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Broadening Our View About the Role of *Mycobacterium tuberculosis* Cell Envelope Components During Infection: A Battle for Survival

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1. Introduction

TB is an ancient disease, however, it is still a major health problem in the world (Centers for Disease Control and Prevention, 2006). The number of new cases of TB worldwide roughly correlates with economic conditions: the highest incidences are seen in those countries with the lowest gross national products (countries mainly in Africa, Asia, and Latin America). WHO numbers indicate that 8 million people are newly infected and nearly 2 million people die of TB every year; translating to one person infected every 4 seconds and one person dying every 18 seconds (WHO, 2007). WHO estimates that by year 2020 up to 36 million people will die of TB every year (WHO, 2010). The current TB burden in the world is strictly associated to *Mycobacterium tuberculosis* (*M.tb*) co-infection with HIV, and the recent emergence of practically untreatable extensive-, extremely-, and totally-drug resistant *M.tb* strains (XDR-, XXDR-, and TDR-) in endemic areas. In this context, a person infected with a XDR/XXDR *M.tb* strain currently has a survival rate between 36-50%, however, for people co-infected with HIV, their survival rate drops to ~15% with a life span of 16 days upon XDR/XXDR *M.tb* infection (Gandhi *et al.*, 2006). XXDR-*M.tb* strains are resistant to all first and second line of drugs currently available, and TDR-*M.tb* strains are even resistant to the new developed drugs currently in clinical trials (Andrews *et al.*, 2010;Basu *et al.*, 2009; Gandhi *et al.*, 2006, 2010). Very little is known about the cell wall composition of XDR/XXDR/TDR *M.tb* strains. The presence of phenolic glycolipids and triglycerides in the *M.tb* cell wall has been directly related to the hypervirulence observed in some strains (Reed *et al.*, 2004, 2007). However, in the case of XDR/XXDR/TDR *M.tb* strains, it still is unknown which bacterial and host factors are involved in the induction of the overwhelming host immune response generated by these strains.

Initial interactions between *M.tb* and the host mark the pathway of infection and the subsequent host inflammatory response that defines disease outcome. Many studies have been performed analyzing the constitution of the cell wall of *M.tb*, where structural-biological function relationships for the majority of the cell wall constituents are still being elucidated. The majority of the cell wall of *M.tb* is comprised of carbohydrates and lipids,

and there is increasing evidence that microbial determinants readily exposed to the host immune system play critical roles in disease pathogenesis. Recent studies have been focused on depicting how *M.tb* adapts to the host by mimicking its cell envelope to mammalian glycoforms. Of particular interest is the fact that some *M.tb* strains are characterized by the presence of mannose-containing biomolecules, whose terminal epitopes closely resemble those on host mannoproteins. In this scenario, it is thought that *M.tb* may use this resemblance to the host to its advantage gaining entrance and establishing its particular intracellular niche within the host; thus, the initial *M.tb*-host interface may dictate the pathway of infection and the successful outcome of the disease. Many factors are involved in this interface. First the constitution of the *M.tb* cell wall, which is strain dependent. Here we will discuss the differences in the cell wall among the widely used laboratory strains (*M.tb* $H_{37}R_v$ and *M.tb* Erdman) and several relevant *M.tb* clinical isolates in endemic TB areas, including hypervirulent, MDR-, XDR, XXDR- and TDR- *M.tb* strains. We will discuss their cell wall constitution in relation to their infection outcome. Second, we will focus on the host cell that acts as a niche, the alveolar macrophage, and we will discuss the innate immunofactors present on the host cell that contribute to control or alternatively can favor the infection. Importantly, we will introduce our new results in an area frequently bypassed in many forums, the host environment and how this may challenge the old dogma of the real constitution of the *M.tb* cell wall during infection. We will discuss the alveolar microenvironment that *M.tb* encounters during infection, and how these may determine/contribute to the pathway of infection and disease outcome.

