cytokines is critical in the host response to *M. tuberculosis.*

TLR-dependent signalling pathways account for the maintenance of this balance. For instance, the receptors TLR2, TLR4 and TLR9 appear to be the main TLRs implicated in the recognition of

mycobacteria and production of antimicrobial effectors and cytokines upon *M. tuberculosis* infection (Kleinnijenhuis et al., 2011). TLR2, 4 and 9-stimulation was interestingly shown to induce maturation of bacterium-containing phagosomes, activate autophagy and increase degradation of bacteria (Delgado et al., 2008, Sanjuan et al., 2007, Xu et al., 2007) by mechanisms dependent on myeloid differentiation primary response gene 88 (MyD88), TIR-domain-containing adapter-inducing interferon-b (TRIF) and MAPK (Delgado et al., 2008, Jo 2013, Shi and Kehrl 2008, Xu et al., 2007). Non-TLR pathways have also been implicated in regulation of autophagy upon *M. tuberculosis* infection. One of the examples is the signalling cascade triggered by NOD-like receptor 2 (NOD2), which is an intracellular receptor that recognizes bacterial molecules (i.e. peptidoglycan) and induces expression of proteins that upregulate autophagy, such as IRGM, LC3 and ATG16L1, contributing to decreased *M. tuberculosis* virulence (Juarez et al., 2012).

Regulation of cytokines production and autophagy activation seem to be mutually regulated by each other; as mentioned before, cytokines regulate autophagy, but the opposite is also true. For instance, autophagy seems to positively regulate the expression and secretion of TNF-α (Crisan et al., 2011, Jo 2013) and to negatively regulate the secretion of several other proinflammatory cytokines, including IL-1α, IL-1β and IL-18 (Crisan et al., 2011, Harris et al., 2011, Jo 2013, Nakahira et al., 2011, Saitoh et al., 2008, Zhou et al., 2011). For the specific case of IL-1β, autophagy was shown to control its expression by different ways: increasing degradation of pro-IL-1β and inhibiting AIM2 and NLRP3 inflammasome, which decreases IL-1β processing and secretion (Bradfute et al., 2013, Harris et al., 2011, Nakahira et al., 2011, Shah et al., 2013, Zhou et al., 2011). Although IL-1 is necessary for protection against mycobacteria, negative regulation of IL-1 by autophagy is likely to have beneficial effects to the infected cells as high levels of IL-1 are associated to excessive inflammation and pathology, suggesting that a precise control of IL-1 expression and release is needed for a successful response against infection (Bradfute et al., 2013). Atg5 deficiency in mice causes indeed an excessive pulmonary inflammatory response characterized by neutrophils infiltration and IL-17 response with increased IL-1α secretion (Castillo et al., 2012) (Figure 3).

In addition to act as a modulator of proinflammatory cytokine secretion, autophagy also plays a role in antigen processing and presentation. In fact, autophagy was shown to be directly associated to

enhanced delivery of intracellular material to major histocompatibility complex (MHC) class II pathway under mycobacteria infection (Jagannath et al., 2009). Rapamycin-induced autophagy enhanced indeed mycobacterial Ag85B presentation by antigen presenting cells (APCs) infected with *M. tuberculosis,* while suppression of autophagy by 3-MA or knockdown of Beclin-1 attenuated this effect (Jagannath et al., 2009).

Association between genetic variants of autophagy-related genes and susceptibility to tuberculosis

Although Crohn's disease is considered to have an autoimmune origin, increasing evidence points to an infectious aetiology involving mycobacteria (Greenstein 2003). Some genome wide-association studies (GWAS) have been suggesting indeed an overlap between genetic susceptibility for inflammatory bowel disease (IBD), such as Chron's disease, and tuberculosis (TB) (Jostins et al., 2012). Therefore, it is not surprising that both diseases share similar profiles of genetic variants and risk factors. *IRGM* polymorphisms are an example, as they were initially identified as an autophagy risk loci for Chron's disease and more recently a TB risk factor in different populations (King et al., 2011, Wellcome Trust Case Control 2007). In fact, genetic variants of the *IRGM* gene are the most consensually associated to TB infection, with at least five different genetic variants identified so far. Although the detailed mechanism by which this gene regulates autophagy is not clear, IRGM was shown to induce clearance of mycobacteria in infected macrophages by inducing phagosomal maturation and autophagy. Most of the variants are associated to increased protection against TB (Bahari et al., 2012, Che et al., 2010, Intemann et al., 2009). However, carriers of the Chron's disease-related T allele of rs10065172 reveal increased susceptibility to TB (King et al., 2011), while the -1208G/-1161T/-947T haplotype is also positively associated with the disease (Che et al., 2010).

TLR2 is another gene that has been associated to TB and, in opposition to IRGM variants, all the TLR2 variants identified so far appear to be risk factors of developing TB (Ben-Ali et al., 2004, Etokebe et al., 2010, Ogus et al., 2004). A variety of cell wall components of mycobacteria are known to activate macrophages through TLR2, suggesting that this innate immune receptor plays a role in the

host response to *M. tuberculosis* infection (Bowdish et al., 2009, Drennan et al., 2004). The direct role of TLR2 activation in the regulation of autophagy is not well ascribed; however, several evidences have been suggesting that TLR2 activation is capable of inducing autophagy by a mechanism dependent on the activation of p38 MAPK (Seto et al., 2012). Interestingly, the R753Q TLR2 polymorphism was shown to render TLR2 incapable of inducing tyrosine phosphorylation and hetero-dimerization with TLR6 upon agonist-binding, ultimately leading to impaired capacity of p38 and autophagy activation (Xiong et al., 2012).

Genetic polymorphisms in *vitamin D receptor* (*VDR*) are also associated with predisposition to TB when combined with 25-hydroxycholecalciferol (calcidiol) deficiency (Wilkinson et al., 2000). Calcidiol is a precursor of calcitriol (1,25-dihydroxyvitamin D3), the active form of vitamin D, which has been shown to protect against *M. tuberculosis* infection via upregulation of autophagy (Campbell and Spector 2012, Fabri et al., 2011, Yuk et al., 2009). Interestingly, regulation of autophagy by TLR2 seems to occur through the activation of VDR by the binding to calcitriol, triggering the expression of the antimicrobial peptide cathelicidin, which induces autophagy and promotes autophagosome-lysosome fusion (Shin et al., 2010b).

These and other autophagy-related genetic variants associated to TB are listed and summarized in Table 1.

Tuberculosis therapeutics based on autophagy anti-mycobacteria responses

The current TB numbers, associated with the HIV epidemic and the growing number of immunocompromised population, under medication of immunosuppressive drugs or due to aging, is a serious cause of concern for public health. When we further combine the portfolio of dated anti-TB drugs in use and the emergence of MDR and XDR strains, the potential threat to public health urges for the development of new and more effective strategies.

As described before, autophagy plays a critical role in the host immune response against *M. tuberculosis* and therefore the development of autophagy-based therapies to combat TB represents an appealing strategy. In fact, it has been established that prolonged use of autophagy inhibitors such as

azitromycin, inhibits intracellular killing of mycobacteria and predisposes cystic fibrosis patients to mycobacterial disease (Renna et al., 2011). Furthermore, autophagy was shown to be determinant in the intracellular killing effect of the first line TB drugs isoniazid and pyrazinamide through a mechanism based on the release of reactive oxygen species (Kim et al., 2012a). Therefore, autophagy inducers can and are being explored as potential new TB therapies.

Rapamycin (sirolimus) and everolimus are potent mTOR inhibitors and autophagy inducers, currently approved for clinical use to prevent transplant rejection (Gutierrez et al., 2004, Ni Cheallaigh et al., 2011). Although these drugs are strong autophagy inducers, they are also immunosuppressing drugs and therefore their direct use in TB therapies is counterproductive (Ni Cheallaigh et al., 2011, Yu et al., 2013). Nevertheless, direct delivery of these drugs to the lungs using a nanoparticle system to enable specific particle uptake by professional phagocytic cells has been proposed in an attempt to minimize the systemic side effects (Ni Cheallaigh et al., 2011). An *in vitro* study recently published showed the potential of investing further in this approach since rapamycin-carrying nanoparticles were efficiently taken up by macrophages and exhibited substantial activity against intracellular *M. tuberculosis* (Gupta et al., 2014). Another possible approach is to enhance rapamycin potency towards autophagy induction, and therefore to reduce the amount of rapamycin that needs to be administered, reducing adverse side effects. To this end, small molecules enhancers of rapamycin (SMERs) in combination with rapamycin have been shown to increase the killing of mycobacteria by primary human macrophages, suggesting a possible application in TB therapy (Floto et al., 2007). Nevertheless, these SMERs were shown to act either independently or downstream of mTOR (Sarkar et al., 2007).

