Mycobacterium tuberculosis and the Macrophage: Maintaining a Balance

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Mycobacterium tuberculosis is a highly efficient pathogen, killing millions of infected people annually. The capacity of *M. tuberculosis* to survive and cause disease is strongly correlated to their ability to escape immune defense mechanisms. In particular, *M. tuberculosis* has the remarkable capacity to survive within the hostile environment of the macrophage. Understanding *M. tuberculosis* virulence strategies will not only define novel targets for drug development but will also help to uncover previously unknown signaling pathways related to the host's response to M. tuberculosis infection.

Mycobacterium tuberculosis is a global health threat, infecting about one third of the human population and resulting in an annual casualty of ~2 million people worldwide (Dye et al., 1999; Yew and Leung, 2008). While antimycobacterial therapy exists, currently available drugs are only partially effective because of the impermeable nature of the mycobacterial cell wall and the propensity of M. tuberculosis to develop resistance (Warner and Mizrahi, 2006). One additional problem is that *M. tuberculosis* has the capacity to remain viable within infected hosts for a prolonged time.

Infection of a host with *M. tuberculosis* is initiated following the inhalation of droplets (aerosols) containing a small number of bacilli (Kaufmann, 2001). Once in the lung, bacilli are internalized through phagocytosis by the resident macrophages of the lung, the alveolar macrophages (Figure 1). Alveolar macrophages activated by the appropriate stimuli can effectively transfer the phagocytosed *M. tuberculosis* to the destructive environment of lysosomes, but some bacilli are able to escape lysosomal delivery and survive within the macrophage (Armstrong and Hart, 1975; Kaufmann, 2001; Russell, 2001). Infected macrophages can then either remain in the lung or are disseminated to other organs in the body. However, only a minority (~10% of infected people [World Health Organization, 2007]) develop tuberculosis, because in most healthy individuals, the immune defense system is sufficient to keep *M. tuberculosis* in check such that disease cannot develop (http://www.who.int/mediacentre/factsheets/ fs104/en/index.html).

The Default Route for Microbes to Macrophage Lysosomes

Phagocytosis of *M. tuberculosis* by macrophages proceeds through a series of membrane invagination, budding, and fusion events, resulting in the formation of the phagosome (Aderem and Underhill, 1999). Apart from its size (>0.5 mm), the membrane and luminal composition in terms of lipids and proteins of the phagosome is similar to that of endosomes, small vesicles utilized by the cell to internalize fluids (that may include microbial derived material) from the extracellular environment (Schmid, 1997).

Material internalized into phagosomes and endosomes can further be distributed within the cell through a series of vesicle trafficking events (Gruenberg and Stenmark, 2004; Schmid, 1997) that may deliver this material to the antigen processing and presentation pathway (Wolf and Ploegh, 1995; Pieters, 1997). This

pathway consists of endosome-related compartments that are dedicated for the degradation of antigens and where antigenic fragments bind to major histocompatibility complex (MHC) class II molecules. From these MHC class II compartments, peptideloaded MHC class II molecules are shuttled to the plasma membrane where they function in the activation of T cells in order to generate an adaptive immune response against the microbe whose material has been digested and presented (Amigorena et al., 1994; Tulp et al., 1994; West et al., 1994). Besides peptide fragments, mycobacterial lipids generated within endosomal/lysosomal organelles are also presented to gd T cells (De Libero and Mori, 2005) (Figure 2). The final destination of both the phagosomal and endosomal cargo is the lysosome, where extensive degradation of the cargo can occur. This results in the clearance of potentially harmful material from circulation through endocytosis and subsequent destruction of pathogens. The reader is referred to several excellent reviews dealing with the molecular processes that govern the transfer of material between different intracellular organelles (Mellman, 1996; Rothman and Wieland, 1996; Schekman, 1994). For this review, it is important to realize that both proteins as well as (glyco)lipids play important roles in the regulation of vesicular traffic (Haucke and Di Paolo, 2007). As will be described below, pathogenic mycobacteria have evolved mechanisms to interfere with both (glyco)lipid and protein-mediated mechanisms that regulate the transfer of cargo to lysosomal organelles for destruction.