2. The cell wall of *M. tuberculosis* and its biological functions

A great effort has been made by many research groups to depict the *M.tb* cell wall structure and biosynthesis. The main distinctive feature of the *M.tb* cellular envelope cited in all books is the thick and waxy cell wall. This complex structure contributes to the main characteristics that distinguish mycobacteria, such as the acid fast staining properties, the low permeability of the cell wall, the resistance to harsh environments, and the intrinsic resistance to many hydrophobic antibiotics (Brennan & Nikaido, 1995;Jarlier & Nikaido, 1994). The properties of the cell wall barrier also contribute to the intracellular survival of the organism by acting as a direct modulator in the immunological reaction between the host and mycobacteria (Barry & Mdluli, 1996;Lederer *et al.*, 1975). *M.tb* is one of a small group of species able to survive inside the phagocytic cells of a host, so it is likely that its cell wall has special properties defending the bacterium against host microbicidal processes. Within the cell wall of *M.tb* may lie all of the elements associated with TB, including the factors responsible for caseation and other features of hypersensitization, the antigens responsible for humoral immunity, the agents of toxicity, and thus, the very antigens implicated in protective immunity (Brennan, 1988). A detailed electron microscopy study has not yet, however, identified any special features in *M.tb* compared to other non-pathogenic mycobacteria. The envelope consists of three distinct parts, the plasma membrane, the wall, and around it, the outer material. These parts are involved in providing mechanical support and osmotic protection plus transport exchange of ions and molecules with the micro-environment(s) surrounding the bacillus during the different stages of infection. Here we will discuss *M.tb* cell wall components in terms of their role in *M.tb* pathogenesis, focusing on the *M.tb* peripheral lipid layer constituents that are involved in *M.tb*-host cell recognition and pathogenesis.

2.1 The plasma membrane

Defined as a classical bilayer (Silva & Macedo, 1983), the *M.tb* membrane does, however, have some distinctive components, notably the lipoglycoconjugates mannose-capped lipoarabinomannan (ManLAM), lipomannan (LM), and phosphatidyl-*myo*-inositol mannosides. Integral membrane proteins embedded in the layers of the cell wall have also been described (Brennan & Draper, 1994). Analyses of the proteome of the plasma membrane of *M.tb* suggests that the plasma membrane of *M.tb* is likely to be rich in proteins comprising several essential enzymes, receptors and transporters (Sinha *et al.*, 2002), like other prokaryotic cell membranes (Sigler & Hofer, 1997). Bioinformatic analysis of the *M.tb* genome predicts more than 600 'putative' membrane-associated proteins with different numbers of transmembrane hydrophobic segments. These proteins undoubtedly play a role in the uptake and effects of various metabolites, peptides, drugs and antibiotics. Nonetheless, the real location, expression patterns, and function for the majority of these transmembrane proteins remain relatively unexplored (Lee *et al.*, 1992;Yokoyama & Shimizu, 2002; D.B. Young & Garbe, 1991).

2.2 The cell wall

The shape-forming properties of the wall are attributable to the peptidoglycan, whose chemical structure in *M.tb* closely resembles that found in other bacteria. Mainly, the cell wall is defined as a skeleton formed by a covalently linked structure of peptidoglycan, with a branched-chain polysaccharide, the arabinogalactan, attached by phosphodiester bonds. The arabinogalactan distal ends are esterified with high-molecular weight fatty acids, the mycolic acids, of sizes and structures unique to mycobacteria. This cell wall skeleton receives the name of the mycolyl-arabinogalactan-peptidoglycan complex (mAGP) (Besra *et al.*, 1995;Daffe *et al.*, 1990). The wall is constructed of three layers. With conventional staining using electron microscopy, their appearance is defined with an inner layer of moderate electron density, a wider electron-transparent layer, and an outer electron-opaque layer of extremely variable appearance and thickness. The outer opaque layer probably contains the outer material. The electron-transparent layer appears to be mycolated arabinogalactan, which forms a large part of the wall. Finally, the inner layer is speculated to contain both peptidoglycan and arabinogalactan (Draper, 1971).