Niclosamide, an approved drug currently used to treat worm infections in the intestinal tract, was also found to be an inhibitor of mTORC1 signalling and a potent stimulator of autophagy (Balgi et al., 2009). Despite its effectiveness in the gastrointestinal tract, its poor absorption precludes any use in TB therapy. However, the niclosamide-derivative drug nitazoxanide, already in use as an anti-protozoal agent and with good intestinal absorption, was also shown to inhibit mTORC1 signalling and potently induce autophagy (Lam et al., 2012). It was demonstrated that nitazoxanide and its active form, tizoxanide, inhibit intracellular *M. tuberculosis* proliferation at the concentration normally

found in the blood after oral administration (Lam et al., 2012). Interestingly, this drug was previously shown to kill both replicating and non-replicating *M. tuberculosis in vitro* by an unknown mechanism (de Carvalho et al., 2009). It is possible that these promising results in TB therapy are the consequence of a dual mode of action involving direct targeting and killing of the bacteria, but also autophagy induction in phagocytic cells, which promotes intracellular clearance of *M. tuberculosis* (Lam et al., 2012). It was also found that this effect was not compromised in the presence of first line anti-tuberculosis drugs, like isoniazid, pyrazinamide, ethambutol, rifampicin and streptomycin (Lam et al., 2012). Furthermore, this drug has been proven safe without significant side effects, in very long treatment regimens (up to 4 years), in AIDS related cryptosporidiosis (Fox and Saravolatz 2005, Rossignol et al., 2006). All of these characteristics make nitazoxanide a drug with very promising applications in TB therapy.

Recently, it was also found that treatment of *M. tuberculosis*-infected macrophages with the AMP-mimetic 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) robustly activates autophagy and inhibits the survival of intracellular bacilli (Yang et al., 2014). This was shown to be dependent on AMPK-induced mTOR inhibition and AMPK-induced peroxisome proliferator-activated receptor-gamma coactivator 1α (PPARGC1A) upregulation, accounting for increased expression of autophagy-related genes via CCAAT/enhancer-binding protein β (CEBPB) and autophagy induction (Yang et al., 2014). Therefore, the development of drugs that activate AMPK may have potential application in TB therapy (Yang et al., 2014).

There are however other methods of inducing autophagy in an mTOR-independent manner. Immuno-adjuvants that induce autophagy have likely applications in TB treatment. INF-γ is a cytokine that has been demonstrated to be absolutely essential to control *M. tuberculosis* infection in both animal models and humans (Jouanguy et al., 1999). Besides activating macrophages to kill bacteria through the production of reactive nitrogen intermediates (Chan et al., 1992), IFN-γ stimulates delivery of mycobacteria to lysosomes by activating autophagy (MacMicking et al., 2003, Singh et al., 2006). A pilot study involving the administration of IFN-γ as an immune adjuvant for drug-resistant TB therapy obtained promising results suggesting the efficacy of IFN-γ in drug resistant TB (Suarez-Mendez et

al., 2004). It is possible that by blocking the Th2 cytokines IL-4 and IL-13, known to restrict autophagy, similar promising results may be achieved, since a high-throughput RNA interference screen in a human monocytic cell line (THP-1) found that these autophagy-negative regulators are absolutely essential for intracellular mycobacterial survival and growth (Kumar et al., 2010). In fact, it has been demonstrated *in vitro* that lactic acid bacteria enhance the bacterial killing ability of mononuclear phagocytes by increasing autophagy-inducing cytokine IFN-γ levels and by reducing IL-4 and IL-13 (Ghadimi et al., 2010). In addition, oral treatment with lactic acid bacteria was sufficient to down-regulate the lung Th2 response (Forsythe et al., 2007, Ghadimi et al., 2010).

Calcitriol is the hormonally active form of vitamin D. It is produced by the kidney but also in human macrophages from its precursor 25-hydroxy vitamin D, by a specific 1-α hydroxylase, and it has been demonstrated to be required for IFN-γ induced autophagy (Fabri et al., 2011). Vitamin D and IFN-γ induced autophagy have been shown to promote lysosomal fusion with phagosomes containing *M. tuberculosis* and to consequently inhibit mycobacterial expansion in the host (Bradfute et al., 2013, Campbell and Spector 2012). It was therefore tempting to test whether vitamin D could be used as a dietary supplement in TB treatment, since historically vitamin D sources like sunbathing and cod liver oil were used to treat TB. Many clinical trials have been performed to test this hypothesis; the results have however been inconclusive, with several studies showing positive results, especially in patients with vitamin D deficiency (Kearns et al., 2014, Salahuddin et al., 2013), but many other major clinical trials showing no benefits overall (Kearns et al., 2014, Martineau et al., 2011, Ralph et al., 2013, Wejse et al., 2009). It is still uncertain whether vitamin D will have any use in TB therapy, with some researchers advocating that better knowledge is needed about vitamin D concentrations for optimal immune response in order to perform adequate clinical trials (Ralph et al., 2013).

Lithium, carbamazepine and sodium valproate are currently approved drugs used to treat mood disorders and epilepsy. They target d-myo-inositol-1,4,5-triphosphate (IP3)-regulated pathway, depleting intracellular inositol, and therefore induce autophagy (Rubinsztein et al., 2007, Sarkar et al., 2005). Tamoxifen is a drug currently used to treat a wide variety of diseases, from breast cancer to

mood disorders and infertility, among others. It was shown to be an inducer of autophagy in a Beclin1-dependent manner (Rubinsztein et al., 2007, Wienecke et al., 2006). Ridaifen-B, a tamoxifen derivative, was also shown to be an inducer of autophagy but through a currently unknown Beclin1-independent mechanism (Nagahara et al., 2013). Gefitinib, an epidermal growth factor receptor (EGFR) inhibitor used to treat breast and other types of cancer, has also been shown to induce autophagy and to be effective in *M. tuberculosis* killing in a mouse model (Stanley et al., 2014). Nortriptyline and fluoxetine, currently used to treat depression, were also reported to promote autophagy and enhance mycobacteria clearance (Stanley et al., 2014, Sundaramurthy et al., 2013). While the mechanisms behind autophagy enhancement by these two drugs are currently unknown, fluoxetine effect is related to increased TNF-α secretion (Stanley et al., 2014). Since all these FDA-approved drugs have been reported as autophagy inducers, they have the potential to be used as complementary treatment(s) to current TB therapies.

Statins are widely used HMG-CoA reductase inhibitors currently approved for clinical use to lower cholesterol levels (Ray et al., 2010). It has been recently documented that statins also have immunomodulatory and anti-inflammatory effects with reports of reduced mortality in patients with bacteraemia (Kwak et al., 2000, Liao and Laufs 2005, Parihar et al., 2014, Tleyjeh et al., 2009). In TB mice models, treatment with statins reduced significantly the bacterial load and the pulmonary pathological effects of TB infection (Parihar et al., 2014). It was further shown in TB-infected macrophages and in mice models that treatment with statins improves the efficacy of first line TB drug regimens and of rifampicin alone (Lobato et al., 2014, Skerry et al., 2014). It was demonstrated that statins improve bacterial clearance by the host and improve the efficacy of TB drugs by promoting autophagy via inhibition HMG-CoA reductase pathway (Parihar et al., 2014). Furthermore, there are some reports claiming that statins also enhance autophagy in an mTOR-dependent way by inhibiting the Rac1-mTOR signalling pathway (Wei et al., 2013). The full mechanisms behind the autophagy inducing effects of statins are still not fully elucidated; however the effects observed from statin administration are promising and may provide another possible complement to TB therapy.

Several TLRs have been shown to be involved in autophagy induction and to play a critical role in the formation of the immune response (Delgado et al., 2008, Sanjuan et al., 2007, Xu et al., 2007). However, prolonged stimulation of TLRs (by abundant TLR-interacting mycobacterial compounds) results in the production of immunosuppressive cytokines, decreased antigen presentation and survival of bacteria inside macrophages (Saraav et al., 2014). Nevertheless, TLR-4-mediated autophagy was found to promote mycobacteria containment in macrophages (Xu et al., 2007). Finding the right equilibrium of different TLRs activation using either drug or vaccine approaches might lead to increased immunogenic response and improved TB therapies.

