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Autophagy in the immune response to tuberculosis: clinical perspectives

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Summary

A growing body of evidence points to autophagy as an essential component in the immune response to tuberculosis. Autophagy is a direct mechanism of killing intracellular *Mycobacterium tuberculosis* and also acts as a modulator of proinflammatory cytokine secretion. In addition, autophagy plays a key role in antigen processing and presentation. Autophagy is modulated by cytokines; it is stimulated by T helper type 1 (Th1) cytokines such as tumour necrosis factor (TNF)-α and interferon (IFN)-γ, and is inhibited by the Th2 cytokines interleukin (IL)-4 and IL-13 and the anti-inflammatory cytokine IL-10. Vitamin D, via cathelicidin, can also induce autophagy, as can Toll-like receptor (TLR)-mediated signals. Autophagy-promoting agents, administered either locally to the lungs or systemically, could have a clinical application as adjunctive treatment of drug-resistant and drug-sensitive tuberculosis. Moreover, vaccines which effectively induce autophagy could be more successful in preventing acquisition or reactivation of latent tuberculosis.

Keywords: autophagy, inflammasome, rapamycin, TNF-α, vaccination, vitamin D

Introduction

Tuberculosis has been declared a global emergency by the World Health Organization (WHO) [1]: the incidence of tuberculosis (TB) has increased dramatically, fuelled by the human immunodeficiency virus (HIV) pandemic, while globalization and migration have ensured that all countries are affected [2]. The rapid spread of drug-resistant strains of TB, with mortality rates from extensively drug-resistant strains of up to 98%, is cause for serious concern [3].

Autophagy is a highly conserved process for the delivery of long-lived cytosolic macromolecules and whole organelles to lysosomes for degradation. During starvation, autophagy acts as a cell survival mechanism, providing essential amino acids [4,5], but autophagy is also important for removing potentially harmful cellular constituents, such as damaged mitochondria, misfolded proteins or protein aggregates [6]. Three distinct types of autophagy have been described; micro-autophagy, in which cytosol is directly engulfed by lysosomes [7]; chaperone-mediated autophagy, in which specific proteins are recognized by a cytosolic chaperone and targeted to the lysosome [8]; and macro-autophagy (hereafter referred to as autophagy), in which an isolation membrane, or phagophore, fuses with itself to form an autophagosome with a distinctive double-membrane, which can then fuse with lysosomes [5].

Evidence is emerging that autophagy plays a key role in promoting a number of critical elements of the host immune responses to infection with *Mycobacterium tuberculosis*. As we start to understand how autophagy is regulated, we may identify potential therapeutic targets in the fight against tuberculosis. Targeting autophagy could lead to effective treatments for drug-resistant tuberculosis, shorter treatments for drug-sensitive tuberculosis and more powerful vaccines, thereby helping to realize the goal of eliminating tuberculosis.

Autophagy and tuberculosis

Considerable evidence now exists of a role for autophagy in immune responses to numerous pathogenic microorganisms, including *Mycobacterium tuberculosis* (*Mtb*) [9,10]. Autophagy may play multiple roles within this response, both as an effector of cytokine/vitamin D-directed killing mechanisms and as a modulator of cytokine secretion (Fig. 1). The importance of autophagy in the host immune response against *Mtb* is highlighted further by the fact that virulent mycobacteria have evolved mechanisms to inhibit autophagy and the production of proinflammatory

