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Interaction of *Mycobacterium tuberculosis* with the Host Cells: A Focus in the Molecular Mechanism Involved in Trafficking and Autophagy

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INTRODUCTION

Tuberculosis (**TB**) is an ancient disease remaining a serious health threat worldwide. It is caused by *Mycobacterium tuberculosis* (**Mtb**), an acid-fast bacilli, non-sporulated, slow-growing, immobile and aerobic. The pathogenesis of the disease is based on its ability to multiply and survive within phagocytic cells of the host, particularly macrophages and monocytes. The majority (90%) of infected humans have a "latent infection", meaning they efficiently contain but do not spread the bacteria; they are infected but asymptomatic and not contagious. However the remaining 10% have a lifetime risk of reactivating the infection and developing active tuberculosis [1].

Human Mtb infections usually begin by inhalation of aerosol droplets containing tubercle bacilli expectorated in the cough of an individual with active pulmonary disease. A single aerosol droplet can contain from 1 to 400 bacilli being the reported infectious dose between 1 and 200 bacilli [2,3]. The bacilli travel to the alveoli, where they are phagocyted by resident macrophages. Once internalized Mtb resides in a phagosome by blocking maturation, lysosomal fusion, and acidification [4]. These infected macrophages are stimulated to produce pro-inflammatory

cytokines and chemokines driving the recruitment of uninfected macrophages and neutrophils, beginning to organize the granuloma formation [5,6]. Finally, a well-organized granuloma develops, consisting of a core of infected macrophages, surrounded by epithelioid macrophages, foam cells, and multinucleated giant cells with peripheral lymphocytes surrounded by a fibrous capsule [7-9]. This structure is a fine balance between host containment of infection and protection of Mtb from IFN-γ-producing lymphocytes.

Granulomas have been seen in latent, active, and reactive tuberculosis [7]. Mtb achieves a persistent infection through rapid changes in its gene expression profile, in order to counteract the biological and immune processes of host cells, such as antigen presentation, pro-inflammatory cytokine secretion and maturation of phagosome [10]. In latent tuberculosis, the bacilli can stay dormant for months to years without causing disease. The immune response can keep the pathogen inactive during this latent period. In active tuberculosis, the granulomas are more numerous and incapable of controlling the infection; bacteria, either extracellular or within macrophages or dendritic cells, then spread throughout the lung or disseminate to other organs, initiating new granuloma formation [11]. Mtb orchestrates a complex set of immune responses in humans, with the most common outcome being lifetime control of the infection. However, when the balance of immune responses is disturbed, primary tuberculosis or reactivation of latent infection can occur.

The great destructive impact on public health, the co-infection with the human immunodeficiency virus (**HIV**) and the appearance of drug resistant strains of Mtb are demanding the development of new tools for prevention and treatment.

During the last decade a greater understanding on the human immune response to Mtb infection as well as the contribution of factors linked to the pathogenesis of the disease has been achieved. Although the knowledge about the human immune response against Mtb as well as the contribution of factors linked to the pathogenesis of the disease have markedly increased in the last year, a deeper understanding of its immunopathogenesis will lead to the identification of new drugs and the development of effective vaccines.

THE ENDOCYTIC PATHWAY IN NON-INFECTED CELLS

The endocytic pathway of mammalian cells consists of distinct membrane compartments, which internalizes molecules from the plasma membrane and recycles them back to the surface (as in early endosomes and recycling endosomes), or sorts them to degradation (as in late endosomes and lysosomes). During these processes, endosomal compartments undergo maturation from early to late endosomes, which involves several changes as decreasing luminal pH, modification of major phosphatidylinositol lipids trough regulation by lipid kinases and phosphatases, and differential recruitment and activation of Rab GTPases [12]. In addition, as part of this maturation process leading to the fusion with the lysosomes, certain proteins of the endosomal membrane are excluded and there are changes in the lipid content [13]. There are two key Rab proteins in the initial steps of the phagosome formation and the subsequent maturation of the phagosome

and fusion with the lysosome: Rab5 and Rab7 [14]. The identity of the endosomal compartment is considered to depend on the Rab recruited to its membrane as well as on their phosphatidyl inositol phospholipid composition. Thus, the vacuole is a phagosome/early endosome when is enriched in the small GTPase Rab5 and the vacuolar-sorting protein-34 (**VPS34**), a lipid kinase that generates phagosomal phosphatidylinositol-3-phosphate (**PtdIns3P**) [15]. The early endosome antigen-1 (**EEA1**) is a Rab5 effector that facilitates endosome fusion [16,17].