Phagocytosis into the Macrophage—A Special Port of Entry for M. tuberculosis to Ensure Intracellular Survival

The entry of *M. tuberculosis* into macrophages can occur via an array of different receptor molecules, including complement receptors, mannose receptor, the dendritic cell-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN), and Fc receptors (Ernst, 1998; Cambi et al., 2005; Greenberg, 1999). The ability of multiple receptor molecules to internalize precise receptor involved in phagocytic entry may have a major impact on the survival chances of *M. tuberculosis* once inside the macrophage. For example, ingestion of particles through Fc receptors results in a respiratory burst and an inflammatory response in macrophages; in contrast, internalization via CR3 re- ceptors prevents the activation of the macrophages (Caron and Hall, 1998). In addition, CR3-mediated uptake of *M. tuberculosis* is strictly dependent on the presence of host plasma membrane cholesterol (Gatfield and Pieters, 2000; Peyron et al., 2000). The presence of cholesterol at the mycobacterial phagosome is also required for the prevention of phagosome-lysosome fusion (Gat- field and Pieters, 2000; Jayachandran et al., 2007). Interestingly, mycobacteria may utilize cholesterol to survive within chronically infected macrophages (Pandey and Sassetti, 2008). Thus, internalization by certain receptors is clearly beneficial to *M. tuberculosis* and mechanisms may exist for selectively binding and internalization by specific receptors, such as CR3, to help avoid immediate and long-term degradation inside macrophages.

Establishment of a Balance: The Granuloma

In a majority of infected individuals, a certain number of macrophages exist that harbor viable mycobacteria, yet most infected individuals do not develop disease. This raises the question of how these bacilli are contained by the host immune system. *M. tuberculosis* residing within macrophages is kept in check within structures termed granulomas (Cosma et al., 2003; Flynn and Chan, 2003). Although the precise biology of granulomas remain only partially understood, it is believed that

granulomas are structured clusters containing *M. tuberculosis* infected macrophages in the center, surrounded by different types of immune cells, in particular macrophages and T lymphocytes (Gordon et al., 1994) (Figure 1). Both nonactivated and activated macrophages coexist within granulomas, with the activated macrophages processing and presenting mycobacterial antigens to the surrounding T lymphocytes (Chan and Flynn, 2004). Following presentation of mycobacterial antigens, the T cells become activated through the triggering of T cell receptors (Kaufmann, 1993; Pieters, 1997). Activated T cells secrete cytokines and chemokines keeping the macrophages in an activated state and ensuring the recruitment of other immune cells to the site of infection. The precise status of *M. tuberculosis* within granulomas is not clear. They may exist as actively dividing bacilli or in a so-called ‘‘dormant’’ state. Also, it cannot be excluded that both actively dividing and dormant bacilli can occur within the same infected individual depending on the stage of the disease (Dannenberg, 2003; Dheda et al., 2005; Karakousis et al., 2004). Regardless, *M. tuberculosis* can persist for a long time, even up to the lifetime of the host (Young et al., 2002), within these structures, and as long as host immunity (in the form of activated macrophages and functional T cells) is effective, there is usually no adverse effect of the *M. tuberculosis* on the host’s health (Saunders and Britton, 2007). Thus, the granuloma structure likely represents a balance between a potentially dangerous pathogen and the host immune system. The delicacy of this balance is illustrated by the observation that any deterioration of host immunity results in a potentially life-threatening condition of an individual harboring live *M. tuberculosis (*Bartlett, 2007). How this balance of *M. tuberculosis* maintenance within macrophages is achieved is a critical issue in understanding *M. tuberculosis* pathogenesis. The rest of this review will examine mechanisms that both macrophages and *M. tuberculosis* utilize to maintain this balance. Particular attention will be paid to how *M. tuberculosis* modulates transport pathways within macrophages to block the delivery of *M. tuberculosis* to lysosomes, bactericidal activities of activated macrophages, and how *M. tuberculosis* can prevent the activation of macrophages.

Mycobacterial Prevention of Phagosome-Lysosome Fusion

Mycobacterial Interference with Lipid-Mediated Signaling Processes in Host Macrophages

M. tuberculosis is an unusual microbe with respect to its cell-wall composition. In contrast to many other bacteria, *M. tuberculosis* harbors a thick cell wall made up of unique lipid and glycolipid moieties (Brennan and Nikaido, 1995). Loss of mycobacterial cell wall components is typically correlated with reduced virulence, suggesting that the pathogen’s cell wall is important for mycobacterial survival inside the host cell (Glickman et al., 2000; Makinoshima and Glickman, 2005). *M. tuberculosis* glycolipids can interfere with phagosome-lysosome fusion through blocking a normal host trafficking event that is regulated by phosphatidylinositol 3-phosphate (PI3P). PI3P is a host membrane component that is essential for phago-lysosome biosynthesis (Roth, 2004). Locally generated by phosphatidylinositol 3-kinase (PI3 kinase) on early endosomal and phagosomal membranes, PIP3 is believed to present a docking site for several proteins involved in the maturation of phagosomes into lysosomes, such as the hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) and the early endosomal autoantigen 1 (EEA1) (Birkeland and Stenmark, 2004; Itoh and Takenawa, 2002; Wurmser et al., 1999).