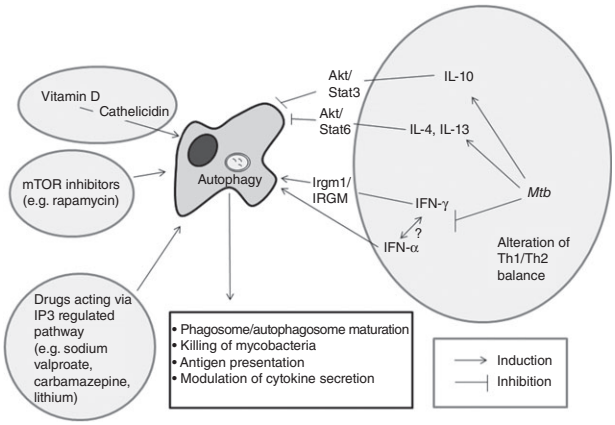

Fig. 1. Modulation of autophagy in macrophages. Autophagy can be induced by mammalian target of rapamycin (mTOR) inhibitors, such as rapamycin, by drugs acting via the D-myo-inositol-1,4,5-trisphosphate (IP3) pathway, such as sodium valproate, by vitamin D (through the induction of the anti-microbial peptide cathelicidin) and by cytokines, including tumour necrosis factor (TNF)-α and interferon (IFN)-γ. Induction of autophagy by IFN-γ is dependent on immunity-related GTPase M (Irgm1/IRGM) and may act, at least partially, through the induction of TNF-α in cells infected with mycobacteria. Other cytokines, including interleukin (IL)-4, IL-13 and IL-10 inhibit autophagy through protein kinase B (Akt), signal transducer and activator of transcription (STAT)6 and STAT3 signalling pathways. In cells infected with *Mycobacterium tuberculosis*, induction of autophagy leads to increased acidification and maturation of mycobacteria-containing phagosomes and increased killing of intracellular bacteria. Autophagy also plays a role in antigen presentation and inhibits the processing and secretion of proinflammatory cytokines, including IL-1β and IL-18.

mediators, such as tumour necrosis factor (TNF)-α [11], which itself induces autophagy [12].

Autophagy and Th1/Th2 polarization

Numerous cytokines, chemokines and growth factors have been shown to modulate autophagy in a variety of cell types (Table 1). However, in the context of *Mtb* infection, it is perhaps the effect of T helper type (Th)1/Th2 polarization on autophagy that is of most interest. Immunity to *Mtb* is reliant on a predominantly Th1-biased response, characterized by the localized secretion of interferon (IFN)-γ, TNF-α and interleukin (IL)-12 [13], while Th2 responses in the lungs and periphery of patients, indicated by increased secretion of IL-4 and high antibody titres, have been associated with more severe disease [14,15]. Infection with *Mtb* results in increased expression of mediators which counteract Th1 responses and promote Th2 responses [16].

Mycobacteria have evolved a number of strategies to circumvent the host immune response, including blocking the fusion of phagosomes with lysosomes (phagosome maturation) [17]. However, treatment of *Mtb*-infected macrophages with IFN-γ can overcome this phagosome maturation block [18,19] and induces autophagy-dependent killing of intracellular mycobacteria [20]. Interestingly, IFN-γ-induced maturation of *Mtb*-containing phagosomes is abrogated by the TNF blockers adalimumab, infliximab and etanercept [21], suggesting that the effects of IFN-γ on phagosome maturation, and possibly autophagosome formation, are directed by TNF-α. Indeed, TNF-α induces both phagosome maturation and autophagy in macrophages [12,21], while pre-treatment of human macrophages with IFN-γ increases TNF-α release in response to infection with *Mtb* [21]. Similarly, ligation of CD40, coupled with TNF-α signalling, induces autophagy-dependent killing of *Toxoplasma gondii* by macrophages [22,23].

While Th1 cytokines have been shown to induce autophagy, the Th2 cytokines IL-4 and IL-13, along with the anti-inflammatory cytokine IL-10 have been shown to inhibit it. IL-4 and IL-13 have been shown to inhibit autophagy through two separate mechanisms; inhibition of starvation-induced autophagy is dependent on signalling through the protein kinase B (Akt) pathway, while inhibition of IFN-γ-induced autophagy is dependent on signal transducer and activator of transcription (STAT)6 activation [24]. In both cases, treatment of *Mtb*-infected macrophages with either IL-4 or IL-13 promotes the intracellular survival of the bacteria [24]. Inhibition of rapamycin-induced autophagy by IL-10 is dependent on both Akt and STAT3 [25], while inhibition of starvation-induced autophagy is dependent on type I PI3K/Akt [26]. We have also found that IL-10 inhibits lipopolysaccharide (LPS)-induced autophagy in murine macrophages (Fig. 2).