As part of endosomal maturation an exchange of Rab5 by Rab7 occurs, modifying the membrane identity that becomes a late endosome [12,18,19]. This process also requires lysosome-associated membrane proteins (**LAMP**) 1 and 2 [20]. Furthermore, as endosomes mature, additional V-type ATPases recruitment decreases the intraluminal pH [12]. Finally, the phagosome/late endosomes fuses with lysosomes in the perinuclear region [21]. The lipid profile of the late endosome and lysosomes is converted to PtdIns (3,5) P2 by the PtdIns3P by PtdIns (3) P5-kinase.

The phagolysosome pH drops even more, to approximately 4.5, generating deadly reactive oxygen species (**ROS**) and activating proteolytic cathepsins [22]. Besides, the lysosome also contain antimicrobial peptides, NO^-, and proteins required to deprive microbes from cofactors and to preserve its low pH [23].

It is widely describe that many intracellular pathogens stop trafficking to elude destruction and create a safe environment to adapt to a life within a host vesicle [24].

THE JOURNEY OF *M. tuberculosis* INTO THE CELL

Recognition and Internalization

Once in the lungs parenchyma, complement-opsonized bacteria are engulfed by resident alveolar macrophages that recognize specific pathogen-associated molecular patterns (**PAMPs**) [25-28]. The internalization occurs in a zipper-like process involving several ligands and phagocytic receptors interacting with a tightly fitting pseudopodia formation that extends around the bacteria [29]. Cholesterol-rich lipid domains may also drive selective entry, and likely have an effect on the signaling response to attachment as well on the formation of Mtb containing phagosome [30]. Generally, after phagocytosis by macrophages, the bacteria-containing phagosome may fuse with LAMP-1-positive lysosomes to generate a phago-lysosome. Intracellular pathogens like Mtb avoid lysosomal fusion through the manipulation of host signal transduction pathways and other cell molecules [31].

Mtb produces and releases antigens common to all bacteria including components of the peptidoglycan cell wall and nucleic acids. However, Mtb cell wall generates unique antigens specific to mycobacterial species. These include lipomannan (**LM**), lipoarabinomannan (**LAM**) and its mannosylated form (phosphatidyl-myo-inositol mannoside: ManLAM), lipoproteins, phthiocerol dimycocerosate (**PDIM**), and mycolic acids [11]. Mycobacterial components are recognized by Toll-like receptors (**TLRs**) in particullary TLR-2 and TLR-4. These receptors

are overexpressed during infection [32,33]. Mtb also secretes effector proteins either via the generalized Sec secretion system or the specialized ESAT-6 (**ESX**) secretion system, and some of these secreted proteins can be recognized by pattern recognition receptors (**PRRs**) [28].

The family of TLRs is characterized by a leucine-rich repeat that recognize a wide variety of PAMPs from all types of infective microorganisms. TLR2 has a main role in detecting Mtb but also TLR4 and TLR9 can detect this pathogen [1,32,33]. Downstream of the Mtb PAMPs- TLRs recognition an intracellular signaling cascade is generated which depends on the recruitment of IL-1 receptor-associated kinases (**IRAK**), TNF receptor-associated factor 6 (**TRAF**), TGF β-activated protein kinase 1 (**TAK1**), and mitogen-activated protein (**MAP**) kinase by the adaptor protein myeloid differentiation primary response protein 88 (**MyD88**) [34]. The pathway leads to the activation of the transcription factor NFκB that translocate to the nucleus to drive the expression of multiple pro-inflammatory cytokines including tumor necrosis factor (**TNF**), interleukin-1β (**IL-1β**) and interleukin-12 (**IL-12**) [31,35]. Following secretion of TNF and IL-12 the production of IFN-γ from neighboring natural killer (**NK**) and T cells is stimulated. Thus, IFN-γ activates macrophages to enhance antigen presentation and promote anti-mycobacterial effector mechanisms such as the production of reactive nitrogen intermediates (**RNI**), reactive oxygen intermediates (**ROI**), phagolysosome fusion and acidification and autophagy [36,37].