There are many models of the mycobacterial cell envelope (Bhamidi *et al.*, 2011;Brennan and Besra, 1997;Crick *et al.*, 2003;Dmitriev *et al.*, 2000;Domenech *et al.*, 2001). Interactions of the asymmetric plasma membrane, peptidoglycan, and covalent attached arabinogalactan together with LAM and PIMs have been speculated, at least some of which are known to be associated with the plasma membrane. Mycolic acids are known to be attached to the majority of the terminal and penultimate arabinose residues of the arabinogalactan (Barry *et al.*, 1998). Since the mycolates possess two hydrocarbon chains of unequal lengths, which form an irregular monolayer, it is proposed that these are complemented by two different classes of polar lipids with medium (*e.g.* mycocerosates) and short (*e.g.* acylglycerols) fatty acyl chains, respectively (Barry *et al.*, 1998). There is also evidence for a small number of porins in the envelope, presumably within the outer hydrophobic bilayer (Senaratne *et al.*, 1998; Trias *et al.*, 1992). A recent study had committed efforts to solve the enigma involving the spatial organization of the mycobacterial cell envelope. This study compared bacteria grown *in vivo* (*i.e. Mycobacterium leprae*) *vs.* grown *in vitro* (*i.e. M.tb*) showing that bacilli

grown *in vivo* had a more compact cell envelope with more mycolic acids and more but shorter arabinogalactan molecules per peptidoglycan (Bhamidi *et al*., 2011). This differential cell envelope spatial conformation may differentially impact the rearrangement of the outer surface exposed cell envelope components that have a critical role in *M.tb*-host recognition.

The barrier for the influx of solutes such as nutrients or drugs is associated with the parallel alignment of mycolic acids (Liu *et al*., 1995;Liu *et al*., 1996). As a consequence, mycobacteria develop aqueous channels formed by porin molecules in the cell wall structure. Other distinguishing cell wall components of *M.tb* include ManLAM, LM, PIMs, and a peripheral layer of lipids such as trehalose mycolates (trehalose dimycolate or TDM, trehalose monomycolate or TMM), lipooligosaccharides (LOSs), phenolic glycolipids [PGLs, described in some *M.tb* clinical isolates (Reed *et al*., 2004;Torrelles *et al*., 2008b)], acyl trehaloses (diacyl- or DAT and triacyl- or TAT), triglycerides and sulfolipids (SLs) (Brennan & Nikaido, 1995; Muñoz *et al*., 1997a, 1997b). Recently, the role for individual components of the cell wall has been elucidated, and much emphasis has been placed on the identification and characterization of various genes that encode enzymes involved in the synthesis of the cell wall constituents. A better understanding of the cell wall components will lead to a better understanding of the relationship/symbiosis between *M.tb* and the infected host, which will lead to the identification of new drug targets and permit the development of new antituberculosis drugs targeting enzymes involved in the biosynthesis/maintenance of the cell wall within the host during infection.

2.2.1 Cell wall core

One distinguishing characteristic of the mycobacterial cell wall is the absence of lipoteichoic acids and lipopolysaccharides, typical structures of gram-positive and -negative bacteria, respectively. Instead, the *M.tb* cell wall has a cell wall core which is a covalently linked skeleton of the mAGP (Daffe *et al*., 1990). This structure is composed of the peptidoglycan, which is covalently linked to AG chains via phosphoryl-*N*-acetyl-glucosaminosyl-rhamnosyl linkage units (-α-L-Rha*p*(1→3)-D-GlcNAc-P-) (Daffe *et al*., 1990; Mikusova *et al*., 1996). AG non-reducing ends are esterified to a variety of α-alkyl, β-hydroxy mycolic acids. Lipoglycans, lipoproteins and especially free lipids are found to be associated with the mAGP complex (Andersen & Brennan P.J., 1994;Brennan & Nikaido, 1995).