Autophagy and the inflammasome

Recent studies have highlighted that autophagy, as well as being modulated by cytokines, can itself regulate secretion of the proinflammatory cytokines IL-1α, IL-1β and IL-18 [27–30]. IL-1β is first produced as a pro-form in response to inflammatory stimuli, including LPS. A second signal, such as ATP or reactive oxygen species (ROS), is then required for this inactive precursor to be cleaved into the bioactive (p17) molecule by caspase 1, following the activation of an inflammasome [31]. Inflammasomes are molecular scaffolds that trigger the activation of caspase 1 and subsequent maturation of IL-1β and IL-18. Typically, inflammasomes are formed from at least one member of the cytosolic innate immune sensor family, the nucleotide oligomerization domain (NOD)-like receptors (NLR), which include NLRP1, NLRP3 and NLRC4 (IPAF), coupled with the adaptor apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC or PYCARD) and caspase 1 [31].

Studies have implicated IL-1β in the immune response to mycobacteria. In humans, IL-1 receptor agonist/IL-1β polymorphisms influence cytokine responses to *Mtb* [32] and polymorphisms in the IL-1 receptor are associated with

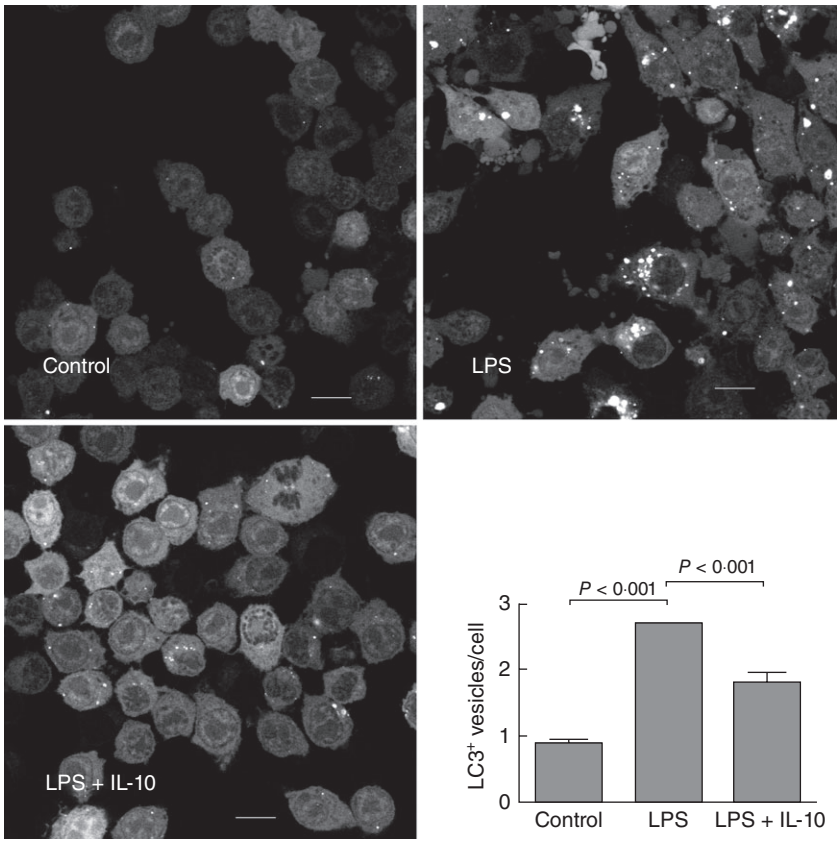

Fig. 2. Interleukin (IL)-10 inhibits lipopolysaccharide (LPS)-induced autophagy in macrophages. Immortalized murine bone marrow-derived macrophages stably transfected with green fluorescent protein-(GFP)-LC3 were treated with LPS (100 ng/ml) in the presence or absence of recombinant murine IL-10 (10 ng/ml) for 24 h. Autophagy induction was determined by counting the number of GFP-LC3$^+$ vesicles per cell. Data are means ± standard error of the mean from three separate experiments. Data were analysed using a one-way analysis of variance, followed by Tukey's honestly least significance post-test. Scale bar = 10 μm.

