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Mycobacterial manipulation of the host cell

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Abstract

Phagosome biogenesis, the process by which macrophages neutralize ingested pathogens and initiate antigen presentation, has entered the field of cellular mycobacteriology research largely owing to the discovery 30 years ago that phagosomes harboring mycobacteria are refractory to fusion with lysosomes. In the past decade, the use of molecular genetics and biology in different model systems to study phagosome biogenesis have made significant advances in understanding subtle mechanisms by which mycobacteria inhibit the maturation of its phagosome. Thus, we are beginning to appreciate the extent to which these pathogens are able to interfere with innate immune responses and manipulate defense mechanisms to enhance their survival within the human host cell. Here, we summarize current knowledge about phagosome maturation arrest in infected macrophages and the subsequent attenuation of the macrophage-initiated adaptive anti-mycobacterial immune defenses.

Keywords: Myobacteria; Tuberculosis; Kinases; Phagosome maturation

Contents

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1. Introduction

Mycobacterium tuberculosis constitutes a growing health concern causing up to 3 million deaths per year and an estimated 1000 million new infections by 2020 [1]. Co-infection with the human immunodeficiency virus makes tuberculosis one of the most common causes of death among people dying with AIDS. Additionally, the emergence of multi drug resistant strains, leading to a more severe infection, continues to raise the death toll. Twelve years have passed since the World Health Organization (WHO) declared tuberculosis (TB) a global health emergency. Since then, intensive efforts to control the spread of TB through supervised medication have been successful, but we are still desperately in need of new means to combat this disease.

Mycobacteria primarily infect host macrophages, which represent the first line of cellular defense against microbial invasion. Pathogenic species of mycobacteria have developed strategies to circumvent the major killing mechanisms employed by macrophages and take advantage of the enclosed environment within its host cell to avoid the antibody and complement mediated humoral immune response. The ability of mycobacteria to survive within macrophages has been one of the main foci of mycobacterial research over the past years. We have gained an increased understanding of how mycobacteria not only have the ability to adapt to a changing host environment [2], but also actively interfere with the signaling machinery within the host cell to counteract or inhibit parts of the killing apparatus employed by the macrophage.

The infected macrophage shows impaired antigen processing [3], reduced responsiveness to interferon-γ (IFN-γ) [4], and reduced production of cytokines as well as reactive oxygen and nitrogen intermediates [5]. The observation that attenuated strains of mycobacteria, such as H37Ra and *Mycobacterium bovis* BCG are significantly more potent inducers of apoptosis than the corresponding virulent strains, H37Rv and wild-type *M. bovis* [6], has suggested that apoptosis functions as a host defense mechanism which is suppressed by virulent strains. Mycobacteria have also been shown to up regulate the production of anti-inflammatory cytokines such as interleukin-10 (IL-10) to suppress host cell apoptosis [7]. Furthermore, virulent strains seem to manipulate the activation of the eukaryotic superfamily of mitogen-activated protein kinases to impair cytokine production or to stimulate anti-inflammatory responses (see [8] for review). This review will focus on mycobacterial interference with host cell defense mechanisms and will elaborate on some of the more recent findings related to mycobacterial-induced phagosomal maturation arrest. As the discussion of mycobacterial uptake coupled with cellular immediate response is a broad and independent field of research by itself, we focus our attention in this review on discussing post-uptake host cellular responses to mycobacterial infection.

2. The mycobacterial phagosome

Normally, macrophage activation results in a series of events specifically designed to induce killing of engulfed microorganisms. These include: (i) the gradual acidification of the phagosome due to the activity of a proton–ATPase pump located in the phagosomal membrane, (ii) phagosome–lysosome fusion, which loads the resulting phagolysosome with proteolytic enzymes, (iii) induction of reactive oxygen and nitrogen intermediates and (iv) antigen processing. The resulting acidic and otherwise lethal environment is effectively designed to eliminate the invader and present the immune system with its antigenic determinants on the surface of the macrophage. Contrasting with this scenario, Armstrong and Hart [9] showed, more than 30 years ago, that phagosomes containing *M. tuberculosis* were resistant to fusion with the later stages of the endosomal–lysosomal pathway. Since then, it has been established that *M. tuberculosis*, the vaccine strain *M. bovis* BCG and *Mycobacterium avium* all reside in compartments secluded from the terminal stages of the endocytic pathway [10–13]. This block in phagosomal maturation is today considered a hallmark of mycobacterial infection and is thought to represent one of the key mechanisms by which mycobacteria are able to avoid host killing and survive within macrophages.