2.2.1.1 The peptidoglycan

Peptidoglycan (PG) classes are identified by the type of peptide cross-linking that they display, the PG structure in *M.tb* is of the common A1γ type, although it does have some distinguishing features (Schleifer & Kandler, 1972). The insoluble PG consists of alternating units of *N*-acetylglucosamine (GLcNAc) and modified muramic acid residues [*N*-acetyl-β-D-glucosaminyl-(1→4)-*N*-acetylmuramic acid]. The typical *N*-acetyl groups in the muramic acid of bacterial PG are further oxidized to *N*-glycolyl groups in *M.tb* [*N*-glycolylmuramic acid]. The muramic acid residues are also modified by tetrapeptide [L-alanyl-D-isoglutaminyl-meso-diaminopimelyl-D-alanine] side chains (Schleifer & Kandler, 1972). Cross-linking can occur between two meso-diaminopimelic acid (DAP) residues as well as between DAP and D-alanine residues (Crick *et al*., 2001). The free carboxyl groups of the glutamate and DAP in the murein peptides from *M.tb* can be amidated in essentially any combination and a small percentage of the D-glutamate residues are substituted with a

glycine. PG serves as a foundation structure forming the backbone of mAGP and provides shape, strength, and rigidity to the *M.tb* cell wall. Although the role of PG in pathogenesis has been shown for other bacterial species (Boneca, 2005), in the case of *M.tb* is still unclear (Table 1). However, recent studies have been focused on the role of nucleotide binding oligomerization domain (NOD)-like receptors (NLRs) (Franchi *et al.*, 2008;Sirard *et al.*, 2007) in the recognition of *M.tb* PG fragments. In particular the NLR Nod2, which resides within the phagocyte cytosolic compartment, is shown to recognize the *M.tb* PG fragment muramyl dipeptide (MDP), where the replacement of the *N*-acetyl group of the muramic acid of MDP with a *N*-glycolyl moiety seem to significantly increase the potency of this compound as a Nod2 agonist (Coulombe *et al.*, 2009). Other studies confirmed that during infection, intra-phagosomal *M.tb* is capable of stimulating the cytosolic Nod2 pathway, and this event requires membrane damage that is actively inflicted by the bacillus (Pandey *et al.*, 2009). Moreover, *M.tb* bacilli recognized by Nod2 trigger NF-kB activation (Ferwerda *et al.*, 2005) and production of IFN-α/β with the subsequent transcription of CCL-5 (or RANTES) via Nod2, Rip2, Tbk-1, Irf3 and Irf5 cascade (Pandey *et al.*, 2009). Recent studies by Brooks *et al.* using human macrophages showed that Nod2 plays a role in controlling pro-inflammation and *M.tb* intracellular growth (Brooks *et al.*, 2011). This is in accordance with recent human polymorphisms studies linking Nod2 mutations to susceptibility to *M.tb* infection.

2.2.1.2 The arabinogalactan

The arabinogalactan (AG) is a heteropolysaccharide chain of furanoid arabinose (Ara*f*) and galactose (Gal*f*) (Daffe *et al.*, 1990;McNeil *et al.*, 1987). The furanosyl residues are arranged into three differential regions; the galactan core, the arabinan, and the non-reducing terminal segments of arabinan. Structural analysis of the AG shows that the galactan core of AG is composed by 5- and 6-linked β-D-Gal*f* residues (Daffe *et al.*, 1990). The arabinan chains consist of linear 5-linked α-Ara*f* residues with branching introduced by 3,5-Ara*f* residues (Daffe *et al.*, 1990). The linkage of arabinan chains to the galactan core occurs at the C-5 of some of the 6-linked β-D-Gal*f* residues. Clusters of four mycolic acids are then attached to the terminal arabinofuranosyl motifs of non-reducing ends of the arabinan chain via ester linkage. Approximately two-thirds of the non-reducing ends of arabinan are mycolated at the 5 position of Ara*f* residues (Daffe *et al.*, 1993), one-third with succinyl and one-third with glucosaminosyl residues (Bhamidi *et al.*, 2008). There are approximately 2-3 arabinan chains attached to the galactan core (Baulard *et al.*, 1998;Brennan and Nikaido, 1995). Finally, the galactan core of AG, in turn, attaches to the C-6-position of muramic acids of the PG via a phosphodiester linkage of α-L-rhamnopyranose (Rha*p*)-(1→3)-D-N-acetylglucosamine (GlcNAc)-(1→phosphate) (McNeil *et al.*, 1990;Mikusova *et al.*, 1996). Although *M.tb* AG has serological activity (Kotani *et al.*, 1971;Misaki *et al.*, 1974), its capacity to generate the innate immune response is unknown (Table 1).