Table 1. Known modulatory effects of cytokines, chemokines and growth factors on autophagy.

Cytokine/chemokine/ growth factor	Effect on autophagy	References
IFN-γ	Induces autophagy in human and murine macrophages: dependent on Irgm1 (IRGM)	[20,97]
TNF-α	Induces autophagosome formation in human and murine macrophages	[12]
TGF-β	Induces autophagy in human hepatocellular carcinoma cell lines	[98]
IL-1α	Induces autophagy in porcine articular chondocytes and human macrophages	[99,100]
IL-1β	Induces autophagosome formation in human macrophages	[100]
IL-2	Induces autophagy in primary murine CD4$^+$ T cells	[101]
IL-3	Withdrawal from IL-3-dependent Bax$^{-/-}$ Bak$^{-/-}$ bone marrow-derived cells induces autophagosome formation	[102]
IL-4	Inhibits starvation-induced autophagy via activation of the Akt pathway	[24]
IL-6	Induces hyperactivation of autophagy in human CD11b$^+$ peripheral blood mononuclear cells	[103]
IL-10	Inhibits rapamycin-induced autophagy via activation of the STAT3 and Akt pathways Inhibits starvation-induced autophagy via type I PI3K/Akt pathway	[25,26]
IL-13	Inhibits starvation-induced autophagy via activation of the Akt pathway and IFN-γ-induced autophagy via STAT6 activation	[24]
IGF1	Inhibits autophagosome formation in HEK293, HeLa, MCF7 and H4 LC3-GFP cells	[104]
FGF2	Inhibits autophagosome formation in HEK293, HeLa, MCF7 and H4 LC3-GFP cells	[104]
LIF	Inhibits autophagosome formation in HEK293, HeLa, MCF7 and H4 LC3-GFP cells	[104]
MCP-1	Induces oxidative stress in cardiac myoblasts, which induces ER stress, autophagy and cell death	[105]
CCL2	Induces hyperactivation of autophagy in human CD11b$^+$ peripheral blood mononuclear cells	[103]
CLCF1	Inhibits autophagosome formation in HEK293, HeLa, MCF7 and H4 LC3-GFP cells	[104]
SDF1 (CXCL12)	Inhibits autophagosome formation in HEK293, HeLa, MCF7 and H4 LC3-GFP cells	[104]

IGF1: insulin-like growth factor 1; IFN: interferon; FGF2: fibroblast growth factor 2; LIF: leukaemia inhibitory factor; MCP-1: monocyte chemotactic protein-1; CCL2: chemokines (C-C motif) ligand 2; CLCF1: cardiotrophin-like cytokine factor 1; SDF1: stromal cell-derived factor 1; GFP: green fluorescent protein; IGRM: immunity-related GTPase M; AKT: protein kinase B; STAT: signal transducer and activator of transcription; ER: endoplasmic reticulum.

increased susceptibility to *Mtb* [33]. Mice deficient in IL-1R1 are more susceptible to pulmonary tuberculosis after infection with *Mtb*, with increased mortality, defective granuloma formation and enhanced mycobacterial growth in the lungs, spleen and liver [34,35]. *Mycobacterium tuberculosis* may suppress secretion of IL-1β and thereby inhibit host bactericidal activity. A mycobacterial gene, *zmp1*, which encodes a putative Zn^{2+} metalloprotease, has been shown to suppress inflammasome activation in infected macrophages [36]. Macrophages infected with zmp1$^{-/-}$ *M. bovis* bacilli Calmette–Guérin (BCG) secreted more IL-1β than those infected with wild-type (WT) BCG. The study demonstrated that IL-1β increases maturation of mycobacteria-containing phagosomes and enhances killing of the bacilli by macrophages. Survival of zmp1$^{-/-}$ BCG was rescued after siRNA knock-down of caspase 1, IL-1β, ASC and IPAF [36].