Phagosomes containing inert particles interact with the endosomal pathway through transient contacts, escribed as a kiss-and-run mechanism where fusion and fission events facilitate the delivery of contents and membrane components between endosomes and phagosomes [14]. The phagosome thereby acquires a membrane composition, which is more or less mirrored by the endosomal compartment with which it interacts at a given time. Purification of endocytic organelles and analysis of coat proteins has highlighted differences in the membrane composition of mycobacterial phagosomes compared to that of early and late endosomes (Table 1) and helped us to better understand the interaction between these compartments. Phagosomes containing viable, virulent mycobacteria show the presence of early endosomal markers such as the transferrin receptor, major histocompatibility complex (MHC)-class II molecules, and the ganglioside GM1 [10,15]. However, in contrast to phagosomes containing killed mycobacteria or the non-pathogenic strain *Mycobacterium smegmatis*, those containing virulent, live organisms exclude late endosomal markers such as the proton ATPase [16], mannose-6-phosphate receptor [13] and the lysosomal protease Cathepsin D [10]. These observations have led to the understanding that mycobacterial

Table 1
Selected list of membrane markers present in different endocytic compartments

Coat proteins	Early endosomes	Late endosomes	Mycobacterial phagosomes	Reference
Actin	–	+	–	[37]
ATPase proton pump	–	+	–	[16]
Calmodulin	–	+	–	[24]
Cathepsin D	–	+	–	[10]
EEA1	+	–	–	[21]
Ganglioside GM1	+	–	+	[15]
LAMP1	–	+	+	[13]
M6PR	–	+	–	[13]
Procathepsin D	+	–	+	[68]
Rab5	+	–	+	[20]
Rab7	–	+	–	[20]
Syntaxin6	–	+	–	[22]
TACO	+	–	+	[17]
Transferin receptor	+	–	+	[68]

EEA1, early endosomal antigen1; LAMP, lysosomal associated membrane protein1; M6PR, mannose-6-phosphate receptor, Rab; ras-assiociated protein; TACO, tryptophan aspartate-containing coat protein; (+), present; (–), not present or reduced.

phagosomes interact with early endosomal compartments, while resisting fusion with later stages. The absence of the proton ATPase is thought to account for the reduced acidification of mycobacterial phagosomes [16], which equilibrate to a pH of 6.2–6.3, compared to a pH of 5.3–5.4 normally associated with endosomal compartments.

The actin-binding protein coronin-1 [17] has been reported to be transiently recruited to the phagosomal membrane. However, the analysis of purified bacillus Calmette–Guérin (BCG) phagosomes by Pieter's laboratory showed that coronin-1 (termed TACO) is actively retained on phagosomes containing live but not killed bacteria [18]. As coronin-1 is normally released prior to phagosome maturation into phagolysosomes [19], its retention on phagosomes was suggested as a marker of phagosome maturation arrest, which might also contribute mechanistically to this process. Conflicting with these findings, further investigations from Young's laboratory showed that coronin-1 is rather associated with early stages of mycobacterial phagocytosis but not with phagosome maintenance [20]. Therefore, more studies are necessary to confirm or refute the retention of TACO/coronin-1 on mycobacterial phagosome and if there is retention of coronin-1, it will be interesting to explain its functional significance.