The existing prophylactic approach to TB, the BCG vaccine, was first tested in humans over 90 years ago and has been used extensively despite its unreliability in terms of averting TB in adults (Colditz et al., 1995, Fine 1995). A new vaccine or an innovative strategy to improve the efficacy of the current BCG vaccine would have a profound impact in current situation of the TB epidemic. Stimulation of autophagy was found to increase the efficacy of attenuated H37rv and BCG vaccines, through enhancement of the ability of macrophage and DCs to present mycobacterial antigens (Jagannath et al., 2009). Rapamycin-treated macrophages exhibited a substantial increase in antigen presentation when infected with the tested TB vaccine strains and wild-type H37rv strain (Jagannath et al., 2009). Furthermore, the results showed that DCs also had enhanced antigen presentation when treated with rapamycin (Jagannath et al., 2009). The increased *in vitro* antigen presentation was observed to translate into *in vivo* protection in a TB mice model (Jagannath et al., 2009). It was further demonstrated that *M. smegmatis* and *M. bovis BCG* strains that were modified in order to overexpress immunogenic antigens targeted by the autophagy-lysosome pathway (such as Ag85B) led to an increase in antigen presentation (Jagannath et al., 2009). This is in agreement with a different study showing that a live BCG strain overexpressing Ag85B is a more efficient vaccine when compared to the wild-type BCG strain (Horwitz et al., 2006).

DNA vaccines used directly or as prime-boost are alternative promising approaches to either improve the efficacy of the current BCG vaccine or to create a new more effective one (Rivas-Santiago and Cervantes-Villagrana 2014). Plasmids containing *M. tuberculosis* DNA (from Ag85, Hsp65 and the

23 members of Esx gene family) used in experimental DNA vaccines have been found to lead to higher INF-γ production and consequent induction of autophagy (Meerak et al., 2013, Villarreal et al., 2014, Zarate-Blades et al., 2013).

These promising TB therapies are listed and summarized in table 2.

Final remarks

Autophagy is a key mechanism in eukaryotic cell resistance to *M. tuberculosis* infection as it plays a vital role in the intracellular clearance of this pathogen. It potentiates the effect of some of the current first line TB drugs, influences antigen presentation and modulates the release of cytokines that are determinant for the infection outcome. Although the knowledge of how autophagy influences immunity is still far from complete, there is a clear potential for autophagy-based therapies in advanced TB treatment strategies. Promotion of autophagy through pharmacological means by administrating autophagy-inducing drugs and cytokines has produced positive results *in vitro*, in TB mice models and even in a human pilot study. Enhancing autophagy also increases the efficacy of the only TB-prophylactic method available, the BCG vaccine. The results of using autophagy-inducing approaches to combat TB are very promising and an autophagy-based therapy for TB may soon be a reality.

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References

Abdallah AM, Gey van Pittius NC, Champion PA, Cox J, Luirink J, Vandenbroucke-Grauls CM, et al. (2007) Type VII secretion--mycobacteria show the way. Nature reviews Microbiology. **5**:883-91.

Bach H, Papavinasasundaram KG, Wong D, Hmama Z, Av-Gay Y. (2008) Mycobacterium tuberculosis virulence is mediated by PtpA dephosphorylation of human vacuolar protein sorting 33B. Cell host & microbe. **3**:316-22.

Bahari G, Hashemi M, Taheri M, Naderi M, Eskandari-Nasab E, Atabaki M. (2012) Association of IRGM polymorphisms and susceptibility to pulmonary tuberculosis in Zahedan, Southeast Iran. TheScientificWorldJournal. **2012**:950801.

Balgi AD, Fonseca BD, Donohue E, Tsang TC, Lajoie P, Proud CG, et al. (2009) Screen for chemical modulators of autophagy reveals novel therapeutic inhibitors of mTORC1 signaling. PloS one. **4**:e7124.

Ben-Ali M, Barbouche MR, Bousnina S, Chabbou A, Dellagi K. (2004) Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. Clinical and diagnostic laboratory immunology. **11**:625-6.

Bento CF, Puri C, Moreau K, Rubinsztein DC. (2013) The role of membrane-trafficking small GTPases in the regulation of autophagy. Journal of cell science. **126**:1059-69.

Bowdish DM, Sakamoto K, Kim MJ, Kroos M, Mukhopadhyay S, Leifer CA, et al. (2009) MARCO, TLR2, and CD14 are required for macrophage cytokine responses to mycobacterial trehalose dimycolate and Mycobacterium tuberculosis. PLoS pathogens. **5**:e1000474.

Bradfute SB, Castillo EF, Arko-Mensah J, Chauhan S, Jiang S, Mandell M, et al. (2013) Autophagy as an immune effector against tuberculosis. Current opinion in microbiology. **16**:355-65.

Briken V, Porcelli SA, Besra GS, Kremer L. (2004) Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response. Molecular microbiology. **53**:391-403.

Campbell GR, Spector SA. (2012) Vitamin D inhibits human immunodeficiency virus type 1 and Mycobacterium tuberculosis infection in macrophages through the induction of autophagy. PLoS pathogens. **8**:e1002689.

Castillo EF, Dekonenko A, Arko-Mensah J, Mandell MA, Dupont N, Jiang S, et al. (2012) Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. Proceedings of the National Academy of Sciences of the United States of America. **109**:E3168-76.

Chan J, Xing Y, Magliozzo RS, Bloom BR. (1992) Killing of virulent Mycobacterium tuberculosis by reactive nitrogen intermediates produced by activated murine macrophages. The Journal of experimental medicine. **175**:1111-22.

Che N, Li S, Gao T, Zhang Z, Han Y, Zhang X, et al. (2010) Identification of a novel IRGM promoter single nucleotide polymorphism associated with tuberculosis. Clinica chimica acta; international journal of clinical chemistry. **411**:1645-9.

Colditz GA, Berkey CS, Mosteller F, Brewer TF, Wilson ME, Burdick E, et al. (1995) The efficacy of bacillus Calmette-Guerin vaccination of newborns and infants in the prevention of tuberculosis: meta-analyses of the published literature. Pediatrics. **96**:29-35.

Collins CA, De Maziere A, van Dijk S, Carlsson F, Klumperman J, Brown EJ. (2009) Atg5-independent sequestration of ubiquitinated mycobacteria. PLoS pathogens. **5**:e1000430.

Cooper AM. (2009) Cell-mediated immune responses in tuberculosis. Annual review of immunology. **27**:393-422.

Court N, Vasseur V, Vacher R, Fremond C, Shebzukhov Y, Yeremeev VV, et al. (2010) Partial redundancy of the pattern recognition receptors, scavenger receptors, and C-type lectins for the long-term control of Mycobacterium tuberculosis infection. Journal of immunology. **184**:7057-70.

Crisan TO, Plantinga TS, van de Veerdonk FL, Farcas MF, Stoffels M, Kullberg BJ, et al. (2011) Inflammasome-independent modulation of cytokine response by autophagy in human cells. PloS one. **6**:e18666.

Davis JM, Ramakrishnan L. (2009) The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell. **136**:37-49.

de Carvalho LP, Lin G, Jiang X, Nathan C. (2009) Nitazoxanide kills replicating and nonreplicating Mycobacterium tuberculosis and evades resistance. Journal of medicinal chemistry. **52**:5789-92.

Delgado MA, Elmaoued RA, Davis AS, Kyei G, Deretic V. (2008) Toll-like receptors control autophagy. The EMBO journal. **27**:1110-21.

Drennan MB, Nicolle D, Quesniaux VJ, Jacobs M, Allie N, Mpagi J, et al. (2004) Toll-like receptor 2-deficient mice succumb to Mycobacterium tuberculosis infection. The American journal of pathology. **164**:49-57.

Ehlers S. (2010) DC-SIGN and mannosylated surface structures of Mycobacterium tuberculosis: a deceptive liaison. European journal of cell biology. **89**:95-101.

Ernst JD. (2012) The immunological life cycle of tuberculosis. Nature reviews Immunology. **12**:581-91.