In another study, Koo *et al.* [37] found that macrophages infected with live, virulent strains of *M. marinum* or *M. tuberculosis* secreted more IL-1β than those infected with attenuated strains or heat-killed bacilli. Secretion, but not synthesis, of IL-1β and IL-18 was dependent on the mycobacterial ESX-1 secretion system and correlated with lysosome exocytosis [37]. In this study, processing and secretion of IL-1β and IL-18 was dependent on caspase 1, ASC and NLRP3, but not IPAF. A more recent study has demonstrated that IL-1β secretion is not only important for host resistance to *Mtb* in mice, but can be generated through a caspase 1-independent mechanism [38]. Thus, while it is clear that IL-1β has an important role to play in host immune responses to *Mtb*, multiple mechanisms for its activation may be induced by the bacilli.

Given that IL-1β clearly has a role to play in immunity against *Mtb*, it is interesting that autophagy has been shown to modulate secretion of the cytokine through at least two separate mechanisms. Saitoh *et al.* [29] found that in the absence of functional autophagic machinery – either through the loss of Atg16L1 or Atg7 – LPS alone was able to drive IL-1β processing or secretion by macrophages, suggesting that autophagy is responsible for the removal either of an endogenous inflammasome-activating molecule or a component of the inflammasome itself. This process is dependent on NLRP3, Toll/IL-1 receptor (TIR) domain-containing adaptor inducing IFN-β (TRIF) and ROS, but is not dependent on the phagocyte nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase NOX2 (gp91 phox) [27–30]. Inhibition of autophagy with 3-methyladenine (3-MA) also increases IL-1α secretion in response to LPS, but this is not dependent on NLRP3 [27]. ROS and mitochondrial DNA (mtDNA) released from mitochondria are responsible for inflammasome activation in autophagy-deficient macrophages treated with LPS and mitophagy (degradation of mitochondria in autophagosomes) regulates this process [28,30]

Autophagy also regulates IL-1β secretion by directly targeting intracellular pro-IL-1β for lysosomal degradation. In murine macrophages treated with LPS, pro-IL-1β can be seen co-localizing with the autophagosomal membrane marker LC3, suggesting that it is sequestered specifically by autophagosomes [27]. Moreover, further induction of autophagy with rapamycin decreases LPS-induced pro-IL-1β expression in macrophages treated with LPS and secretion of mature IL-1β in macrophages and dendritic cells (DCs) treated with LPS and ATP, alum or chitosan [27]. Similarly, rapamycin reduces serum levels of IL-1β in a murine model of LPS-induced sepsis [27], suggesting that autophagy may play a pivotal role in regulating inflammation and may, in turn, be a useful target for therapeutic intervention. In the context of *Mtb* infection, following early IL-1β secretion, autophagy might act to limit further production of the cytokine, thus preventing excessive inflammation, while itself acting as a potent anti-mycobacterial response.

Autophagy, vitamin D and TB immunity

Vitamin D treatment has been proposed as a tuberculosis 'cure' since the 19th century [39], but recent research has firmly established a role for the vitamin D receptor in macrophage responses to *Mtb* infection. Moreover, a number of vitamin D polymorphisms have been associated with susceptibility to tuberculosis [40–43]. Similarly, low serum levels of vitamin D have been associated with tuberculosis reactivation and treatment with vitamin D can enhance TB immunity in an *ex vivo* whole blood assay [44,45]. More recently, however, a double-blind randomized placebo-controlled trial failed to demonstrate improvement in treated tuberculosis patients who took vitamin D supplements [46]. Beneficial effects of vitamin D may be limited to those with a certain vitamin D receptor genotype [47], or it may be that vitamin D is best employed in the prevention of progression from latent tuberculosis infection (LTBI) to reactivation tuberculosis. A trial of vitamin D treatment in this setting has yet to be addressed.