Thus, while in uninfected cells, the generation of PI3P regulates the delivery of phagocytosed cargo to lysosomes, M. tuberculosis interferes with this trafficking event by actively preventing PI3P accumulation on phagosomal membranes (Fratti et al., 2003). This is achieved via a 2-fold strategy. First, *M. tuberculosis* interferes with the activity of the PI3 kinase hVP34, thereby preventing the generation of PI3P on the phagosomal membrane and blocking phagosome-lysosome fusion. This inhibitory activity was proposed to be mediated by the mycobacterial cell-wall component lipoarabinomannan (LAM), since the addition of purified LAM was sufficient to prevent lysosomal delivery (Vergne et al., 2003). However, since killed mycobacteria fail to block lysosomal transfer yet possess LAM, it remains unclear how LAM-mediated blockage of trafficking is regulated. A second strategy relies on the prevention of PI3P accumulation on phagosomes by the activity of a phosphatase, termed SapM, a eukaryotic-like acid phosphatase secreted by *M. tuberculosis* (Saleh and Belisle, 2000). SapM is presumably released within the host cell cytosol upon infection, where it may hydrolize PIP3 on phagosomal membranes (Vergne et al., 2005). Apart from lipid phosphatases, *M. tuberculosis* also possesses protein phosphatases (PtpA and B [Grundner et al., 2005]) that interfere with host trafficking processes, possibly by modulating vacuolar sorting proteins (Bach et al., 2008).

Interfering through the Production of Host-like Signaling Molecules: The Mycobacterial Eukaryotic-like

Protein Kinases

Typically, prokaryotes regulate their signal transduction through two component systems (Stock et al., 2000), and until recently a function for serine/threonine kinases in bacteria was not known. However, when the genome of *M. tuberculosis* was sequenced, 11 members of the eukaryotic-like serine/threonine kinase family were identified (Cole et al., 1998). Horizontal gene transfer may be a mechanism by which mycobacteria have acquired this set of eukaryotic-like serine/threonine kinases, which are likely to be involved in the modulation of signal transduction cascades (Av-Gay and Everett, 2000; Nguyen and Pieters, 2005).

Interestingly, most prokaryotic serine/threonine kinases as well as the phosphatases appear to be modulators of developmental changes or involved in the host-pathogen interaction (Greenstein et al., 2005; Nguyen and Pieters, 2005). Indeed, of the 11 kinases analyzed from *M. tuberculosis*, most appear to be involved either in processes such as cell division and stress responses, or, alternatively, in the modulation of host cell responses. Consistent with a function in the regulation of myco- bacterial physiology, the majority of the mycobacterial kinases are transmembrane molecules, displaying the kinase domain in the bacterial cytosol. Only two of these kinases, PknG and PknK, are soluble molecules, and of these, PknG is the only soluble kinase maintained in the genome of all pathogenic mycobacteria, including *Mycobacterium leprae*. This is important, since *M. leprae* possesses only one-third of the genes present in *M. tuberculosis* and has therefore been referred to as the mycobacterial pathogen having the minimal set of genes needed for pathogenicity (Cole et al., 2001).

Interestingly, PknG is only required for the survival of *M. tuberculosis* once the bacteria have been internalized inside macrophage phagosomes and both wild-type and PknG-deficient bacteria survive equally well in in vitro cultures (Nguyen et al., 2005). In macrophages, PknG-deficient mycobacteria show an attenuated phenotype, cannot resist lysosomal transfer, and are rapidly degraded (Majlessi et