3. Mycobacterial interference with macrophage trafficking events

The process of phagosomal maturation requires the assembly of a proper fusion machinery, which in part is determined by specific phagosomal coat proteins as well as cytoskeletal dynamics. The Rab-family of small GTPases plays an important role in this process by recruiting and facilitating fusion events between early and late organelles of the endocytic pathway. Two of the Rab proteins, Rab5 and Rab7, are normally only transiently associated with, respectively, early and late endosomal compartments (see [21] for review). Normally, Rab5 facilitates endocytosis and homotypic fusion between early endosomes while Rab7 regulates transport from early to late endosomes. Paradoxically, studies using murine macrophages transfected with dominant negative Rab5, Rab5(S34N), showed an increase in *M. avium* colocalization with markers of late endosomes/lysosomes and rapid mycobacterial killing [22]. Such a phenotype was reversed in cells supplemented with exogenous iron [22] suggesting that fusion with early endosomes is required for mycobacterial retention in early phagosomal compartments and that an inadequate supply of iron is one factor in mycobacteria's inability to prevent the normal maturation process in macrophages expressing inactive Rab5. In another line of investigations, Deretic and colleagues identified two Rab5 effectors, phosphatidylinositol-3 kinase (PI3K) and the tethering protein early endosomal antigen1 (EEA1) as necessary for maturation of phagosomes into late endosomes [23,24]. *M. tuberculosis* as well as the cell wall glycolipid, mannose-capped lipoarabinomannan (ManLAM) have been found to mediate the exclusion of EEA1 from the phagosomal membrane in correlation with reduced maturation [23]. By using brefeldin A, a component that disrupts the Golgi network and inhibits Golgi-derived traffic to the endosomal pathway, it was shown that phagosomes acquire components from the Golgi network through a route independent of endosomes [25]. In this context, it was shown that the ManLAM-induced EEA1 exclusion resulted from an inhibition of the sorting pathway from the *trans*-Golgi-network to phagosomes, and was seen in connection with inhibition of a PI3-kinase dependent pathway [25]. A block in this pathway can also explain the absence of phagosomal markers, such as the

vacuolar ATPase proton pump, Cathepsin D and the mannose-6-phosphate receptor on the mycobacterial phagosome [23].

Calcium is an important regulator of signaling pathways involved in phagosome–lysosome fusion and has recently been shown to affect phagosomal maturation [26,27]. Macrophages infected with live *M. tuberculosis* demonstrated a decrease in the elevation of calcium normally associated with phagocytic uptake [26]. Mycobacterial-induced inhibition of two principal calcium effectors, sphingosine kinase and calcium/calmodulin dependent protein kinase II (CaMII kinase) was associated with this observation [28]. Furthermore, inhibition of calcium was associated with reduced acidification of the phagosomal compartment. It was recently shown that also purified ManLAM from *M. tuberculosis* serves as a potent inhibitor of calcium increase during the early stages of infection [29]. Based on these observations, it was hypothesized that the inhibition of calcium increase is connected to the inhibition of EEA1-recruitment to phagosomal membranes [29]. In support of this, several observations were made: (1) calmodulin, necessary for the activation of CaMII kinase, promotes the association of phagosomes with EEA1; (2) the effects of calmodulin are PI3-kinase dependent; and (3) calcium is necessary for the interaction of PI3-kinase with calmodulin. Consequently, LAM-induced inhibition of intracellular calcium increase abrogates the activity of PI3-kinase, resulting in a block in the sorting pathway between the *trans*-Golgi-network and phagosomes. A recent report from the same group has suggested a role for the LAM precursor phosphatidylinositol mannoside (PIM) in facilitating fusion events between the mycobacterial phagosome and the early endocytic pathway [30]. In this manner, mycobacteria ensure delivery of nutrients to the phagosome, such as iron, that are essential for intracellular survival [31]. Together, these reports provide compelling evidence to suggest active mycobacterial interference with host signaling pathways to prevent the delivery of components critical for proper acidification and maturation of the phagosome, while at the same time ensure delivery of contents necessary for intra-phagosomal survival (Fig. 2). Screening of *M. tuberculosis* mutants revealed a series of mutants impaired in their ability to cause phagosome maturation arrest [32]. This, together with previous reports, including the involvement of a mycobacterial kinase [33] in blocking phagosome–lysosome fusion, suggest that this process involves more than a single mycobacterial factor.