The human macrophage, unlike the mouse macrophage, is sensitive to vitamin D binding to the vitamin D receptor, which works in concert with Toll-like receptor (TLR) signals to increase the expression of cathelicidin, an anti-microbial peptide capable of killing the intracellular bacillus [48]. Subsequent investigations have suggested that vitamin D, via cathelicidin, can also induce autophagy One study has shown that vitamin D_3 specifically induces autophagy in human monocytes and macrophages via cathelicidin [49], and that cathelicidin comes into direct contact with mycobacteria within the autophagosome. Vitamin D supplementation in patients deficient in vitamin D did not, however, increase circulating cathelicidin [50]. None the less, localized increases of this anti-microbial peptide may be achievable in the granuloma – which might not be detectable by peripheral sampling. Further studies are needed to assess the true benefits, if any, of vitamin D in the immune

response to tuberculosis and what role autophagy might play in this.

Autophagy and antigen presentation

Autophagy assists with antigen processing of intracellular and extracellular material for major histocompatibility complex (MHC) class I and class II presentation, and has also been shown to be important for efficient cross-presentation to $CD8^+$ T lymphocytes. Autophagosomes containing pathogens, including mycobacteria, converge with endosomes and thus deliver antigens for loading in MHC class II compartments. Autophagy can also deliver endogenous antigens to the MHC II pathway [51] enhancing presentation to $CD4^+$ T cells [52–56]. These studies showed a direct association of autophagy with enhanced delivery of endogenous proteins to the MHC class II pathway and suggest that autophagy is a mechanism by which the peptide repertoire presented by MHC class II molecules may be extended from exogenous to endogenous antigens. There is evidence that autophagy-associated proteins, including LC3, gain access to MHC II compartments [57] and coupling of antigens to Atg8/LC3 enhanced their presentation on MHC class II [58]. Moreover, the induction (with rapamycin or starvation) or suppression (with 3-MA or RNAi knock-down) of autophagy have been shown to have direct effects on MHC II-peptide presentation [59,60]. *In vivo*, autophagy has also been shown to be important for MHC class II presentation of self-proteins during central tolerance induction [61]. In the context of mycobacteria, autophagy also enhances MHC class II presentation. Vaccination with rapamycin-treated DC enhanced MHC class II presentation of Ag85B and was associated with the induction of potent protective $CD4^+$ responses in mice [62].

Autophagy may also contribute to the generation of MHC class I-restricted responses. English *et al.* demonstrated that autophagy contributed to processing of herpes simplex virus-1 antigens for MHC class I presentation [63]. Autophagy may also influence antigen presentation to $CD8^+$ T cells via degradation of the MHC class I molecules themselves [64]. Autophagy induction resulted in reduced MHC class I surface expression, consistent with the presence of MHC I in autophagosomes, but this was reversed by IFN-γ. These data suggest that in the presence of IFN-γ autophagy contributes significantly to the loading of peptides onto MHC class I and the induction of $CD8^+$ T cell responses.

Finally, autophagy may facilitate cross-presentation of antigens on MHC class I molecules. Li and colleagues demonstrated that autophagy plays an important role in antigen sequestration and delivery to DCs for cross-presentation of tumour antigens [65]. This study also showed that isolated autophagosomes could be used as an antigen source for cross-presentation after being loaded into DCs, suggesting potential in vaccine development, where cross-presentation of antigen to $CD8^+$ T lymphocytes is required.

Modulation of autophagy by pathogen-derived factors

Mycobacterial lipoproteins and cytidine phosphate guanosine (CpG)-containing DNA are known agonists for TLR-2 (dimerized with either TLR-1 or TLR-6) and TLR-9, respectively, while TLR signalling through myeloid differentiation primary response gene 88 (MyD88) and TRIF results in proinflammatory, anti-mycobacterial responses [66]. TLR-2 knock-out mice have increased susceptibility to tuberculosis [38,67] and TLR-2 polymorphisms are associated with TB susceptibility in humans [33,68]. Engagement of TLRs has been shown to induce autophagy in macrophages. Treatment of macrophages with LPS induces autophagy and enhances anti-mycobacterial responses in murine macrophages [52]. This effect was found to be MyD88-independent and TRIF-dependent, although another study has shown TLR-induced autophagy to be both MyD88- and TRIF-dependent [69]. Activation of MyD88 or TRIF results in the recruitment of beclin 1 (Atg6) to the TLR-4 signalling complex [69]. A role for both MyD88 and TRIF in TLR-dependent autophagy is suypported further by the observation that numerous different TLR agonists induce autophagy in macrophages, including the TLR-3 agonist poly I:C and the TLR-7 agonist imiquimod [69,70].