M. tuberculosis Interaction with the Endocytic Pathway

Phagolysosome fusion is a very important mechanism host cells use to combat infection and to hamper the survival of intracellular pathogens. Mtb avoids digestion by lysosomal enzymes by halting its progression along the phagosomal pathway at the early endosome stage [13,38,39]. The Mtb-containing vacuoles acquire endosomal markers but consequently inhibit phagosomal maturation or lysosomal degradation. The V-ATPase is specifically excluded from its vacuole and the fusogenic capacity with late endosomes and lysosomes is disabled [40]. The resulting parasitophorous vacuole has near-neutral pH and is deprived of reactive oxygen species and degradative enzymes.

The advantaje of *Mycobacterium* on residing in the phagosomal compartment is that the interaction with early endosomes is not affected allowing it to acquire essential nutrients for replication while evades fusion with degradative compartments. Mtb orchestrates its trafficking arrest through the production of several key virulence factors that interfere with calcium flux, host membrane fusion events, and recruitment of inducible nitrous oxide synthase (**iNOS**) [41].

Soon after entry, the early endosome containing Mtb acquires Rab5 and facilitates the recruitment of its effector proteins EEA1 (early endosomal autoantigen 1) and PI3K [42,43]. In addition, Rab34, Rab22 and Rab23 GTPases are recruited as well, the first one in high percentage respect to the others. Rab22 and Rab23 are specific to early endosomes/phagosomes and, their downregulation indicates maturation of the compartment [44]. In Mtb containing-phagosomes, Rab7 is altered or absent because of inhibition of Rab5-Rab7 switch [39,45]. Thus, the Mtb

containing-phagosome recruits and maintains Rab5 and Rab22a to avoid Rab7 acquisition and inhibiting phagolysosome biogenesis [46] (Figure 1).