Etokebe GE, Skjeldal F, Nilsen N, Rodionov D, Knezevic J, Bulat-Kardum L, et al. (2010) Toll-like receptor 2 (P631H) mutant impairs membrane internalization and is a dominant negative allele. Scandinavian journal of immunology. **71**:369-81.

Fabri M, Stenger S, Shin DM, Yuk JM, Liu PT, Realegeno S, et al. (2011) Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Science translational medicine. **3**:104ra2.

Fine PE. (1995) Variation in protection by BCG: implications of and for heterologous immunity. Lancet. **346**:1339-45.

Floto RA, Sarkar S, Perlstein EO, Kampmann B, Schreiber SL, Rubinsztein DC. (2007) 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. **3**:620-2.

Forsythe P, Inman MD, Bienenstock J. (2007) Oral treatment with live Lactobacillus reuteri inhibits the allergic airway response in mice. American journal of respiratory and critical care medicine. **175**:561-9.

Fox LM, Saravolatz LD. (2005) Nitazoxanide: a new thiazolide antiparasitic agent. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. **40**:1173-80.

Fratti RA, Chua J, Vergne I, Deretic V. (2003) Mycobacterium tuberculosis glycosylated phosphatidylinositol causes phagosome maturation arrest. Proceedings of the National Academy of Sciences of the United States of America. **100**:5437-42.

Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, et al. (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nature cell biology. **12**:119-31.

Ghadimi D, de Vrese M, Heller KJ, Schrezenmeir J. (2010) 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. International immunopharmacology. **10**:694-706.

Gomes LC, Dikic I. (2014) Autophagy in antimicrobial immunity. Molecular cell. **54**:224-33.

Greenstein RJ. (2003) Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease. The Lancet Infectious diseases. **3**:507-14.

Gupta A, Pant G, Mitra K, Madan J, Chourasia MK, Misra A. (2014) Inhalable particles containing rapamycin for induction of autophagy in macrophages infected with Mycobacterium tuberculosis. Molecular pharmaceutics. **11**:1201-7.

Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. (2004) Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell. **119**:753-66.

Harboe M, Oettinger T, Wiker HG, Rosenkrands I, Andersen P. (1996) Evidence for occurrence of the ESAT-6 protein in Mycobacterium tuberculosis and virulent Mycobacterium bovis and for its absence in Mycobacterium bovis BCG. Infection and immunity. **64**:16-22.

Harris J. (2011) Autophagy and cytokines. Cytokine. **56**:140-4.

Harris J, De Haro SA, Master SS, Keane J, Roberts EA, Delgado M, et al. (2007) T helper 2 cytokines inhibit autophagic control of intracellular Mycobacterium tuberculosis. Immunity. **27**:505-17.

Harris J, Hartman M, Roche C, Zeng SG, O'Shea A, Sharp FA, et al. (2011) Autophagy controls IL-1beta secretion by targeting pro-IL-1beta for degradation. The Journal of biological chemistry. **286**:9587-97.

Harris J, Hope JC, Keane J. (2008) Tumor necrosis factor blockers influence macrophage responses to Mycobacterium tuberculosis. The Journal of infectious diseases. **198**:1842-50.

Horwitz MA, Harth G, Dillon BJ, Maslesa-Galic S. (2006) A novel live recombinant mycobacterial vaccine against bovine tuberculosis more potent than BCG. Vaccine. **24**:1593-600.

Huang J, Brumell JH. (2014) Bacteria-autophagy interplay: a battle for survival. Nature reviews Microbiology. **12**:101-14.

Intemann CD, Thye T, Niemann S, Browne EN, Amanua Chinbuah M, Enimil A, et al. (2009) Autophagy gene variant IRGM -261T contributes to protection from tuberculosis caused by Mycobacterium tuberculosis but not by M. africanum strains. PLoS pathogens. **5**:e1000577.

Ishikawa E, Ishikawa T, Morita YS, Toyonaga K, Yamada H, Takeuchi O, et al. (2009) Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. The Journal of experimental medicine. **206**:2879-88.

Jagannath C, Lindsey DR, Dhandayuthapani S, Xu Y, Hunter RL, Jr., Eissa NT. (2009) Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. Nature medicine. **15**:267-76.

Jo EK. (2013) Autophagy as an innate defense against mycobacteria. Pathogens and disease. **67**:108-18.

Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, et al. (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. **491**:119-24.

Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondaneche MC, Dupuis S, et al. (1999) A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nature genetics. **21**:370-8.

Juarez E, Carranza C, Hernandez-Sanchez F, Leon-Contreras JC, Hernandez-Pando R, Escobedo D, et al. (2012) NOD2 enhances the innate response of alveolar macrophages to Mycobacterium tuberculosis in humans. European journal of immunology. **42**:880-9.

Kearns MD, Alvarez JA, Seidel N, Tangpricha V. (2014) Impact of Vitamin D on Infectious Disease: A Systematic Review of Controlled Trials. The American journal of the medical sciences.

Kim JJ, Lee HM, Shin DM, Kim W, Yuk JM, Jin HS, et al. (2012a) Host cell autophagy activated by antibiotics is required for their effective antimycobacterial drug action. Cell host & microbe. **11**:457-68.

Kim KH, An DR, Song J, Yoon JY, Kim HS, Yoon HJ, et al. (2012b) 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. **109**:7729-34.

King KY, Lew JD, Ha NP, Lin JS, Ma X, Graviss EA, et al. (2011) Polymorphic allele of human IRGM1 is associated with susceptibility to tuberculosis in African Americans. PloS one. **6**:e16317.

Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R. (2011) Innate immune recognition of Mycobacterium tuberculosis. Clinical & developmental immunology. **2011**:405310.

Kumar D, Nath L, Kamal MA, Varshney A, Jain A, Singh S, et al. (2010) Genome-wide analysis of the host intracellular network that regulates survival of Mycobacterium tuberculosis. Cell. **140**:731-43.

Kwak B, Mulhaupt F, Myit S, Mach F. (2000) Statins as a newly recognized type of immunomodulator. Nature medicine. **6**:1399-402.

Lam KK, Zheng X, Forestieri R, Balgi AD, Nodwell M, Vollett S, et al. (2012) Nitazoxanide stimulates autophagy and inhibits mTORC1 signaling and intracellular proliferation of Mycobacterium tuberculosis. PLoS pathogens. **8**:e1002691.

Lang R. (2013) Recognition of the mycobacterial cord factor by Mincle: relevance for granuloma formation and resistance to tuberculosis. Frontiers in immunology. **4**:5.

Lee J, Repasy T, Papavinasasundaram K, Sassetti C, Kornfeld H. (2011) Mycobacterium tuberculosis induces an atypical cell death mode to escape from infected macrophages. PloS one. **6**:e18367.

Liao JK, Laufs U. (2005) Pleiotropic effects of statins. Annual review of pharmacology and toxicology. **45**:89-118.

Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, Ioerger T, et al. (2014) Sterilization of granulomas is common in active and latent tuberculosis despite within-host variability in bacterial killing. Nature medicine. **20**:75-9.

Lobato LS, Rosa PS, Ferreira Jda S, Neumann Ada S, da Silva MG, do Nascimento DC, et al. (2014) Statins increase rifampin mycobactericidal effect. Antimicrobial agents and chemotherapy. **58**:5766-74.

MacMicking JD, Taylor GA, McKinney JD. (2003) Immune control of tuberculosis by IFN-gamma-inducible LRG-47. Science. **302**:654-9.

Manzanillo PS, Ayres JS, Watson RO, Collins AC, Souza G, Rae CS, et al. (2013) The ubiquitin ligase parkin mediates resistance to intracellular pathogens. Nature. **501**:512-6.

Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, et al. (2011) High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. Lancet. **377**:242-50.

Meerak J, Wanichwecharungruang SP, Palaga T. (2013) Enhancement of immune response to a DNA vaccine against Mycobacterium tuberculosis Ag85B by incorporation of an autophagy inducing system. Vaccine. **31**:784-90.

Mira MT, Alcais A, Nguyen VT, Moraes MO, Di Flumeri C, Vu HT, et al. (2004) Susceptibility to leprosy is associated with PARK2 and PACRG. Nature. **427**:636-40.

Mizushima N. (2011) Autophagy in protein and organelle turnover. Cold Spring Harb Symp Quant Biol. **76**:397-402.