4. Mycobacteria suppress MHC class II expression in macrophages

One of the most important functions of the macrophage is to initiate an adaptive immune response by presenting antigenic peptides to helper T cells. Recognition of the MHC class II associated antigens by the $CD4^+$ T-cell population induces cell activation, proliferation, and the subsequent secretion of various cytokines, which in turn amplify macrophage responses and recruit pro-inflammatory leukocytes. Antigen processing, transportation, and final presentation of the antigen in combination with MHC class II molecules involve a complex process that initially is induced by INF-γ, the principal activator of macrophage resistance to intracellular pathogens (see [34] for review). Macrophages infected with *M. tuberculosis* show a markedly reduced expression of MHC class II molecules [10,35–37]. A model in which mycobacteria interfere with the INF-γ signaling pathway to suppress the expression of MHC class II molecules has been proposed. Although results from several groups indicate that IFN-γ signaling from receptor binding to signal transducers and activators of transcription (STAT) phosphorylation, dimerization and nuclear translocation remains unaffected, there is evidence to suggest a mycobacterial-induced inhibition at the transcriptional level. For example, long-term exposure (48 to 72 h) to the purified mycobacterial 19-kDa antigen, has the capacity to partially inhibit IFN-γ-induced surface expression of MHC class II [38]. Moreover, recent findings suggest that this may be related to attenuation of MHC gene transcription by p19 [39]. Several regulatory mechanisms are involved in the expression of functional class II molecules. After transcription, MHC molecules are assembled in the endoplasmatic reticulum. In combination with the chaperone molecule, invariant chain, and the class II-associated invariant chain peptide (CLIP), the class II dimer is transported from the *trans*-Golgi-network to the MHC class II compartment. Although mycobacterial phagosomes show a "relatively intense staining for MHC class II molecules" [10], these class II molecules are likely already loaded with peptide and derived from the plasma membrane and not from the *trans*-Golgi network [40]. Degradation of the invariant chain and removal of CLIP depend on the activity of specific cysteine proteases and is necessary for proper antigen loading of the class II molecule prior to membrane presentation (see [41] for review). In this context, we have shown that *M. tuberculosis* infection of the human monocytic cell line THP-1 inhibited MHC class II expression by blocking the transport and processing of MCH class II molecules through the enodosomal/lysosomal system [35]. Additional findings from our laboratory showed that maturation of class II dimers – dependent upon removal of invariant chain and peptide loading – was reduced in infected cells by a mechanism dependent, at least in part, on alkalization of class II compartments by mycobacterial urease [42]. This finding indicates that MHC class molecules and

the associated invariant chain are successfully transported to the endocytic pathway and that the inhibitory effect exerted by *M. tuberculosis* on MHC class II expression occurs during their maturational processing rather than at earlier steps. The recent findings that mycobacteria specifically block a transportation pathway between the *trans*-Golgi network and the endocytic pathway has provided a likely explanation for the absence of the proton ATPase and certain lysosomal proteases on the mycobacterial phagosome [25]. Since maturation and peptide loading of MHC class II molecules are dependent on proteolytic activity of lysosomal proteases [43] a defect in the expression of these enzymes in class II compartments may also occur in infected cells. Indeed, recent findings in our laboratory (personal observation) showed that BCG inhibited IFN-γ induced cathepsin S activity in macrophages. Cathepsin S is a cysteine protease that plays a major role in the maturational processing of MHC class II, which precedes their export to the macrophage cell surface [44,45]. In this context we have observed that, inhibition of cathepsin S in infected cells correlated with a significant reduction in the expression of mature, peptide loaded, class II molecules, and increased the export of an immature (associated with invariant chain) class II population to the surface of macrophages.

5. Manipulation of host cell actin dynamics

Pathogenic species of mycobacteria have recently been found to interfere with the host cell's actin filament network [46,47]. Actin forms part of the eukaryotic cytoskeletal network and serves many cellular processes ranging from migration and membrane ruffling through phagocytosis and organ motility to the formation of stress fibers and cellular adherence. Several intracellular pathogens including *Salmonella*, *Yersinia*, *Shigella*, and enteropathogenic *Escherichia coli* depend on manipulation of the host cell cytoskeleton for their intracellular survival [48]. On this note, it is not surprising to find that species of mycobacteria interfere with cytoskeletal elements during intracellular infection of macrophages. Murine macrophages infected with *M. avium* displayed a time-dependent disruption of actin filaments in a similar manner to that observed after treatment of macrophages with the actin-depolymerizing agent, cytochalasin D [46]. This was the first report to propose actin filaments as a target for pathogenic species of mycobacteria. In a follow-up study, the mycobacterial-induced disruption of actin filaments was seen in connection with a delayed acquisition of phagosomal markers, indicating a resulting inhibitory effect on the endocytic transportation system [49].