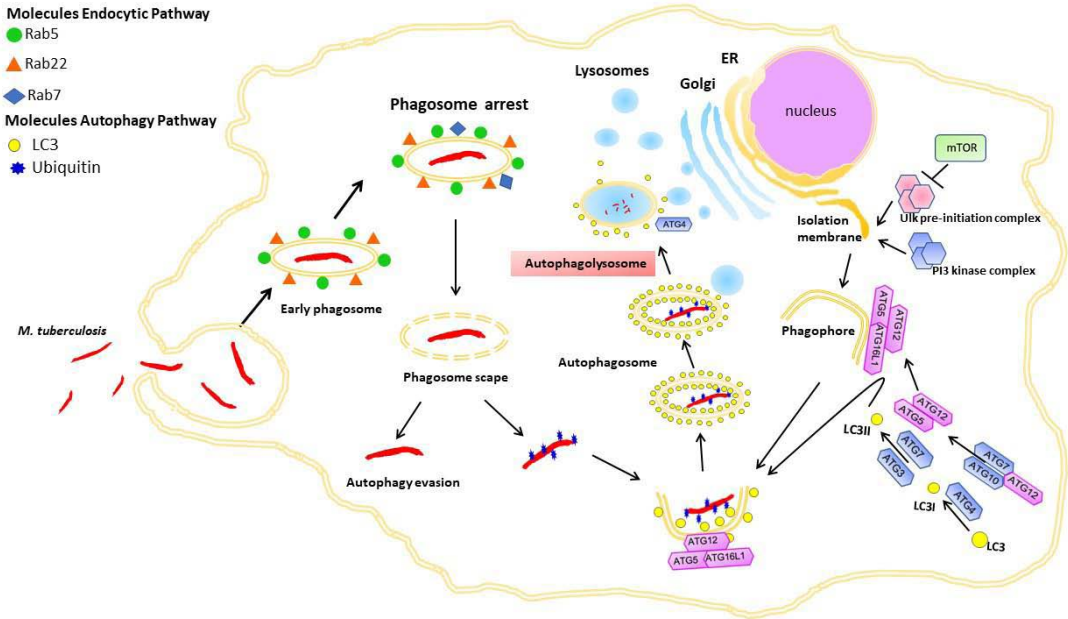

Figure 1: Interaction of *M. tuberculosis* with the host cell. Once Mtb is phagocytosed by macrophages, there are several scenarios the mycobacteria may face. a) Mtb resides within the host cell by interfering with phagosome maturation blocking Rab5 (early endosome)-Rab7 (late endosome) conversion and maintaining Rab22 labelled early phagosomes (phagosome arrest). On the other hand, a few Mtb-containing phagosomes carrying Mtb may fuse with lysosomes, eliminating the bacteria (not shown). c) Mtb may disrupt the phagosomal membrane and escape from the phagosomal compartment triggering xenophagy. d) Xenophagy begins with the isolation of the ER membrane by the action of both Ulk1 pre-initiation complex and PI3 kinase complex. As a result of the action of ATG12-ATG5-ATG16L1 the phagophore is formed, which then, will be modified with LC3II to capture Mtb. Subsequently, the autophagosomes fuse with lysosomes to form autophagolysosomes that finally remove the bacteria.

The block on the canonical trafficking pathway is the result of two converging pathways: calcium signaling and recruitment of EEA1, both regulated by the Mtb LAM [47]. The LAM of Mtb has a cap of mannose, and is therefore referred to as ManLAM [48]. In macrophages, the levels of cytosolic Ca^{2+} are increased after phagocytosis to activate calmodulin that binds with Ca^{2+}/calmodulin protein kinase CaMKII, and stimulate maturation of the phagolysosome [49]. The output of this signaling cascade is to recruit VPS34 to the phagosome, this produce PtdIns3P required for EEA1 recruitment [50]. EEA1 is necessary to bind syntaxin 6, a SNARE that delivers V-ATPase and cathepsins from the trans-Golgi network to endosomes [43]. Through inhibition of the cytosolic Ca^{2+} increase, caused by Mtb infection, and through p38 mitogen-activated protein

(**MAP**) kinase activation, ManLAM contributes to privation of VPS34 on the Mtb containing-phagosomes and reduction in Rab5 levels on early endosomes and EEA1, and likely preventing EEA1 association [43,50,51]. The negative effect that ManLAM has on the activities of both signaling pathways results in the exclusion of V-ATPase of the, preventing from acidification and acquisition of lysosomal hydrolases [13,48].

One process required for the host defense in phagocytes involves the intracellular trafficking of hydrolases to the phagosome from several organelles. Sortilin, also known as neurotensin receptor 3 (**NTR3**) is a transmembrane receptor that transports lysosomal proteins from the trans-Golgi network into lysosomes, as an alternative route to mannose-6-phosphate receptors. In that regards, it has been described a role of the proneurotrophin receptor sortilin during phagosome maturation and mycobacterial killing, showing that the phagosomal association of sortilin is critical for the delivery of acid sphingomyelinase and required for efficient phagosome maturation. Furthermore, *in vitro* and *in vivo* assays revealed to sortilin as a pathway required for optimal intracellular mycobacteria control and lung inflammation [52].

In addition, patients with active Tb have increased levels of Rab20 GTPase. According to [53], Rab20 upregulation allows to generate spacious phagosomes that will be able to fuse with late endocytic compartments generating proteolytic phagolysosomes. Together with the production of IFN-γ maintains the integrity of the Mtb phagosome and controls Mtb replication to achieve an effective bacterial clearance [53].

M. tuberculosis Resources to Inhibit Phagosomal Maturation

In addition to the expression of ManLAM, Mtb employs other virulence factors in an effort to preserve the early endosomal characteristics of the Mtb containing-phagosomes [28]. Another lipid produced by Mtb can delay phagosomal acidification via an unknown mechanism [54]. In addition to lipids, a variety of protein "effectors" have been identified, via transposon library screens, as being involved in arresting phagosomal maturation. Ndk is a nucleoside diphosphate kinase, which is secreted into growth medium and is cytotoxic to macrophages [55]. Based on *in vitro* and biochemical data, Ndk appears to dephosphorylate cellular Rab7-GTP and Rab5-GTP, thereby preventing Rab7-dependent heterotypic fusion of the Mtb containing-phagosomes . PtpA is a low-molecular weight tyrosine phosphatase that dephosphorylates VPS33B, a host protein involved in regulation of membrane fusion in the endocytic pathway. PtpA also binds to the H subunit of V-ATPase, inhibiting its ability to acidify vacuoles [56]. The above examples of Mtb products that participate in the regulation of intracellular trafficking of the Mtb containing-phagosomes are only a subset of the identified bacterial factors that may play a role in this complex process [28].