Nagahara Y, Takeyoshi M, Sakemoto S, Shiina I, Nakata K, Fujimori K, et al. (2013) Novel tamoxifen derivative Ridaifen-B induces Bcl-2 independent autophagy without estrogen receptor involvement. Biochemical and biophysical research communications. **435**:657-63.

Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, et al. (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nature immunology. **12**:222-30.

Ni Cheallaigh C, Keane J, Lavelle EC, Hope JC, Harris J. (2011) Autophagy in the immune response to tuberculosis: clinical perspectives. Clinical and experimental immunology. **164**:291-300.

Nobre A, Alarico S, Maranha A, Mendes V, Empadinhas N. (2014) The molecular biology of mycobacterial trehalose in the quest for advanced tuberculosis therapies. Microbiology. **160**:1547-70.

Ogus AC, Yoldas B, Ozdemir T, Uguz A, Olcen S, Keser I, et al. (2004) The Arg753GLn polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. The European respiratory journal. **23**:219-23.

Parihar SP, Guler R, Khutlang R, Lang DM, Hurdayal R, Mhlanga MM, et al. (2014) Statin therapy reduces the mycobacterium tuberculosis burden in human macrophages and in mice by enhancing autophagy and phagosome maturation. The Journal of infectious diseases. **209**:754-63.

Philips JA. (2008) Mycobacterial manipulation of vacuolar sorting. Cellular microbiology. **10**:2408-15.

Philips JA, Ernst JD. (2012) Tuberculosis pathogenesis and immunity. Annual review of pathology. **7**:353-84.

Pilli M, Arko-Mensah J, Ponpuak M, Roberts E, Master S, Mandell MA, et al. (2012) TBK-1 promotes autophagy-mediated antimicrobial defense by controlling autophagosome maturation. Immunity. **37**:223-34.

Ralph AP, Lucas RM, Norval M. (2013) Vitamin D and solar ultraviolet radiation in the risk and treatment of tuberculosis. The Lancet Infectious diseases. **13**:77-88.

Ravikumar B, Futter M, Jahreiss L, Korolchuk VI, Lichtenberg M, Luo S, et al. (2009) Mammalian macroautophagy at a glance. Journal of cell science. **122**:1707-11.

Ray KK, Seshasai SR, Erqou S, Sever P, Jukema JW, Ford I, et al. (2010) Statins and all-cause mortality in high-risk primary prevention: a meta-analysis of 11 randomized controlled trials involving 65,229 participants. Archives of internal medicine. **170**:1024-31.

Renna M, Schaffner C, Brown K, Shang S, Tamayo MH, Hegyi K, et al. (2011) Azithromycin blocks autophagy and may predispose cystic fibrosis patients to mycobacterial infection. The Journal of clinical investigation. **121**:3554-63.

Rivas-Santiago B, Cervantes-Villagrana AR. (2014) Novel approaches to tuberculosis prevention: DNA vaccines. Scandinavian journal of infectious diseases. **46**:161-8.

Rossignol JF, Kabil SM, el-Gohary Y, Younis AM. (2006) Effect of nitazoxanide in diarrhea and enteritis caused by Cryptosporidium species. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. **4**:320-4.

Rubinsztein DC, Gestwicki JE, Murphy LO, Klionsky DJ. (2007) Potential therapeutic applications of autophagy. Nature reviews Drug discovery. **6**:304-12.

Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, et al. (2008) Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature. **456**:264-8.

Salahuddin N, Ali F, Hasan Z, Rao N, Aqeel M, Mahmood F. (2013) Vitamin D accelerates clinical recovery from tuberculosis: results of the SUCCINCT Study [Supplementary Cholecalciferol in recovery from tuberculosis]. A randomized, placebo-controlled, clinical trial of vitamin D supplementation in patients with pulmonary tuberculosis'. BMC infectious diseases. **13**:22.

Sanjuan MA, Dillon CP, Tait SW, Moshiach S, Dorsey F, Connell S, et al. (2007) Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature. **450**:1253-7.

Saraav I, Singh S, Sharma S. (2014) Outcome of Mycobacterium tuberculosis and Toll-like receptor interaction: immune response or immune evasion? Immunology and cell biology. **92**:741-6.

Sarkar S. (2013) Regulation of autophagy by mTOR-dependent and mTOR-independent pathways: autophagy dysfunction in neurodegenerative diseases and therapeutic application of autophagy enhancers. Biochemical Society transactions. **41**:1103-30.

Sarkar S, Floto RA, Berger Z, Imarisio S, Cordenier A, Pasco M, et al. (2005) Lithium induces autophagy by inhibiting inositol monophosphatase. The Journal of cell biology. **170**:1101-11.

Sarkar S, Perlstein EO, Imarisio S, Pineau S, Cordenier A, Maglathlin RL, et al. (2007) Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. Nature chemical biology. **3**:331-8.

Schafer G, Jacobs M, Wilkinson RJ, Brown GD. (2009) Non-opsonic recognition of Mycobacterium tuberculosis by phagocytes. Journal of innate immunity. **1**:231-43.

Seto S, Tsujimura K, Koide Y. (2012) Coronin-1a inhibits autophagosome formation around Mycobacterium tuberculosis-containing phagosomes and assists mycobacterial survival in macrophages. Cellular microbiology. **14**:710-27.

Shah S, Bohsali A, Ahlbrand SE, Srinivasan L, Rathinam VA, Vogel SN, et al. (2013) Cutting edge: Mycobacterium tuberculosis but not nonvirulent mycobacteria inhibits IFN-beta and AIM2 inflammasome-dependent IL-1beta production via its ESX-1 secretion system. Journal of immunology. **191**:3514-8.

Shi CS, Kehrl JH. (2008) MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. The Journal of biological chemistry. **283**:33175-82.

Shin DM, Jeon BY, Lee HM, Jin HS, Yuk JM, Song CH, et al. (2010a) Mycobacterium tuberculosis eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. PLoS pathogens. **6**:e1001230.

Shin DM, Yuk JM, Lee HM, Lee SH, Son JW, Harding CV, et al. (2010b) Mycobacterial lipoprotein activates autophagy via TLR2/1/CD14 and a functional vitamin D receptor signalling. Cellular microbiology. **12**:1648-65.

Shui W, Petzold CJ, Redding A, Liu J, Pitcher A, Sheu L, et al. (2011) Organelle membrane proteomics reveals differential influence of mycobacterial lipoglycans on macrophage phagosome maturation and autophagosome accumulation. Journal of proteome research. **10**:339-48.

Singh SB, Davis AS, Taylor GA, Deretic V. (2006) Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science. **313**:1438-41.

Skerry C, Pinn ML, Bruiners N, Pine R, Gennaro ML, Karakousis PC. (2014) Simvastatin increases the in vivo activity of the first-line tuberculosis regimen. The Journal of antimicrobial chemotherapy. **69**:2453-7.

Stanley SA, Barczak AK, Silvis MR, Luo SS, Sogi K, Vokes M, et al. (2014) Identification of host-targeted small molecules that restrict intracellular Mycobacterium tuberculosis growth. PLoS pathogens. **10**:e1003946.

Suarez-Mendez R, Garcia-Garcia I, Fernandez-Olivera N, Valdes-Quintana M, Milanes-Virelles MT, Carbonell D, et al. (2004) Adjuvant interferon gamma in patients with drug - resistant pulmonary tuberculosis: a pilot study. BMC infectious diseases. **4**:44.

Sundaramurthy V, Barsacchi R, Samusik N, Marsico G, Gilleron J, Kalaidzidis I, et al. (2013) Integration of chemical and RNAi multiparametric profiles identifies triggers of intracellular mycobacterial killing. Cell host & microbe. **13**:129-42.

Tleyjeh IM, Kashour T, Hakim FA, Zimmerman VA, Erwin PJ, Sutton AJ, et al. (2009) Statins for the prevention and treatment of infections: a systematic review and meta-analysis. Archives of internal medicine. **169**:1658-67.

Tobin DM, Ramakrishnan L. (2008) Comparative pathogenesis of Mycobacterium marinum and Mycobacterium tuberculosis. Cellular microbiology. **10**:1027-39.

van der Wel N, Hava D, Houben D, Fluitsma D, van Zon M, Pierson J, et al. (2007) M. tuberculosis and M. leprae translocate from the phagolysosome to the cytosol in myeloid cells. Cell. **129**:1287-98.