Autophagy can also be induced by NOD-like receptor 2 (NLR-2). Intracellular NLR-2 has been shown to play a non-redundant role in recognition of *Mycobacterium tuberculosis* [71], and has also been shown to be involved in regulation of IL-1β secretion [72]. Engagement of NLR-2 by muramyl dipeptide activates autophagy, promotes bacterial trafficking to the autophagolysosome and enhances antigen presentation [73]. NOD2 also recruits ATG16L1 to the plasma membrane on bacterial entry [74].

Modulation of autophagy by other factors

Host immune responses determine the outcome of infection with *Mtb*. The majority of individuals infected with *Mtb* mount an immune response which contains, but does not eliminate, the bacteria: this is termed latent tuberculosis infection (LTBI). Over time, some of these individuals will lose control of the infection and develop active tuberculosis disease. A number of medical conditions and host risk factors have been identified which greatly increase the risk of developing active tuberculosis disease [75]. The most potent of these is HIV infection, particularly if untreated and advanced, which causes as much as a 10-fold increase in risk [76]. Exposure to cigarette smoke carries a two- to threefold increase in risk [77]. Treatment with TNF-blockers and diabetes mellitus also confer increased risk. It is interesting to note that all these conditions are associated with impaired autophagy: HIV-infected cells block autophagy in bystander macrophages via HIV-1 Tat and IL-10 in a Src-Akt and STAT3-dependent process [25]; cigarette smoke causes a

defect in autophagy in alveolar macrophages [78] and TNF induces autophagy [12]. Moreover, early type 2 diabetes is characterized by hyporesponsiveness to insulin and excessive levels of insulin and insulin has been shown to inhibit autophagy [79].

Clinical applications of autophagy

As increasing evidence emerges that autophagy plays a critical role in host immune responses to tuberculosis, the modulation of autophagy either directly or via upstream targets may result in improved outcomes for the millions of individuals infected with *Mtb*.

Vaccine development. A more effective TB vaccine is needed to achieve global TB elimination. The current TB vaccine is a live attenuated stain of *M. bovis*: BCG. BCG has variable efficacy, is only 50% effective in preventing tuberculous disease [80] and is not useful as a therapeutic vaccine in promoting the elimination of latent infection. One of the main barriers in designing an effective vaccine is that, as an intracellular organism, *Mtb* is hidden inside the macrophage, and antigens must be presented to T cells to elicit a response. Autophagic mechanisms for intracellular antigen processing onto MHC class I and class II for enhanced presentation to T cells have been identified. Thus, a vaccine designed specifically to elicit a strong autophagic response may prove more effective at preventing infection and/or promoting elimination or improved control of latent infection with *Mtb*.

Immunotherapy: targeting autophagy. Recent years have seen an explosive growth in the incidence of drug resistant *Mtb*. In some parts of eastern Europe, up to 50% of TB cases are multi-drug resistant (MDR-TB) [3]. Worldwide, almost one in four cases of MDR-TB results in death [81]. Recent years have also seen numerous outbreaks of extensively drug-resistant TB (XDR-TB), associated with up to 98% fatality rates [82]. The anti-microbials used to treat MDR and XDR-TB are toxic, slow-acting and often ineffective. Immunotherapy which stimulates autophagy could be an answer to the difficulty of treating patients with disease for which there are no good anti-microbial drugs.

Adjunctive immunotherapy could also prove useful in shortening the duration of tuberculosis treatment. The current treatment regimen for active tuberculosis is a course of three or four antibiotics, given for a minimum of 6 months. Side effects are common, and up to half of patients fail to adhere to this protracted course of treatment [83]. A minimum of three anti-tuberculous antibiotics are used to treat tuberculosis. Rifampicin is the most potent antibiotic, but interacts with other medications; rifampicin interactions with anti-retrovirals used to treat HIV are particularly problematic. Adjunctive immunotherapy with autophagy-promoting agents could potentially shorten the duration of treatment and improve adherence. It could also enable the use of rifamycin-sparing regimens, which would not affect HIV medications.