The primary survival strategy of Mtb is to quickly stall the progression of its internalized phagosome before it becomes too hostile to inhabit. It orchestrates a delicate balance between blocking of phagosomal fusogenicity with the ability to interact with early endosomes as a source

of essential nutrients (Figure 1). While the arrested phagosome is free of degradative cathepsins and excludes acidifying V-ATP ase, the niche that Mtb occupies is not devoid of host defenses. Mtb counters the vigorous oxidative burst of the infected macrophage by direct detoxification and indirectly through the expression of protective chaperones.

Mycobacterial phagosomes are also prevented from association with inducible nitric oxide synthase (**iNOS**), thereby limiting exposure to damaging nitrogen radicals [1,57]. This protective environment also prevents effective antigen processing of Mtb [1,58].

M. tuberculosis Scape to Cytosol

Although still controversial a growing body of evidence supports the fact that under certain circumstances Mtb can escape from the phagosome to reside in the cytoplasm and induce immune responses. Furthermore, it has been demonstrated that vacuole rupture by Mtb elicits host cell death [59].

Mtb rely on types of specialized protein export system: the ESX systems and the accessory SecA2 system [60,61]. The ESX-1 secretion systems is responsible not only to block phagosome maturation in Mtb-infected macrophages but also the cytosolic contact (early) and phagosomal scape (late) require of this specialized secretion system [62,63]. Mtb uses the ESX-1 to translocate its effectors into the host to modulate host-cell functions. The ESX-1 specialized secretion system of Mtb is encoded by RD1 genes (region of difference 1) and its surrounding region and together constitute the extRD1 (extended RD1). RD1 contains nine genes in *M. tuberculosis* (Rv3871-Rv3879c) that are removed from the vaccine strain *M. bovis* BCG. Two proteins encoded within RD1, CFP-10 and ESAT-6, are known to be secreted by this specialized secretion system. There is continuo proof that ESAT-6 perturbs membranes by producing regulated perforation of the lipid bilayer early after infection to generate "holes" in phagosomal membranes (Figure 1). However, the precise mechanism of pore formation by ESAT-6 remains unknown.

Recent findings from Jamwal et al suggest that phospholipase A2 (**cPLA2**) activity contributes to ESAT-6 function in regulating the escape of Mtb from the phagosome. This goes in agreement with the key role that cytosolic cPLA2 enzymes play in both phagosomal trafficking and cargo export from the various endocytic compartments [64-66]. The contribution of cPLA2 helps to explain the specific differences between Mtb strains on how they resist against phagosomal stresses according to their distribution (vesicle versus cytosolic), number of bacteria (per compartment) and the temporal space of the process (kinetics) [67].

Works from the groups of Rao and Lerm allows envision that certain strains of mycobacteria are capable to undergo a phenotype switch as alternative strategy to counteract the phagosomal stresses in order to survive, replicate, and spread [67,68]. Conversely, macrophages rely on autophagy (see below) to eliminate intracellular bacteria [69-73]. The cornerstone of Mtb-cytosolic localization is related to an enhanced capacity to resist autophagy. Then, the capability

of translocation is not other than an effective adaptation of Mtb to subsist within the macrophage. It is widely known that some intracellular pathogens have the ability to move within the cell as an important mechanism for cell-cell transmission along with autophagy evasion (see below) [127-129].

ROLE OF AUTOPHAGY IN *M. tuberculosis* PATHOGENESIS

The eukaryotic organisms rely on several degradative pathways for cellular components. An example is the autophagy, a dynamic process that delivers cytoplasmic portions or specific cytosolic targets to lysosomes for degradation or removal [74]. There are different types of autophagy, clasified as selective or non-selective, that depends on the inducing signals, the temporal aspects of the induction, type of cargo and mechanism of sequestration [75]. Autophagy, also known as macroautophagy, is characterized by the use of ATG proteins that are encoded by autophagy-related (Atg) genes and constitute the core of the molecular machinery of autophagy [76]. In morphological terms it is characterized by exhibiting in the cytoplasm double membrane organelles called autophagosomes that capture cytosolic components and fuse with lysosomes for their processing and elimination [77].

Autophagy is involved in several physiological and pathological processes. Moreover, there is experimental evidence supporting that autophagy is a genuine immunological process [78,79]. In particular, xenophagy, a type of selective macroautophagy, specifically targets intracellular pathogens to lysosomes, restricting their replication and survival [80]. This is induced by cytoplasmic PAMPs or damage-associated molecular patterns (**DAMPs**), once a bacterial pathogen either escapes into the cytosol or is exposed to the cytosol through damage of the vesicle in which it resides [70,81-84].