al., 2007; Walburger et al., 2004; Cowley et al., 2004). Whereas a PknG homolog present in *Corynebacteriium glutamicum* was suggested to be involved in glutamine metabolism (Niebisch et al., 2006), a careful analysis of mycobacteria lacking PknG revealed similar cell growth, glutamine uptake processes, and intracellular glutamine concentrations (Nguyen et al., 2005), arguing against a role for mycobacterial PknG in the regulation of glutamine metabolism. As a soluble molecule, PknG is released from mycobacteria inside the macrophage phagosomes and accesses the cytosol (Walburger et al., 2004). The mechanism of cytosolic translocation of PknG and the precise action of PknG on host trafficking machinery remains largely unknown.
The kinase activity of PknG is essential for the prevention of phagosome-lysosome fusion, since overexpression of a dominant negative mutant form of PknG results in lysosomal delivery of the bacteria (Walburger et al., 2004). As several factors that are involved in the regulation of intracellular transport reactions require phosphorylation (Morgan and Burgoyne, 2004; Turner et al., 1999), it is likely that PknG acts by phosphorylating a host molecule, thereby preventing the activity of this host factor in carrying out phagosome-lysosome fusion.
The requirement of the PknG kinase for mycobacterial survival inside macrophages has spurred the search for specific kinase inhibitors, since kinases are attractive targets for the develop- ment of inhibitory compounds (Huse and Kuriyan, 2002; Noble et al., 2004). Most drugs currently used to treat tuberculosis act to interfere directly with mycobacterial physiology. In contrast, blocking the activity of PknG may allow the macrophage to carry out its innate antimicrobial activity, by redirecting intracellularly residing mycobacteria from phagosomes to lysosomes, thereby inducing their destruction. An additional advantage of targeting PknG may be that as a secreted molecule, inhibitors do not need to acquire access to the extremely impermeable mycobacterial cell wall. Indeed, a highly selective inhibitor for PknG has been recently identified (Scherr et al., 2007). Although PknG is highly homologous to eukaryotic serine/threonine kinases, it possesses a unique domain not found in eukaryotic kinases. The inhibitor interacts with this unique domain of PknG, explaining its specificity (Scherr et al., 2007).

Interfering with Host Cell Signaling: Hijacking the Calcineurin Pathway
The prolonged survival of *M. tuberculosis* within their mammalian host cells suggested that these pathogens, apart from producing virulence factors such as SapM and PknG, have evolved mechanisms to utilize host molecules for their own survival. An analysis of the protein composition of the mycobacterial phagosome showed the exclusive presence of a protein that was strongly retained on phagosomes harboring viable mycobacteria, but this protein did not associate to any other subcellular organelle (Ferrari et al., 1999; Hasan et al., 1997). This protein, initially termed TACO (for Tryptophan aspartate containing coat protein, also known as P57), is now referred to as coronin 1 and is recruited to phagosomes containing live bacilli, but rapidly released from phagosomes containing killed mycobacteria, suggesting that coronin 1 is an important host factor that specifically prevents the lysosomal delivery and death of mycobacteria inside macro- phages (Ferrari et al., 1999; Gatfield et al., 2005).
Coronin 1 is a member of the WD repeat-containing protein family of coronins. The founding member of this protein family was identified in the amoeba *Dictyostelium discoideum* and is involved in the regulation of actin-based dynamics (de Hostos, 1999). In mammalian cells, up to seven coronin isoforms can be expressed, and based on the homology to Dictyostelium coronin, mammalian family members were

thought to regulate the F-actin-dependent cytoskeleton (Uetrecht and Bear, 2006). However, no defects in the F-actin cytoskeleton or F-actin-mediated cellular processes is apparent in coronin 1-depleted macrophages cell lines or in cells from coronin 1-deficient mice (Jayachandran et al., 2007, 2008; Mueller et al., 2008). Instead of modulating the actin cytoskeleton, coronin 1 prevents phagosome-lysosome fusion by regulating calcium-dependent signaling processes. When macrophages are infected with mycobacteria, they respond with a sustained calcium flux that is dependent of the presence of coronin 1. The coronin 1-dependent cytosolic influx of calcium activates the calcium-dependent phosphatase calcineurin. In the absence of coronin 1, influx of calcium does not occur and therefore calcineurin is not activated. Strikingly, the coronin 1-dependent activation of calcineurin is required for blocking phagosome-lysosome fusion (Jayachandran et al., 2007). Activated calcineurin links up to several downstream signaling cascades, ranging from the transfer of transcription factors to the nucleus to dephosphorylation of proteins involved in endocytosis (Cousin and Robinson, 2001; Winslow et al., 2003).