We have argued for a role of the actin-binding protein α-adducin in mycobacterial infection based on increased α-adducin phosphorylation in cells exposed to whole bacteria (*M. bovis* BCG) or purified mannose-capped lipoarabinomannan [50]. Infection of cells leads to an increased distribution of phosphorylated adducin in the cytocolic fraction (Fig. 1A). This was further supported by western analyses showing a translocation of phosphorylated adducin from insoluble to soluble fraction [51]. These observations are in agreement with other reports [52,53] and indicate a dissociation of phosphorylated adducin from the actin–spectrin network [52]. Due to the role of adducin in cytoskeletal rearrangements [54], it was of interest to determine the distribution of actin in infected cells. Cells infected with live or heat-killed *M. bovis* BCG for 24 hours were stained for actin and demonstrated that heat-killed but not live bacteria co-localized with actin (Fig. 1B). In agreement with the latter result, fluorescence microscopy analyses of mycobacterial phagosomes have demonstrated that macrophages containing live pathogenic mycobacteria are refractory to phagosomal actin assembly [47]. In contrast, phagosomes containing the non-pathogenic *M. smegmatis* or killed *M. tuberculosis* assemble actin on their phagosomes in a manner very similar to that observed for phagosomes containing latex beads [47,55]. Furthermore, it was shown that several lipids, such as arachidonic acid, phosphatidlyoinositide (4,5)-bisphosphate ($PI(4,5)P_2$), and ceramide were able to activate and restore actin assembly resulting in phagosomal maturation and enhanced killing of pathogenic mycobacteria [47].

The specific role of actin filaments in endosomal biogenesis has long been unclear, but the analysis of latex-bead phagosomes [56,57] has significantly contributed to our current understanding of this interaction. In vitro analyses have shown that actin nucleation is an inherent property of phagosomal membranes that depends on the presence of certain phosphoinositides and actin-binding proteins [58,59]. The actin filaments are nucleated with the fast-growing end localized at the membrane while the slow growing end extends outward. This orientation provides the right polarity for potential fusion partners to move along and has been suggested to act as tracks for endosomal organelles such as lysosomes (Fig. 2). Treatment of cells with agents that interfere with actin filament "treadmilling" (the process of polymerizing or de-polymerizing), such as cytochalasin D and latrunculin A, inhibits phagosomal transport and fusion along the endosomal pathway [56]. A closer examination of this phenomenon has recently shown that actin filaments act as scaffolds that facilitate clustering of phagosomes and endocytic organelles [60]. Furthermore, it was shown that only late endocytic organelles and not early endosomes, are able to nucleate actin [60]. Based on these observations, the data showing that mycobacterial