Xenophagy involves the following steps: initiation, elongation, substrate targeting, and maturation/lysosomal fusion resulting in degradation of cargo [76,80]. The initiation of xenophagy begins with the sequestration of bacteria into a membranous structure denominated phagophore (Figure 1) [76,85]. Ubiquitin is recognized and bound by autophagy receptors such as p62/SQSTM1, NBR1 (neighbor of BRCA1 gene 1), NDP52 (nuclear dot protein 52 kDa), and optineurin (**OPTN**) and then, these adaptors will interact with LC3 to recruit the bacteria to autophagosomes [79]. Interestingly, it has been reported that these adaptors could be phosphorylated by several bacteria and thus regulate autophagy [86-88].

Phagophore formation is mediated by translocation of the ULK1 complex (ULK1/ULK2, ATG13, FIP200, ATG101) from the cytoplasm to the endoplasmic reticulum (**ER**) thought to be the source of the autophagosome membrane. The ULK1 complex recruits the autophagosome-specific phosphatidylinositol 3- kinase (**PI3K**) complex consisting of ATG14L, BECLIN1, VPS15, and VPS34 [76,85]. Phosphatidylinositol 3-phosphate (**PI3P**), (which is not usually located in the ER) is produced by the PI3K complex, being this essential for canonical autophagy [76,79,89] (Figure 1).

Two ubiquitin-like conjugation systems are reponsible for the autophagosomal double membrane elongation. In the first system, ATG12 (ubiquitin-like protein) is synthesized with a glycine exposed on its C9 terminal, activated by ATG7 (E1-like), and moved to ATG10 (E2-like), which promotes its final ligation to ATG5 [76,79,89]. The interaction of ATG5 with ATG16L1, results in an ATG5-ATG12-ATG16L1 complex that is present on the phagophore and elongating membrane, after completion of the autophagosome formation this complex is dissociated [76,90,91]. The second ubiquitin-like component is LC3 (microtubule-associated protein 1 light chain 3) [76,79,89]. The LC3 is produced as a pro-protein (pro-LC3) that is rapidly processed by ATG4 (a cysteine protease) generating a cytoplasmic form named LC3-I. Then, by the action of ATG7 (E1-like) and ATG3 (E2-like) proteins, LC3-I is conjugated to phosphatidylethanolamine (**PE**), producing LC3-II (membrane-bound form). Lipidation of LC3 is facilitated by ATG5-ATG12 through interaction with ATG3; while ATG16L1 specifies the localization of LC3 conjugation to the autophagosome membrane. Upon completion of the autophagosome membrane, ATG4 facilitates autophagosome maturation and LC3 recycling by removal of external LC3, being LC3 on the interior of the autophagosome inaccessible to ATG4 and degraded. Autophagosome maturation is associated with the participation of several SNAREs [92-95]. For example, the SNARE Syntaxin 17 induces the fusion with lysosomes, generating an autolysosome (terminal degradative organelle) where the cargo is captured and finally degraded [78,94,95] (Figure 1).

AUTOPHAGY AND IMMUNE MEDIATORS DURING *M. tuberculosis* INFECTION

It has been shown that xenophagy plays an important role during Mtb infection. This type of selective autophagy targets intracellular pathogens to lysosomes, damaging their replication and survival [80]. Once the bacterium invades the host cells, can replicate in infected cells by arresting phagosome maturation and then potentially escaping into the cytosol inducing autophagy [96]. The PAMPs recognition through specific receptors called pathogen recognition receptors (**PRRs**) by host cells, induce signaling cascades that lead among other responses to the activation of autophagy [70,97-100]. For example, the second messenger 20-50 cyclic GMP-AMP (**cGAMP**) generated by mammalian cGAMP synthase in response to the presence of mycobacterial cytosolic DNA or secreted bacterial 30-50 cyclic-di-GMP or cyclic di-AMP can stimulate autophagy and ULK1 [82,101].