The mechanism of coronin 1 recruitment to the mycobacterial phagosome and the precise activity of calcineurin required to prevent lysosomal delivery of mycobacteria are unknown. However, mycobacterial proliferation inside macrophages is fully blocked by the presence of the calcineurin blockers cyclosporin A and FK506, further providing support for the role of activated calcineurin in inhibiting phagosome-lysosome fusion (Jayachandran et al., 2007). Since these calcineurin inhibitors are clinically used as immunosuppressive agents, it remains to be established whether blocking this pathway will allow an inhibition of *M. tuberculosis* growth within infected individuals. However, these results do illustrate the importance of the coronin 1-dependent activation of calcineurin for the survival of *M. tuberculosis* within macrophages (Figure 3) (Jayachandran et al., 2007).

The Consequences of Macrophage Activation and Its Subversion by M. tuberculosis

It is important to point out that the capacity of *M. tuberculosis* to block their transfer to lysosomes is operational almost exclusively in nonactivated macrophages. Once macrophages have become activated (which is the condition under which microbicidal activities are upregulated, usually following stimulation by cytokines), *M. tuberculosis i*s rapidly transferred to lysosomes where they are destroyed by bactericidal activities such as the generation of reactive oxygen and nitrogen, which are also upregulated upon macrophages activation. Macrophage activation can occur in response to several cytokines, of which interferon-g and tumor necrosis factor alpha (TNF-a) are probably the most important (Adams and Hamilton, 1984; Bogdan and Schleicher, 2006; Flynn and Chan, 2001). Depressing the interferon-g or TNF signaling pathways in either animal models or human hosts dramatically enhances the risk of developing tuberculosis, illustrating the importance of these cytokines (Jouanguy et al., 1999; Stenger, 2005).

Generation of reactive oxygen and nitrogen intermediates upon macrophage activation via interferon-g is damaging to M. tuberculosis (MacMicking et al., 1997b). Despite the high toxicity of these reagents and their continuous generation upon macrophage activation, *M. tuberculosis* can persist within this environment. Mycobacterial resistance against reactive oxygen and nitrogen intermediates is based on multiple strategies that these bacilli have developed. First, mycobacteria produce KatG, a cata- lase-peroxidase that can inactivate reactive oxygen within

phagosomes (Li et al., 1998). Second, subunits of the mycobacterial proteasome are essential for coping with nitric-oxide stress (Darwin et al., 2003). In eukaryotes, the proteasome's major function is to degrade proteins, while in prokaryotes its function is far less clear (Pearce et al., 2006). The mycobacterial proteasome may provide resistance against reactive nitrogen intermediates, for example by functioning in the elimination or refolding of proteins damaged by reactive nitrogen intermediates (Darwin et al., 2003; Pieters and Ploegh, 2003). The importance of nitric-oxide production in the control of tuberculosis is well established for mouse models (MacMicking et al., 1997a). However, the situation in humans is less clear, and is probably related to the difficulties in establishing systems to study human macrophage functions in vitro (Nathan, 2006).

In addition, the diverse array of Toll-like receptor (TLR) ligands (discussed in Review by Ishii et al.) can also cause macrophage activation and thereby assist in the control of tuberculosis (Merdzhitov and Janeway, 1998; Takeda et al., 2003). Although the extent to which phagosome-lysosome fusion depends on the precise type of ligand being internalized via TLRs is not clear, regulation of phagosome maturation was recently shown to occur through the TLR adaptor protein myeloid differentiation factor 88 (MyD88) and the mitogen-associated protein kinase (MAPK) p38 protein (Blander and Medzhitov, 2006). Accordingly, infection of mice lacking MyD88 with *M. tuberculosis* results in rapid death of the host (Fremond et al., 2004), highlighting the importance of the TLR pathway in the control of *M. tuberculosis*. Finally, recent studies in human macrophages have linked the TLR pathway to a cascade upregulating the vitamin D receptor and vitamin D-1-hydroxylase genes. This activation leads to the induction of cathelicidin, an antimicrobial peptide that effectively blocks *M. tuberculosis* proliferation (Figure 2) (Liu et al., 2006, 2007). Although earlier results linking the vitamin D signaling pathway and resistance to tuberculosis were reported (Hill, 1998), exactly how this is achieved was unknown. Since vitamin D levels are also upregulated upon ultraviolet (UV) light exposure, these findings explain the observed benefits of sanatoria visits for tuberculosis patients in high-altitude areas (Zasloff, 2006).