2.2.1.3 Mycolic acids

Mycolic acids are complex hydroxylated branched-chain fatty acids with characteristic carbon numbers (60-90 carbon atoms)(Barry *et al.*, 1998). They may also contain diverse functional groups such as methoxy, keto, epoxy ester groups and cyclopropane rings (Asselineau & Lederer, 1950). Mycolic acids found in *M.tb* are composed of an α-branch and a meromycolate branch, where the latter defines the heterogeneity in the mycolic acids, although the α-branch length frequently also generates variation (Barry *et al.*, 1998). The

possible functional groups (unsaturations, methyl branches and cyclopropanes) and polar moieties (ketones, methoxy groups) are localized only in the meromycolate branch (Barry *et al*., 1998). In particular for *M.tb*, unsaturated mycolates containing cyclopropanes (either *cis*/*trans* with sometimes an adjacent methyl branch), are known as α-mycolic acids. Mycolic acids containing a methoxy group with double bond or cyclopropane ring are known as methoxymycolic acids; similarly, mycolic acids containing an α-methyl-branched ketone are known as ketomycolic acids (Takayama *et al*., 2005). The α-mycolate is the most abundant form found in the *M.tb* cell wall [65-70%] followed by methoxy- and keto-mycolates [8-15%] (Qureshi *et al.*, 1978). The majority of the mycolic acids are localized in the inner leaflet of the *M.tb* cell wall covalently bound via carboxylate ester to form the non-extractable tetramycolyl-pentarabinosyl unit (McNeil & Brennan, 1991). However, mycolic acids can also be found loosely forming extractible lipids mainly in the form of TDM and TMM (Minnikin, 1982). In this case, it is thought that TDM and TMM stabilize their position within the cell wall by associating their mycolic acid lipid tails with the AG-covalent linked mycolates. In general, mycolic acids are involved in maintaining a rigid cell shape but they also contribute to the resistance to chemical injury and to the protection of the *M.tb* bacillus against hydrophobic antibiotics (Barry *et al*., 1998). The importance of the mycolic acids in the *M.tb* cell wall is defined by the action of isoniazid, which inhibits their biosynthesis and is an efficient antimycobacterial agent (Winder & Collins, 1970). Furthermore, looking at the biological functions described for mycolic acids (Table 1), it is noticable to point out that these were the first known CD1-presented antigens capable of stimulating and activating CD1b-restricted T cells (Beckman *et al*., 1994;Montamat-Sicotte *et al*., 2011;Moody *et al*., 1999). Moreover, mycolic acids *per se* are shown to be immunomodulatory, where their structural nature (*i.e.* presence of determined functional groups) may determine the degree of virulence of a *M.tb* strain (Barry, *et al*., 1998).

2.2.2 The peripheral lipid layer in the *M. tuberculosis* cell wall

The study of mycobacterial lipids was initiated more than 70 years ago under the direction of Anderson in 1939 (Anderson, 1938). This field is still an active source of research due to the fascinating diversity of their structures and biological activities. The glycolipids are major *M.tb* cell wall constituents, known for their toxic or immunological properties (Brennan and Nikaido, 1995). They comprise the acyl trehaloses [mono- and dimycolyl trehalose (TMM, TDM), di- and triacyltrehalose (DAT, TAT) and sulfolipid (SL)], oligosaccharides containing lipids (lipooligosaccharides (LOSs), phenolglycolipid (PGL), apolar lipids such as the phthiocerol dimycocerosate (DIM), and the glycosyl derivatives of phosphatidyl-*myo*-inositol.