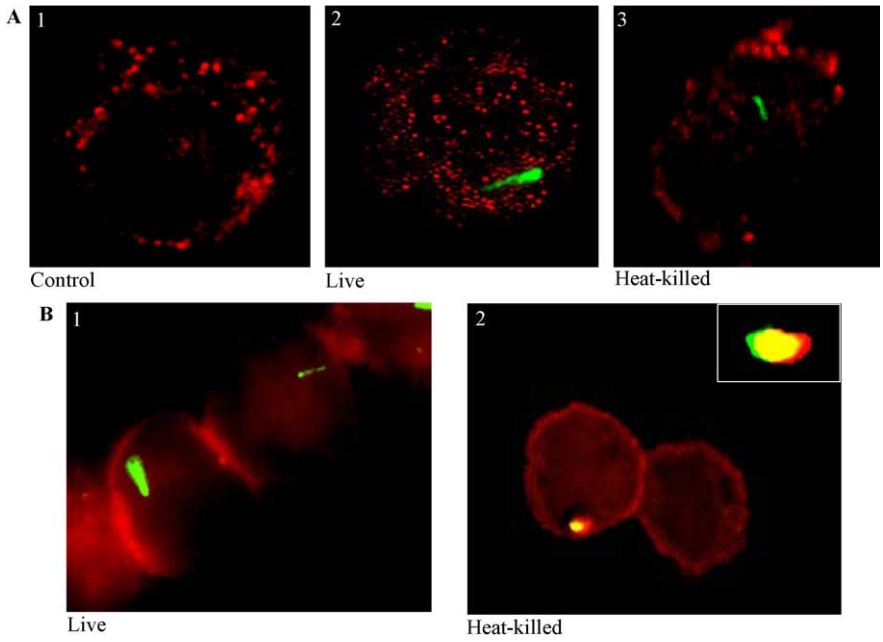

Fig. 1. (A) Intracellular distribution of adducin in infected cells. Untreated control cells showed a punctuate pattern of adducin staining mainly localized to the outskirts of the cell (Panel 1). Macrophages infected with live *M. bovis* BCG (Panel 2) showed an increased distribution of phosphorylated adducin in the cytosolic fraction. In contrast, cells infected with heat-killed bacteria (Panel 3) displayed a pattern of phospho-adducin distribution resembling that of control untreated cells. Red, Alexa-red conjugated secondary antibody for anti-phospho-adducin. Green, *M. bovis* BCG expressing the green fluorescent protein. (B) Phagosomal actin assembly. No colocalization between actin and mycobacteria-containing phagosomes was observed in macrophages infected with live *M. bovis* BCG (Panel 1). In stark contrast, infection with heat-killed bacteria showed that a significant proportion of engulfed bacteria were coated with actin (Panel 2). Panel 2 inset, shows area of colocalization enlarged. Red, Alexa-red conjugated phalloidin. Green, *M. bovis* BCG expressing the green fluorescent protein.

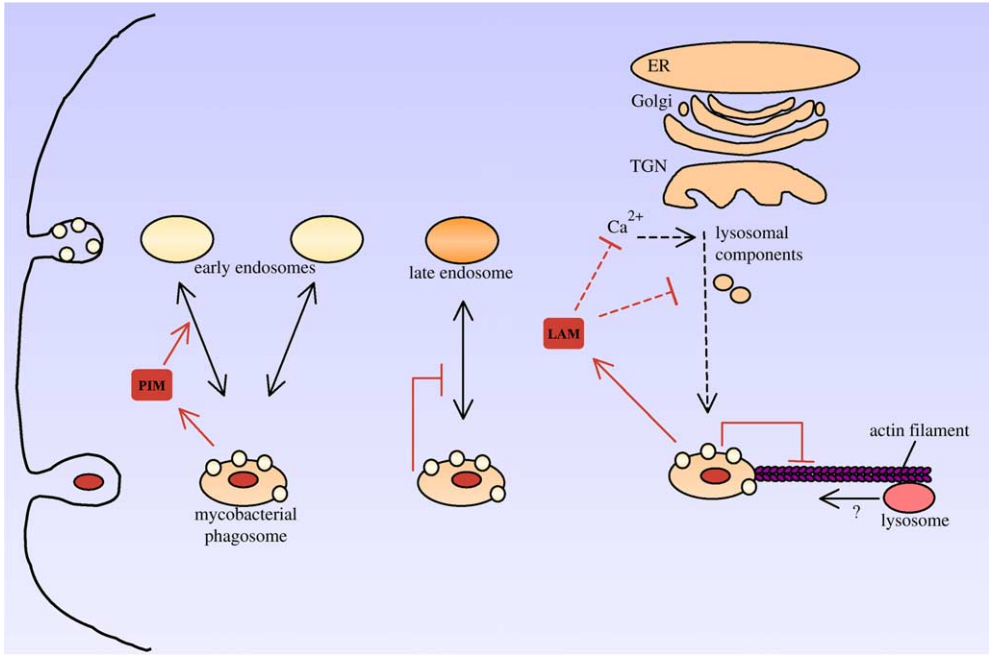

Fig. 2. Mycobacteria reside within phagosomes that retain early endosomal properties and resist fusion with later stages of the endosomal/lysosomal pathway. Mycobacteria-induced inhibition of phagosomal actin filament nucleation can possibly explain the inability to fuse with late endocytic vesicles. LAM induced inhibition of calcium elevation is linked to the inhibition of transportation pathways between the TGN and the phagosome and can account for the absence of the ATPase hydrogen pump and certain proteases on the mycobacterial phagosome. Mycobacterial lipids such as ManLAM and PIM can be responsible for some of these events. ER, endoplasmatic reticulum; ManLam, mannose-capped lipoarabinomannan; PIM; Phosphatidylinositol mannoside; TGN, *trans*-Golgi network; TR, transferrin receptor.

phagosomes resist fusion with late endosomes may be related to the ability of mycobacteria to inhibit phagosomal actin assembly. As suggested by Anes et al. [47], the addition of lipids may activate signaling cascades involved in actin regulations that had been suppressed by mycobacteria. The in depth analysis of lipid signaling and implications for mycobacterial infections is well beyond the scope of this review, but it is interesting to note the central role of phosphoinositides and phosphoinositide kinases in the regulation of membrane trafficking events (see [61] for review). PI(4,5)P_2 forms part of most endo-membranes and is a necessary component for initiation of membrane actin nucleation and vesicle membrane fusion [56]. Furthermore, the transportation of protein cargo from the *trans*-Golgi-network is dependent on PI(4,5)P_2 and its effectors consisting of actin, spectrin and ankyrin[62]. Re-establishment of the actin filament network through the addition of lipids may therefore not only allow for specific actin-mediated fusion events to occur, but also restore transportation pathways between Golgi and the endosomal pathway that are blocked by pathogenic mycobacteria [25].