Mycobacterial glycolipids also play an important role in the subversion of macrophage activation. The abundant cell-wall component LAM as well as its glycosylated forms (manLAM) can modulate signaling pathways that can be initiated to induce macrophage activation, including interferon-g-mediated gene expression, TLR activation, and phagosome-lysosome fusion (Chan et al., 1991). In addition, LAM can block the activation of an important kinase (MAPK) that is downstream of various stimuli that cause macrophage activation (Knutson et al., 1998). The fact that a large part of the M. tuberculosis genome is devoted to the production of molecules involved in lipid synthesis (Cole et al., 1998) may be related to the many functions lipids play in the survival of *M. tuberculosis* inside hosts.

In addition, modulation of bacterial metabolic pathways can ensure a long-term survival within macrophages. For example, when bacteria enter a nutrient-poor environment such as can occur within macrophage phagosomes, they become restricted to fatty acids as their carbon source. This then activates the glyoxylate shunt pathway, which facilitates carbon retention through de novo synthesis of carbohydrate (Honer zu Bentrup and Russell, 2001). The first step in this pathway is catalyzed by the enzyme isocitrate lyase. *M. tuberculosis* contains two genes that encode for isocitrate lyase. A deletion mutant lacking both genes is fully impaired in its intracellular replication and is rapidly eliminated from the lungs (Munoz-Elias and McKinney, 2005). This suggests that the isocitrate lyase-based pathway is essential

for *M. tuberculosis* to be able to establish a persistent infection. Several of the above-mentioned mechanisms require the transfer of proteins into the host environment. The complexity of the mycobacterial cell wall poses constraints on protein secretion, but pathogenic mycobacteria have evolved several systems to deal with secretion. These include the generic Sec-dependent secretion pathway to transport N-terminal signal sequence containing proteins across the cytosolic membrane and a twin-arginine transporter (Tat) system that is used to transport-folded molecules across the membrane. A more recently described secretion system is the early secretory antigenic target of 6 kD (ESAT-6) system 1 (ESX-1) (Abdallah et al., 2007; DiGiuseppe Champion and Cox, 2007), which is responsible for the secretion of ESAT-6, CFP-10 (culture filtrate protein of 10 kD), and possibly other molecules (Gao et al., 2004; McLaughlin et al., 2007). Interestingly, both ESAT-6 and CFP-10 are important T cell antigenic targets and essential for the virulence of *M. tuberculosis* (Gao et al., 2004; Guinn et al., 2004; Hsu et al., 2003). Mycobacteria encode five ESX-1-related secretion systems, named ESX 1–5, that appear to have independent roles, some of which are essential for mycobacterial growth (Abdallah et al., 2007). How this secretion system functions remains unknown. In addition, the precise role in virulence of ESX secretion systems and the substrates they secrete remains to be determined.

Apart from surviving within macrophage phagosomes, pathogenic mycobacteria have also been suggested to escape into the cytosol following phagocytosis in an ESX-1-dependent manner (McDonough et al., 1993; van der Wel et al., 2007). Analysis of cytosolic translocation within infected hosts may help to shed light on the importance of this phenomenon during an in vivo infection (Jordao et al., 2008; Mwandumba et al., 2004).

From the above description, it seems that *M. tuberculosis* has evolved a strategy to counter virtually any bactericidal activity of the macrophage. However, there are several host signaling pathways that, when activated, result in the destruction of *M. tuberculosis*, for which at present no clear subversion mechanisms has been defined. One of these relies on the induction of the immunity-related GTPase (IRG) members that are upregulated following interferon-g treatment, promoting lysosomal delivery (Feng et al., 2004; MacMicking et al., 2003, MacMicking, 2005; Martens and Howard, 2006), possibly via the autophagosomal pathway (Singh et al., 2006). However, genetic screens aimed at analyzing the mode of M. tuberculosis interference with such pathways may allow the definition of additional strategies used by mycobacteria to counteract these host immune defense mechanisms (Hisert et al., 2004).

Conclusions

The prolonged coevolution of *M. tuberculosis* with its human hosts and specifically within macrophages has clearly resulted in a number of survival strategies. These strategies range from producing eukaryotic-like signaling molecules such as kinases and phosphatases that interfere with host trafficking, the pro- duction of modulators of macrophage activation, to the activation of eukaryotic Ca2+-dependent signaling molecules such as calcineurin. Current efforts in understanding these virulence strategies should not only allow the development of better strategies to treat tuberculosis, but also contribute to unraveling the cell biology of the host immune system.