Trehalose-containing glycolipids share a common α-D-Glc*p*(1→1') α-D-Glc*p* unit and are the class of *M.tb* lipids that have been most extensively studied and still fascinate the majority of lipidologists and mycobacteriologists. TDM (or cord factor) was first obtained by Bloch (Bloch, 1950) after a petroleum ether extraction from cells of a virulent strain of *M.tb.* The resulting extract was toxic when injected into mice and a drastic disorganization of the cords that *M.tb* formed at the culture medium surface was also observed. The toxic compound present in the extract was shown to be 6,6'-dimycoloyl-α-D-trehalose (Noll, 1956). TDM toxicity is due to an increase of the tissue specific nicotinamide adenine dinuclease activity decreasing the levels of NAD in several tissues by blocking the electron flow along the

mitochondrial respiratory chain and thus oxidative phosphorylation (Artman *et al.*, 1964;Barry *et al.*, 1998;Brennan, 2003). During infection and when inside of the phagosome, *M.tb* is shown to produce large quantities of TDM (Fischer *et al.*, 2001). From many studies done on TDM, it is remarkable that TDM induces lung granulomas and has immunostimulating properties (Bekierkunst, 1968) that are probably at the origin of its antitumoral activity (Bekierkunst *et al.*, 1971a) (Table 1). TDM has also been shown to have adjuvant properties generating an optimal antibody response (Bekierkunst *et al.*, 1971b) and a non-specific immune response against bacterial infections and parasitic infections (Bekierkunst, 1968;Parant *et al.*, 1977, 1978;Ribi *et al.*, 1976;Yarkoni & Bekierkunst, 1976). Recent studies uncover that TDM is actively participating in blocking the mycobacterial phagosome maturation (Indrigo *et al.*, 2003). Inhibition of the phagosome maturation is observed after phagocytosis of virulent strains of *M.tb*, allowing the bacillus to survive within the phagocyte (Schlesinger LS *et al.*, 2008). Recently, Mincle (macrophage-inducible C-type lectin) (Yamasaki *et al.*, 2008) on the macrophage surface, has been shown to recognize *M.tb* TDM, and working together with the Fcγ receptor transmembrane segment induces pro-inflammation (Ishikawa *et al.*, 2009; Schoenen *et al.*, 2010). In the case of *M.tb* TMM, this is shown to be used by the bacillus to transfer mycolic acids towards molecules like the wall-linked AG. In agreement with this fact, the known secreted immunogenic Ag 85 complex has been identified as a trehalose mycolyltransferase in *M. smegmatis* (Sathyamoorthy & Takayama, 1987) and later in *M.tb* (Belisle *et al.*, 1997). TMM is also shown to have lethal toxicity, adjuvant activity, and capable of stimulating tumour necrosis factor via activation of the protein kinase C pathway (Numata *et al.*, 1985)(Table 1).

The sulfated trehaloses (sulfolipids, designated by SL) (Middlebrook *et al.*, 1959) are also present in virulent strains of *M.tb*, specifically SL-1 (Goren, 1970a,1970b). SL-1 can be acylated by 2 to 4 very long (up to C_{64}) saturated and unsaturated, highly branched fatty acids. Sulfate derivatives are rare in natural substances, and some of the acyl chains of SL-1 are also uncommon, since they are mainly highly branched in their carboxyl end (Goren & Mor, 1990;Leigh & Bertozzi, 2008). The SLs have attracted much interest since it was shown that, like TDM, they seem to inhibit phagosome-lysosome fusion in macrophages (Goren *et al.*, 1976), and are cytotoxic (Kato & Goren, 1974a, 1974b). However, the role of SL-1 in *M.tb* pathogenesis seems to be dependent of the model system studied. In this context, results obtained from *in vitro* and *in vivo* studies (the latter using different animal models and *M.tb* strains) dispute the role of SL-1 in *M.tb* pathogenesis (Brozna *et al.*, 1991;Gangadharam *et al.*, 1963;Goren *et al.*, 1974, 1982;Pabst *et al.*, 1988;Rousseau *et al.*, 2003;L. Zhang *et al.*, 1988, 1991). While SL-1 has been shown to induce specific host cell responses, such as inhibition of phagocyte priming/activation (Brozna *et al.*, 1991;Pabst *et al.*, 1988), its mechanism of action is still unclear; although a role of a guanine nucleotide binding protein in both priming and direct activation of neutrophils by SL-1 has been suggested (L. Zhang *et al.*, 1991) (Table 1).