Interestingly, *Mycobacterium marinum*, a species of mycobacteria which causes a tuberculosis-like disease in frog and fish and occasionally in humans, has recently been show to manipulate the host actin filament network in a way very similar to that described for the intracellular pathogen, *Listeria monocytogenes* [63]. Intraphagosomal *M. marinum* induced the formation of actin rocket tails that enabled an escape from the phagosome into the cytosol and, in some cases, further into neighboring cells. This behavior has never before been observed in mycobacteria and can be a unique feature of *M. marinum*, which reflects inherent differences between mycobacterial species.

6. Mycobacterial mediators of host cell interactions

The recent years' advances within the study of mycobacterial pathogenesis has provided compelling evidence suggesting that mycobacteria have the ability to actively interfere with host cell defense mechanisms through inhibition or counteraction. One of the main questions is: what are the mycobacterial mediators accountable for these effects?

6.1. Mycobacterial lipids

A survey of the heterogeneity in gene expression among various clinical isolates of *M. tuberculosis* [64] revealed a significant variability in the expression of genes involved in lipid metabolism. Earlier, studies from Barry's laboratory [65] identified a polyketide synthase-derived phenolic glycolipid (PGL) as a highly biologically active lipid species in a subset of *M. tuberculosis* isolates belonging to the hypervirulent W-Beijing family. The functional relevance of PGL was further proven by the demonstration that disruption of PGL synthesis results in an increase in the release of the pro-inflammatory cytokines from infected cells and that infection with *M. tuberculosis* overproducing PGL inhibited the release of these pro-inflammatory mediators [65]. Therefore, differences in the mycobacterial lipid metabolism may modify the host cellular immune response, thereby contributing to the observed diversity of clinical outcomes.

Lipids represent also an abundant feature of the mycobacterial cell wall and make ideal mediators for subverting host microbicidal mechanisms. Several reports indicate that the mycobacterial phagosome membrane is disrupted in a manner that may allow for entry of the organism or its products into the host cell cytosol [66,67]. Indeed, analysis of infected macrophages revealed that lipid-containing moieties are actively trafficking out of the mycobacteria-containing phagosome [68]. The cell wall glycolipid, ManLAM, of *M. tuberculosis*, has long been considered a virulence factor. As discussed above, several lines of evidence have implicated ManLAM to be involved in a wide array of immunomodulatory functions. In addition to a direct role in phagosomal maturation arrest [25], ManLAM has been implicated in: (i) inhibition of IFN-γ signaling, (ii) scavenging of oxygen free radicals, and (iii) inhibition of protein kinase C [5]. Other glycolipids of the mycobacterial cell wall have also been shown to inhibit macrophage microbicidal functions. The 19-kDa-lipoprotein of *M. tuberculosis* has been found to inhibit several functions associated with host defense such as MHC class II expression and antigen processing [36]. Contradictory findings on the role of the 19-kDa lipoprotein with regard to apoptosis have shown either inhibition [69] or induction associated with release of interleukin-1 beta [70]. The 19-kDa lipoprotein of *M. tuberculosis* belongs to a family of bacterial lipoproteins characterized by a distinctive *N*-terminal lipo-amino acid, *N*-acyl *S*-diacylglycerol. Although deacylation abrogates the effect of ManLAM, the same is not true for the 19-kDa lipoprotein where the non-acylated recombinant polypeptide component is sufficient for apoptosis induction [69]. Contrasting with these findings, ManLAM, another abundant lipidic molecule on the bacterial surface, does not induce apoptosis although the LAM precursor lipomannan (LM) is a potent inducer of apoptosis [71]. Rather, ManLAM was shown to promote cell survival via Bad phosphorylation dependent upon activation of the PI3K/Akt signaling pathway [72]. This is likely one of the virulence-associated mechanisms by which pathogenic mycobacteria would block host cell apoptosis dependent anti-mycobacterial activities. Thus, the relative abundance of p19 and ManLAM on the surface of *M. tuberculosis* would be a determinant for the outcome-survival versus intracellular killing of mycobacteria. In this context, studies of mycobacterial

p19 knockout strains, and p19 distribution studies failed to demonstrate that the p19 molecules are needed for *M. tuberculosis* pathogenicity [73,74]. Intriguingly, the effects of both LAM and 19-kDa lipoprotein have been shown to be mediated by toll-like receptor 2 (TLR2) [75]. Thus, how two antagonistic effects could be signaled via TLR2 is an important question to be elucidated in view to better understand macrophage-dependent innate immunity.