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Figures

Figure 1:

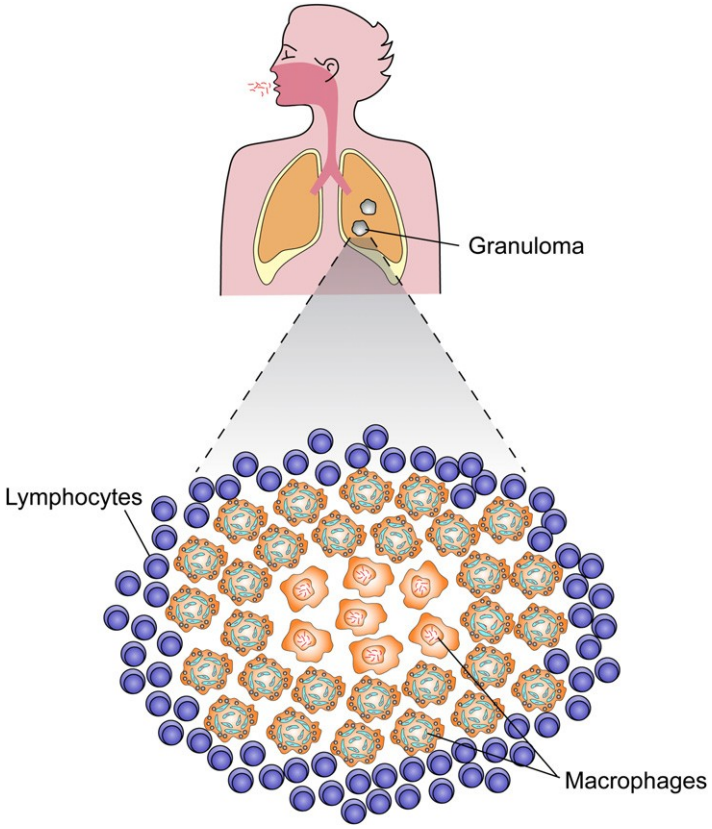

Figure 1. Infection with *M. tuberculosis*: Establishment of a Balance
M. tuberculosis is inhaled via aerosols into the lung, where they are internalized within alveolar macrophages. Inside nonactivated macrophages (those within the center of the granuloma), mycobacteria (indicated in red) resist destruction, although macrophage activation by T cell-dependent cytokines can overcome this resistance (activated macrophages are believed to reside at the periphery of the granuloma). In case of an immunodeficiency, for example when T cell function is compromised, unrestricted growth within macrophages causes dissemination of tubercle bacilli. Modified with permission from (Kaufmann, 2001).

Figure 2:

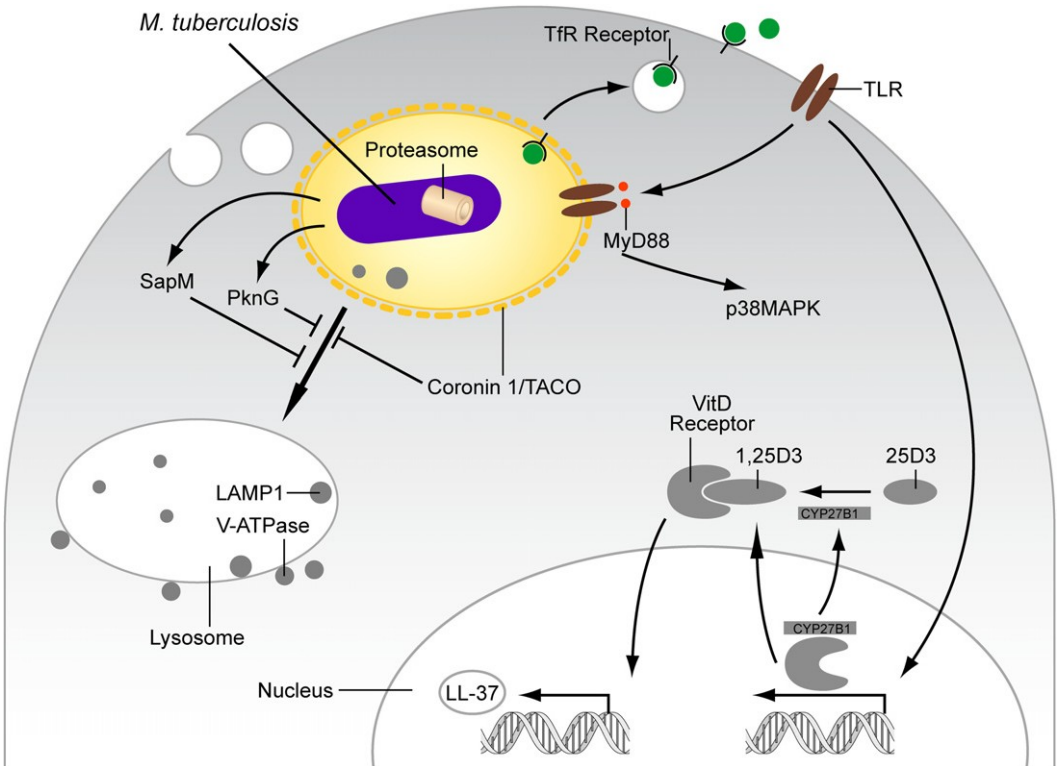

Figure 2. Methods of Macrophage Antimicrobial Activity and Evasion by *M. tuberculosis*

The mycobacterial phagosome is formed upon entry of mycobacteria and remains accessible to the extracellular environment, e.g., for the acquisition of iron through the transferrin receptor (TfR). Once inside phagosomes M. tuberculosis secretes the phosphatase SapM and the serine/threonine kinase PknG to prevent phagosome-lysosome fusion. The mycobacterial phagosome (light-yellow circle) is also prevented from fusing with lysosomes by the active recruitment of the host protein coronin 1/TACO (yellow). Lysosomal-associated membrane protein 1 (LAMP1) and vacuolar type H+-ATPase (V-ATPase) are components of the lysosome. Furthermore, the *M. tuberculosis* proteasome neutralizes the effect of nitric oxide generated within macrophages. The Toll-like receptor (TLR) signaling pathway can modulate phagosome-lysosomal fusion via the activation of p38MAPK, although it is unclear whether mycobacteria interfere with this pathway. In addition, the antimicrobial peptide cathelicidin (also referred to as LL-37) is induced through TLR signaling via activation of the vitamin D receptor by 1,25- dihydroxyvitamin D3 (1,25D3) converted from vitamin D3 (25D3) by 1-hydroxylase (CYP27B1). See text for further details. Reprinted with permission from Pieters (2008b).

Figure 3:

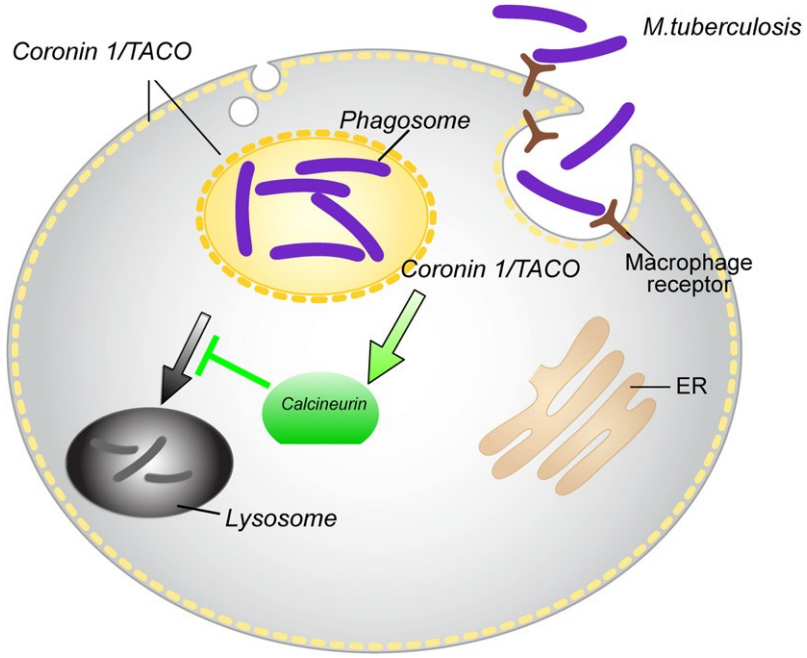

Figure 3. Model for the Activity of Coronin 1 in Macrophages
Upon mycobacterial entry into macrophage phagosomes mediated by macrophage receptors, coronin 1 (also known as TACO) is recruited to the mycobacterial phagosome. Coronin 1 retention leads to the activation of the Ca2+-dependent phosphatase calcineurin, which is responsible for blocking phagosome-lysosome fusion, thereby allowing *M. tuberculosis* survival. The endoplasmic reticulum (ER) of the macrophage is shown as well. Reprinted with permission from Pieters (2008a).