Autophagy responds to innate immunity signals and cytokine stimulation during immune responses [78]. In particular, Th1 cytokines activate autophagy to kill intracellular Mtb [102]. In this regard, it has been described that the INF-γ production induces autophagy activation in cultured macrophages to promote trafficking of Mtb to the lysosome and subsequent killing [73]. Interestingly, it has also been demonstrated that in cells from patients with active tuberculosis there is a high correspondence between the levels of IFN-γ as well as IL-17 with the autophagy response. In both cases, there is a correlation between the levels of these pro-inflammatory

cytokines with the severity of the disease [103,104]. Also, it has been studied that autophagy activation by IL-1β through its receptor signaling, named IL-1 Receptor, together with the Myd88 adapter protein is critical for the early control of Mtb infection [87,105-111].

Several studies have demonstrated that autophagy factors including LC3 are targeted to a subset of intracellular Mtb bacteria [80]. Also, there are reports that demonstrate that cells knockdown for different genes related to autophagy may result in improved Mtb survival [82,112,113]. In this context, mice lacking Atg5 in myeloid derived cells (Atg5fl/fl LysM-Cre) died to Mtb infection within the first 40 days of infection, associated with higher bacterial burden and more severe inflammation in lungs than in control mice [77,114]. The role of PARKIN, an ubiquitin ligase important for mitophagy in controlling Mtb infection has been described. Approximately 12% of the protein colocalizes with Mtb and loss of the gene encoding PARKIN resulted in an increase in Mtb survival both *in vitro* and *in vivo* [83].

M. tuberculosis NEGATIVELY MODULATES AUTOPHAGY

Although the eukaryotic cells have mechanisms to control Mtb infection, several virulence factors of this bacteria can modify cell signaling, vesicle trafficking and autophagy death, contributing to evade the host defense [115].

In cultured murine macrophages, it has been observed that ManLAM besides interfering with phagolysosome fusion, can alter mammalian target of rapamycin (**mTOR**) signaling and ULK1-activation through to the inhibit Ca^{2+} influx [50]. This observation is in agreement with experimental evidence that demonstrate that ManLAM-coated beads decrease LC3-targeting in cultured murine macrophages [116].

The reactive oxygen species (**ROS**) activate autophagy in cultured murine macrophages and Mtb can interfere with the ROS formation. In that regard, Eis, a N-acetyltransferase of Mtb, decrease the ROS by inactivating JUN N-terminal kinase (**JNK**) blocking the autophagy activation [117,118]. Also, it has been described that Mtb alters the autophagic machinery through ESAT-6 impairing autophagy at the step of autophagosome-lysosome fusion. While Mtb H37Rv blocks the autophagy flux, attenuated strains that have a functional inhibition of the ESAT-6 as Mtb H37Ra or BCG were unable to hamper autophagosome maturation. This ability to inhibit autophagy was restored in recombinant BCG and Mtb H37Ra strains with ESAT-6, demonstrating that the capacity to inhibit autophagy is highly dependent on ESAT-6 expression [119,120].

It has been also established that Mtb is an intracellular bacterium that can survive within macrophages, associating this survival with the host factor called Coronin 1a [71,121,122] a member of the coronin family associated with F-action [121]. Later, it has been described that autophagy was involved in the inhibition of mycobacterial survival in Coronin1a knockdown macrophages. In fact, Mtb can recruit Coronin1a to phagosomes inhibiting autophagosome formation within cultured murine macrophages [123].

ANTITUBERCULOSIS THERAPY

The treatment against tuberculosis consists of a cocktail of first-line drugs, including isoniazid and pyrazinamide [124,125]. Because Mtb is a major threat to human health and the increasing worldwide prevalence of antibiotic-resistant strains, it has raised significant interest in developing host-directed therapies that harness the immune response to control bacterial infection as an alternative therapeutic strategy that together with antibiotic therapy, allow the control of Mtb infections. One attractive cellular target for immunotherapy is the stimulation of autophagy [126]. Thus, it has been demonstrated that isoniazid and pyrazinamide promote autophagy activation and phagosomal maturation in Mtb infected host cells. In addition, *atg7* mutant flies (autophagy-defective) infected with *Mycobacterium marinum* exhibited decreased survival rates and could not be rescued by antimycobacterial treatment, indicating that autophagy is required for effective antimycobacterial drug action *in vivo* [118]. The autophagy activation by anti-tuberculosis drugs is an interesting therapeutic alternative to generate complementary treatments that, in combination with antibiotic therapies, will counteract more efficiently this ancient disease.

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