Vergne I, Chua J, Lee HH, Lucas M, Belisle J, Deretic V. (2005) Mechanism of phagolysosome biogenesis block by viable Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences of the United States of America. **102**:4033-8.

Vergne I, Fratti RA, Hill PJ, Chua J, Belisle J, Deretic V. (2004) Mycobacterium tuberculosis phagosome maturation arrest: mycobacterial phosphatidylinositol analog phosphatidylinositol mannoside stimulates early endosomal fusion. Molecular biology of the cell. **15**:751-60.

Via LE, Deretic D, Ulmer RJ, Hibler NS, Huber LA, Deretic V. (1997) Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by rab5 and rab7. The Journal of biological chemistry. **272**:13326-31.

Villarreal DO, Walters J, Laddy DJ, Yan J, Weiner DB. (2014) Multivalent TB vaccines targeting the esx gene family generate potent and broad cell-mediated immune responses superior to BCG. Human vaccines & immunotherapeutics. **10**.

Watson RO, Manzanillo PS, Cox JS. (2012) Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell. **150**:803-15.

Wei J, Dahl JL, Moulder JW, Roberts EA, O'Gaora P, Young DB, et al. (2000) Identification of a Mycobacterium tuberculosis gene that enhances mycobacterial survival in macrophages. Journal of bacteriology. **182**:377-84.

Wei YM, Li X, Xu M, Abais JM, Chen Y, Riebling CR, et al. (2013) Enhancement of autophagy by simvastatin through inhibition of Rac1-mTOR signaling pathway in coronary arterial myocytes. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. **31**:925-37.

Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, et al. (2009) Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. American journal of respiratory and critical care medicine. **179**:843-50.

Wellcome Trust Case Control C. (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. **447**:661-78.

Wienecke R, Fackler I, Linsenmaier U, Mayer K, Licht T, Kretzler M. (2006) Antitumoral activity of rapamycin in renal angiomyolipoma associated with tuberous sclerosis complex. Am J Kidney Dis. **48**:e27-9.

Wilkinson RJ, Llewelyn M, Toossi Z, Patel P, Pasvol G, Lalvani A, et al. (2000) Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. Lancet. **355**:618-21.

Xiong Y, Song C, Snyder GA, Sundberg EJ, Medvedev AE. (2012) R753Q polymorphism inhibits Toll-like receptor (TLR) 2 tyrosine phosphorylation, dimerization with TLR6, and recruitment of

myeloid differentiation primary response protein 88. The Journal of biological chemistry. **287**:38327-37.

Xu Y, Jagannath C, Liu XD, Sharafkhaneh A, Kolodziejska KE, Eissa NT. (2007) Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. Immunity. **27**:135-44.

Yang CS, Kim JJ, Lee HM, Jin HS, Lee SH, Park JH, et al. (2014) The AMPK-PPARGC1A pathway is required for antimicrobial host defense through activation of autophagy. Autophagy. **10**:785-802.

Youle RJ, Narendra DP. (2011) Mechanisms of mitophagy. Nature reviews Molecular cell biology. **12**:9-14.

Yu X, Li C, Hong W, Pan W, Xie J. (2013) Autophagy during Mycobacterium tuberculosis infection and implications for future tuberculosis medications. Cellular signalling. **25**:1272-8.

Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, et al. (2009) Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell host & microbe. **6**:231-43.

Zarate-Blades CR, Rodrigues RF, Souza PR, Rios WM, Soares LS, Rosada RS, et al. (2013) Evaluation of the overall IFN-gamma and IL-17 pro-inflammatory responses after DNA therapy of tuberculosis. Human vaccines & immunotherapeutics. **9**:1093-103.

Zhou R, Yazdi AS, Menu P, Tschopp J. (2011) A role for mitochondria in NLRP3 inflammasome activation. Nature. **469**:221-5.

Legend of figures

Figure 1. The autophagy pathway and its main regulators. Autophagy is typically subdivided in different steps: (i) vesicle nucleation/initiation, (ii) phagophore elongation, (iii) autophagosome maturation, (iv) autophagosome-lysosome fusion and (v) cargo degradation. Following AMPK activation and/or mTORC1 inhibition (by factors such as nutrient depletion and energetic stress), the complex formed by ULK1/2, FIP200 and ATG13 is activated, which is turn activates the VPS34 complex by phosphorylation. Both complexes regulate the nucleation/initiation step of autophagy, with VPS34 providing PI3P to the phagophore, which is likely to assist the recruitment of WIPI to the phagophore. On the other hand, membrane expansion depends on ATG9, which is postulated to supply lipid bilayers to the phagophore, and on two ubiquitin-like conjugation systems that conjugate LC3 and ATG12 to PE and ATG5, respectively. ATG12-ATG5 complex further interacts with ATG16, presumably at the surface of the autophagosome membrane. LC3-II seems to be involved in the elongation and closure of the autophagosome membrane, as well as in the recruitment of cargo to the phagophore. Subsequently, the autophagome fuses with the lysosome, forming the autolysosome, where degradation of the autophagosomal contents occurs.

Figure 2. *M. tuberculosis* clearance by autophagy. *M. tuberculosis* (Mtb) invades macrophages by phagocytosis and arrests the maturation of the phagosome by excluding late endosome and lysosome markers (i.e. RAB7, V-ATPase, VPS33b, LAMP-1) from the phagosome and by promoting the retention of early endosome markers (i.e. RAB5) in the phagocytic compartment. Host cells have developed ways of overcoming the evasion of *M. tuberculosis* from the phagocytic pathway by taking advantage of some intrinsic *M. tuberculosis* mechanisms. For instance, phagosomal permeabilization induced by the bacterial ESX-1/ESAT-6 system allows the host protein STING to recognize extracellular bacterial DNA, which then promotes ubiquitin marking of bacteria (mostly via K63-linkage chains formation by the E3 ligase Parkin). Ubiquitin is then recognized by autophagy adaptors, such as P62 that deliver the bacilli to autophagosomes. TBK-1-induced phosphorylation of

Ser403 of P62 increases the affinity of P62 to ubiquitin. Autophagosomes are subsequently fused to lysosomes, where degradation of mycobacteria occurs.

Figure 3. Crosstalk between autophagy and inflammation during *M. tuberculosis* infection. Autophagy activation in macrophages is controlled by membrane- and intracellular-innate immune receptors, as well as by several inflammatory cytokines released by T helper 1 (Th1) and T helper 2 (Th2) cells upon *M. tuberculosis* infection. The receptors TLR2, TLR4, TLR9 and NOD2, and the pro-inflammatory cytokines TNF-α, IFN-γ, IL-2, IL-6 and CCL2 promote autophagy activation. On the other hand, the anti-inflammatory cytokines IL-4 and IL-13 appear to inhibit IFN-γ-induced autophagy activation. However, cytokines expression and secretion are also regulated by autophagy. For instance, IL-1α, IL-1β and IL-18 are negatively regulated by autophagy, while TNF-α is upregulated by this mechanism.