6.2. Mycobacterial proteins

Sequencing of the *M. tuberculosis* genome revealed the presence of eukaryotic-like protein kinases and protein phosphatases. An analysis of this family of phosphoproteins showed that at least six of them could be phosphorylated in vitro [76]. This clearly indicates the presence of functional protein kinases in *M. tuberculosis*. An earlier report demonstrated the existence of a major 55-kDa tyrosine phosphorylated protein [77] and so the presence of a functional tyrosine kinase in the *M. tuberculosis* genome was predicted. Surprisingly, no such kinase was identified. This poses the question as to the source and function of these tyrosine-phosphorylated proteins. If the virulence of *M. tuberculosis* is dependent on the activity of mycobacterial phospho-proteins, secretion of these proteins into the phagosome, and possibly through the phagosomal membrane into the host cell cytoplasm, is likely. Secretion of bacterial protein into the host cell is critical for the intracellular survival of pathogens such as *Salmonella* and *Yersinia* [78]. The recent identification of a specified secretion system in mycobacteria [79,80] raises the possibility of active transport of mycobacterial proteins into the host cell. Significantly, the culture medium of *M. tuberculosis* shows the presence of a protein tyrosine phosphatase [81] and a protein kinase [82], indicating an active secretion of these proteins.

Recently, *M. bovis* BCG mutant in a serine/threonine kinase PknG, was suggested to be essential for the survival of mycobacteria inside macrophages [33]. Walburger et al., demonstrated that PknG is secreted within the mycobacterial phagosome and involved in blocking phagosome–lysosome fusion. However, *M. tuberculosis* PknG knockout mutants are impaired in growth both in the in vitro stationary phase and in mice [82]. This, together with earlier reports suggesting that PknG is essential for mycobacterial growth under certain in vitro conditions [83], provide an alternative explanation to the reason behind PknG requirement for growth in macrophages.

7. Concluding remarks

The macrophage represents a highly specialized cell of the immune system, yet fails to eliminate the *M. tuberculosis* bacillus, one of the most successful pathogens of our time. Through years of cohabitation and mutual interaction, *M. tuberculosis* has managed to establish itself within the macrophage, avoiding the host killing mechanisms.

The ability of *M. tuberculosis* to infect and persist within these specialized cells is a result of the bacteria's ability to adapt to a fluctuating host environment and to utilize the sources available to it within the host. Sequencing of the *M. tuberculosis* genome [84] and post-genomic research has allowed for the identification of genes important in growth and survival [83] and has significantly contributed to our understanding of the interaction between *M. tuberculosis* and its host environment. Mycobacterial proteins such as respiratory enzymes, stress-related products, metabolic enzymes and proteins involved in fatty-acid metabolism have been identified as essential for the survival of the bacteria within the host cell [see [2] for review]. Moreover, a critically important part of the mycobacterial survival lies in its ability to respond to the environment in which it resides. However, another- and probably equally important-part lies in the ability of mycobacteria to actively interfere with and manipulate host defense mechanisms.

Cell signaling controls virtually every cellular event and is regulated by two principal classes of enzymes: protein kinases and protein phosphatases. These enzymes represent key control points within the host cell and possible targets for mycobacterial interference. It is intriguing to hypothesize a model of interference with host cell signaling machinery in which mycobacterial mediators in the form of lipoproteins, protein kinases and protein phosphatases are transported out of the phagosomal membrane, into the host cell cytoplasm.

The past decades' advances in genomics, immunology, microbiology and biotechnology have increased our knowledge and taken us several steps further in our understanding of the interaction between *M. tuberculosis* and its host cell. However, the knowledge we have must be seen in the light of what methods we use to achieve it. The experimental work in macrophages employs a variety of cell lines and primary cells of murine and human origin. Results from different research laboratories have revealed considerable differences in responses to stimuli between primary cells and cell lines and also between the different cell lines. These differences especially apply to the study of signaling mechanisms where differences between cell lines are often manifested through variation in specific pathways.

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