Given the potent effect of induction of autophagy in promoting the intracellular killing of Mtb *in vitro* [20], therapy with an inducer of autophagy may prove valuable as a therapeutic strategy for infection with *Mtb*. Options would include mTOR inhibitors, including rapamycin (sirolimus) and everolimus, both of which are currently licensed for clinical use to prevent transplant rejection. Aerosolized administration of these drugs, possibly in combination with nanoparticles to enable targeting to macrophages, could maximize efficacy and minimize systemic side effects. Another option would be to target the mTOR-independent, D-myo-inositol-1,4,5-trisphosphate (IP3)-regulated pathway which induces autophagy. Lithium, carbamazepine and sodium valproate, used to treat mood disorders and epilepsy, activate this pathway [84], and may be amenable to use as adjunctive treatment of tuberculosis [85]. Alternatively, targeted administration of autophagy-promoting cytokines, such as TNF-α and IFN-γ, could prove effective. Indeed, adjunctive immunotherapy for drug-resistant TB with aerosolized IFN-γ has been trialled with some success [86]. Suppression of IL-10 or the Th2 cytokines IL-4 and IL-13 is another potential approach to promoting autophagy. Ghadimi *et al.* demonstrated that infection of peripheral blood mononuclear cells treated with heat-killed *Mtb* with lactic acid bacteria (LAB) resulted in decreased secretion of IL-4, IL-13 and IL-10 and increased secretion of IFN-γ, along with increased autophagosome formation [87]. *In vivo*, oral treatment with lactobacilli may be sufficient to down-regulate the Th2 response, as this has been shown to down-regulate the lung Th2 response in mice [88] and has been found to improve lung immunity in humans [89]. Other approaches to suppressing Th2 cytokines include helminth-derived immunomodulators [90].

Paradoxically, when tuberculosis is treated, patients' symptoms may worsen, due possibly to increased proinflammatory responses to dead mycobacteria [91,92]. This 'paradoxical reaction' can cause serious clinical complications, such as compression of the airways in patients with tuberculosis in neck lymph nodes. The inflammatory response to *Mtb* is particularly problematic in patients with TB meningitis, and can cause stroke and death. Steroids are used to treat paradoxical reaction and TB meningitis, but are not very effective [93] Autophagy-promoting treatments could potentially limit the production of proinflammatory IL-1β [29] yet promote the clearance of dead mycobacteria, and thereby reduce the overactive inflammatory response.

Autophagy as a predictor of LTBI to TB progression? Latent TB infection (LTBI) represents a reservoir from which active disease and subsequent transmission can propagate, particularly when the immune system is compromised. It is estimated that rates of LTBI in the community need to be less than 1% to allow TB elimination [94]. At present, there are

no accurate tests to predict which of the 2 billion individuals with LTBI will fail to contain the infection and progress to active tuberculosis. Individual testing for genotypes associated with a reduced risk of active tuberculosis, such as autophagy gene variant immunity-related GTPase M (IRGM)-261T [95] and Mal S180L [96], may enable clinicians to target treatment for LTBI to patients at a higher risk of progression.

Conclusion

Autophagy plays a key role in immune responses to mycobacteria; it kills intracellular mycobacteria, enhances antigen presentation and modulates the secretion of important cytokines. Moreover, genomewide analysis of host responses to infection with *Mtb* indicates that survival of the bacilli hinges on its ability to modulate autophagy. Thus, autophagy offers an attractive therapeutic target. Agents that promote autophagy might prove efficacious as an adjunctive treatment for drug-resistant and drug-sensitive tuberculous disease. They might also be used to target latent tuberculosis. In addition, vaccines which specifically stimulate autophagy could prove more effective in protecting against tuberculosis. Effective treatment for tuberculosis could save as many as 1·7 million lives every year: the stakes are high, and autophagy could be a trump card.

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