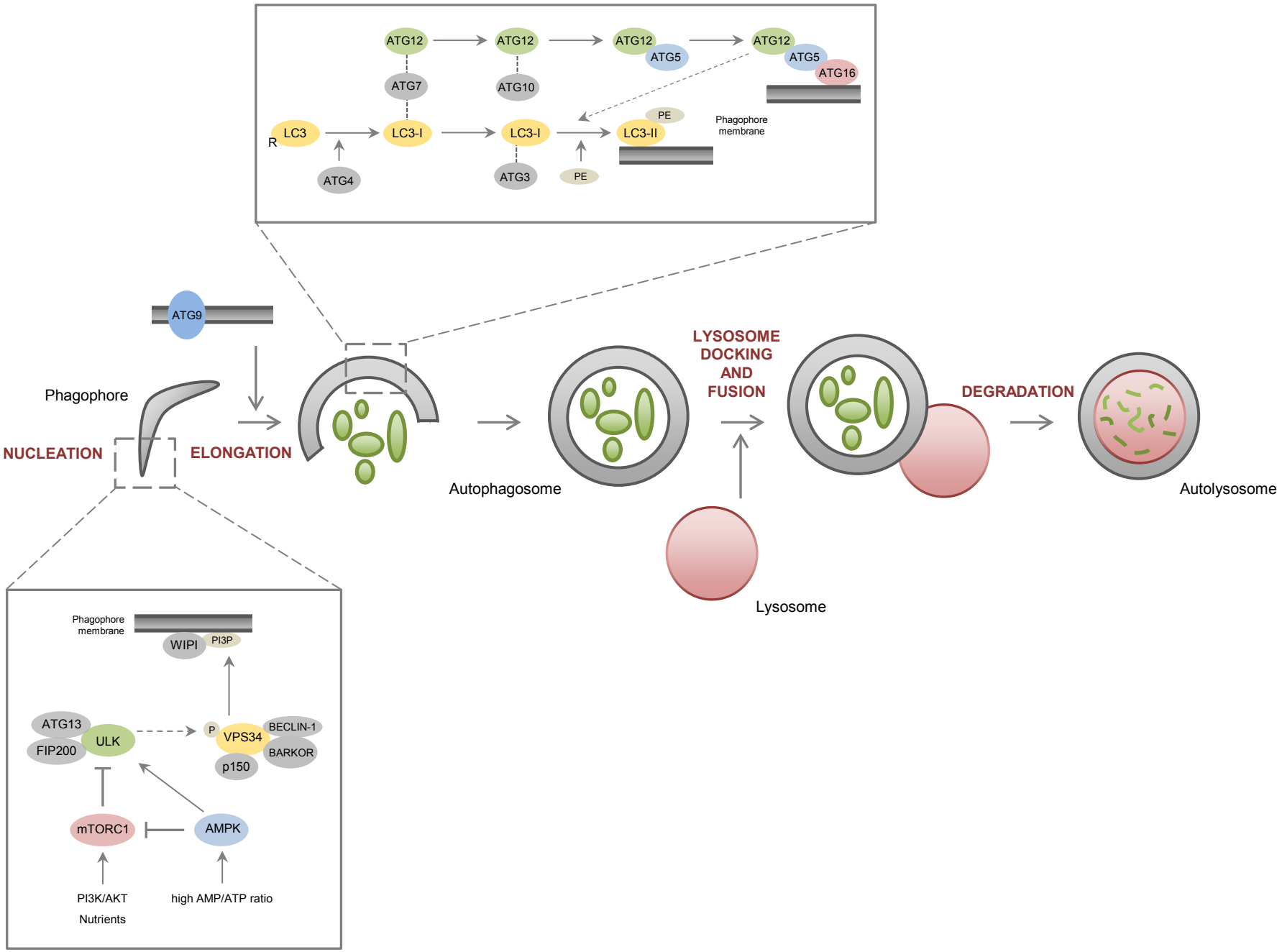
ATG12
ATG12
ATG12
ATG12
ATG5
ATG5
ATG16
ATG7
ATG10
PE
Phagophore membrane
LC3
R
LC3-I
LC3-I
LC3-II
ATG4
ATG3
PE
ATG9
Phagophore
NUCLEATION
ELONGATION
Autophagosome
LYSOSOME DOCKING AND FUSION
DEGRADATION
Autolysosome
Lysosome
Phagophore membrane
WIPI
PI3P
ATG13
ULK
FIP200
P
VPS34
BECLIN-1
BARKOR
p150
mTORC1
AMPK
PI3K/AKT
Nutrients
high AMP/ATP ratio

Figure 1

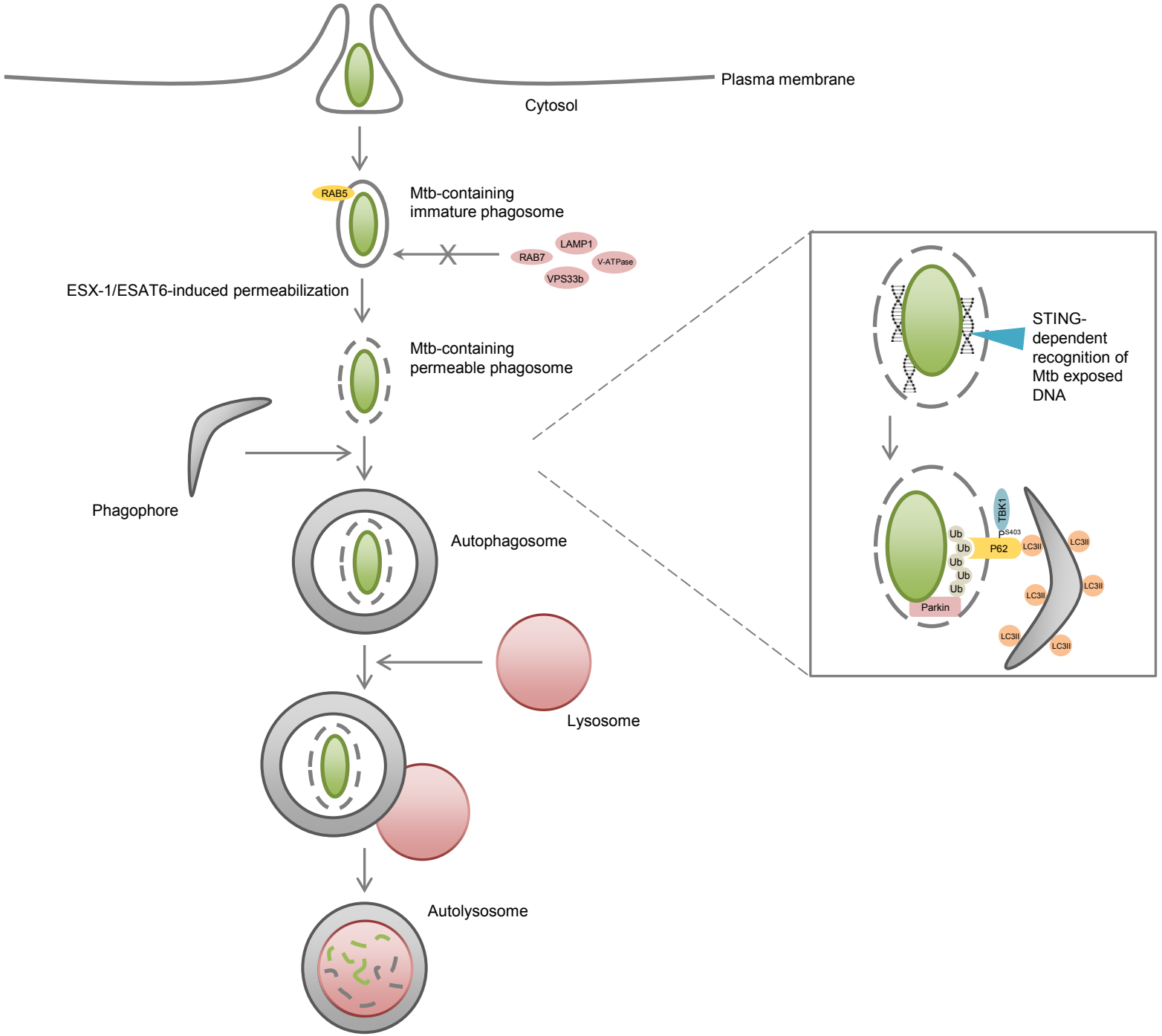
Plasma membrane
Cytosol
RAB5
Mtb-containing
immature phagosome
RAB7
LAMP1
V-ATPase
VPS33b
ESX-1/ESAT6-induced permeabilization
Mtb-containing
permeable phagosome
Phagophore
Autophagosome
Lysosome
Autolysosome
STING-
dependent
recognition of
Mtb exposed
DNA
TBK1
Ub
P62
LC3II
Parkin

Figure 2

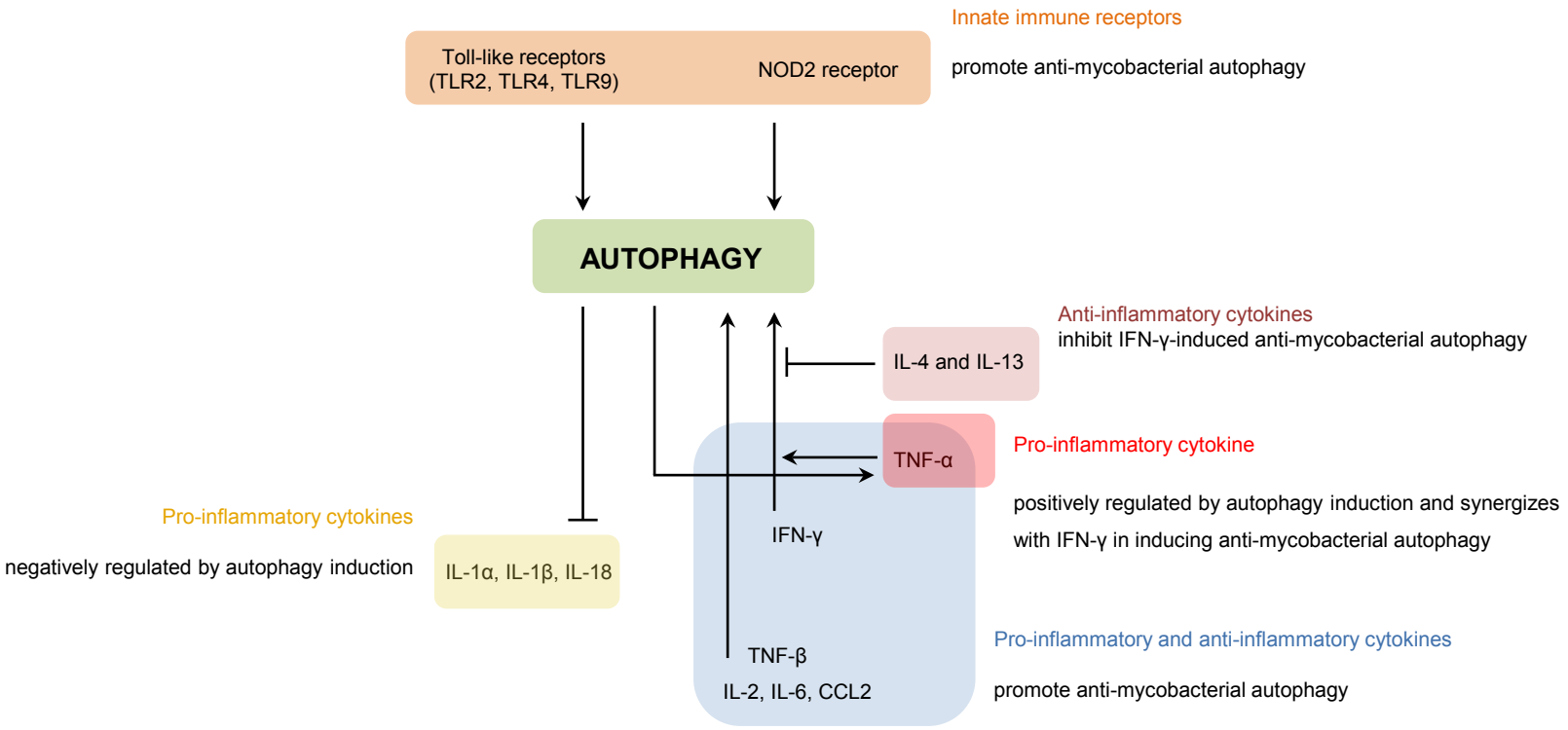
Innate immune receptors
Toll-like receptors (TLR2, TLR4, TLR9)
NOD2 receptor
promote anti-mycobacterial autophagy
AUTOPHAGY
Anti-inflammatory cytokines
inhibit IFN-γ-induced anti-mycobacterial autophagy
IL-4 and IL-13
TNF-α
Pro-inflammatory cytokine
positively regulated by autophagy induction and synergizes with IFN-γ in inducing anti-mycobacterial autophagy
IFN-γ
Pro-inflammatory cytokines
negatively regulated by autophagy induction
IL-1α, IL-1β, IL-18
TNF-β
IL-2, IL-6, CCL2
Pro-inflammatory and anti-inflammatory cytokines
promote anti-mycobacterial autophagy

Figure 3

Table 1. Autophagy-related genetic variants associated to tuberculosis

Gene	Variant	TB-association	Reference *
VDR	Combination of TT or Tt genotype (Taq1) with 25-hydroxycholecalciferol deficiency; ff (Fok1) genotype	The combination of genotype *TT/Tt* and 25-hydroxycholecalciferol deficiency, and the genotype *ff* are positively associated with TB (individuals of Hindu and Gujarati origins).	Wilkinson et al, 2000
TLR2	Arg753Gln (AA genotype)	Carriers of this polymorphism present higher risk of developing TB (Turkish individuals).	Ogus et al, 2004
TLR2	Arg677Trp (C2029T)	This polymorphism is associated to increased susceptibility to TB (Tunisian individuals).	Ben-Ali et al, 2004
IL-1β	-31position genotypes	A significant difference of IL-1 beta -31 genotypes was found between 98 tuberculosis patients and healthy controls.	Sun et al, 2007
IRGM	-261TT	It confers protection against TB (Ghanaian individuals).	Intemann et al, 2009
TLR2	P631H	It is significantly overrepresented in tuberculosis when TB patients were compared to controls, indicating a possible low-risk predisposition (Croatian individuals).	Etokebe et al, 2010
IRGM	-1208AA genotype and -1208G/-1161T/-947T haplotype	The -1208AA genotype is associated to decreased susceptibility to TB, while the -1208G/-1161T/-947T haplotype is positively associated to TB (Chinese individuals).	Che et al, 2010
IRGM	rs10065172C/T	The carriers of the Chron's disease-related rs10065172C/T SNP present increased susceptibility to TB (African American individuals).	King et al, 2011
LAMP1* and *MTOR	Rs9577229 (*LAMP1*) Rs6701524 (*MTOR*)	Associations were observed between SNPs in LAMP1, MTOR and infection with *M. tuberculosis* Beijing genotype, but statistical significance was lost after correction for multiple testing (Indonesian individuals).	Songane et al, 2012
IRGM	-1161C/T and -947C/T	Both polymorphisms are associated to decreased susceptibility to TB (Iranian individuals).	Bahari et al, 2012

* Note: Genes are listed according to the respective date of publication (from earliest to latest).

Table 2. Potential tuberculosis therapies based on autophagy manipulation

Therapy	Effects	Reference
Sirolimus (Rapamycin), Everolimus	mTORC1 inhibitor and autophagy inducer; increases BCG vaccine efficacy	Gutierrez et al, 2004 Jagannath et al, 2009 Gupta et al, 2014
SMERs	Synergistic effect with rapamycin on autophagy, acting independently or downstream of mTOR; autophagy inducer	Floto et al, 2007
Nitazoxanide (tizoxanide)	Inhibitor of mTORC1 signalling and autophagy inducer; kills mycobacteria directly by an unknown mechanism	de Carvalho et al, 2009 Lam et al, 2012
IFN-γ	Increases production of reactive nitrogen species in macrophages and induces autophagy	Chan et al. 1992 MackMicking et al, 2003 Singh et al, 2006
Lactic acid bacteria	Increase production of IFN-γ and decrease the autophagy negative regulators IL-4 and IL-13; induce autophagy	Ghadimi et al, 2010
Vitamin D	Required for IFN-γ induced autophagy	Campbell & Spector 2012
Lithium, Carbamazepine, Sodium valproate	Targets d-myo-inosiltol-1,4,5 triphosphate (IP3)-regulated pathway, depletes intracellular inositol and induces autophagy in a mTOR-independent way	Sarkar et al, 2005
Tamoxifen	Inducer of autophagy in a Beclin1-dependent manner	Wienecke et al, 2006
Ridaifen-B	Tamoxifen derivative that induces autophagy through an unknown Beclin1-independent mechanism	Nagahara et al, 2013
Gefitinib	Induces of autophagy (although it is not clear, the authors postulate that gefitinib-induction of autophagy relies on p38 MAPK inhibition)	Stanley et al, 2014
Nortriptyline	Induces autophagy by an unknown mechanism	Sundaramurthy et al, 2013
Fluoxetine	Induces autophagy possibly due to enhanced TNF-α secretion	Stanley et al, 2014
Statins	Promote autophagy by inhibiting HMG-CoA reductase pathway and in a mTOR-dependent way; improves the efficacy of first line TB drugs	Wei et al, 2013 Parihar et al, 2014 Skerry et al, 2014 Lobato et al, 2014
Ag85-overexpressing strains for vaccines	Ag85 is targeted by the autophagy-lysosome pathway and increases antigen presentation	Horwitz et al, 2006 Jagannath et al, 2009
DNA vaccines (Ag85, Hsp65, Esx genes)	Potentiate INF-γ production and induce autophagy	Meerak et al, 2013 Zarate-Blades et al, 2013 Villarreal et al, 2014