Diacyltrehalose (DAT, a 2, 3-diacyltrehalose) and triacyltrehalose (TAT, a 2, 3, 6-triacyltrehalose) (Gautier *et al.*, 1992), who's acyl groups are mainly branched polymethyls, are also present in the *M.tb* cell wall. The main use of these glycolipids is in *M.tb* sero-diagnosis (Muñoz *et al.*, 1997a). However, a study recently showed that *M.tb* mutants lacking DATs and sulphoglycolipids cannot block phagosome maturation and thus, revealing the importance of these molecules in the *M.tb* pathogenesis (Table 1)(Brodin *et al.*, 2010). In *M.tb*, other trehalose-based lipids are lipooligosaccharides (LOSs), which contain a polyacylated trehalose with long chain fatty acids and an oligosaccharide. It contains 2 or 3

straight or methyl-branched chains. In some LOSs, acyl residues can be distributed between the two glucose residues of the trehalose end of the polymer. Depending on the species, an oligosaccharide (2 to 6 sugar residues) is linked either on carbon 3, 4 or 6 of the trehalose end (Gilleron *et al.*, 1994;Hunter *et al.*, 1985). Pyruvic acid residues (carboxyethylidene) can also be present, giving an anionic character to the molecule. LOSs have been considered to be immunogenic and also phage receptors (Besra & Chatterjee, 1994), suggesting that they are located in the *M.tb* cell wall surface. Other *M.tb* lipids containing oligosaccharides are the phenolic glycolipids (PGLs). These have been extensively studied in *Mycobacterium leprae* (PGL-I) (Hunter *et al.*, 1983;Hunter & Brennan, 1981). Leprosy patients have antibodies against this molecule, and therefore it is a useful diagnostic tool (Cho *et al.*, 1983). PGLs have been found in the Canetti strain of *M.tb* (Daffe *et al.*, 1987) (PGL-Tb), however, serodiagnostic studies have shown that there were large variations among tuberculous patients in the response to this antigen (Daffe *et al.*, 1991). This is likely due to large differences in phenolglycolipid amounts produced by different *M.tb* strains (Cho *et al.*, 1992; Torrelles *et al.*, 2008b). Recently, the hypervirulent phenotype observed in several strains of *M.tb* (*i.e.* strain HN878) has been associated with the presence of PGL in their cell wall (Reed *et al.*, 2004).

There are many apolar lipids described forming part of the cell wall of *M.tb*, however, of particular importance is the phthiocerol dimycocerosate (or PDIM/DIM). PDIM is a major apolar lipid present in the cell wall of *M.tb*. PDIM is considered a wax containing multiple methyl and/or methylene groups (Brennan, 2003). Several studies have tied, as in the case of TDM and SL-1, the presence of PDIM to *M.tb* virulence (Goren & Brennan, 1980). *In vivo* studies using *M.tb* strains depleted of PDIM show attenuation in the growth of these strains in mice (Cox *et al.*, 1999;Ferwerda *et al.*, 2007), in accordance with previous studies in Guinea pigs using a *M.tb* clinical isolate lacking PDIM in its cell wall (Goren & Brennan, 1980) (Table 1). Recent studies using a genetically engineered *M.tb* PDIM mutant concluded that PDIM inserts into the host membrane and participates both in the receptor-dependent phagocytosis of *M.tb* and the prevention of phagosomal acidification (Astarie-Dequeker *et al.*, 2009). Using PDMI mutants also is shown that these are required for *M.tb* resistance to an IFN-γ-mediated immune response that is independent of NOS2 (Kirksey et al., 2011).

Glycosyl derivatives of phosphatidyl-*myo*-inositol in the *M.tb* cell wall are the phosphatidyl-*myo*-inositol (PI) and its mannosylated derivatives known as phosphatidyl-*myo*-inositol mannosides (PIMs), phosphatidylethanolamine, phosphatidylglycerol, diphosphatidyl-glycerol, cardiolipin, and glycosylphosphopolyisoprenols. PI and its mannosylated derivatives are important lipids in the cell wall of *M.tb*, both as key membrane constituents and as participant in essential *M.tb*-host interactions and metabolic processes. PI is an acidic (anionic) phospholipid that in essence consists of a phosphatidic acid backbone, linked via the phosphate group to inositol (hexahydroxycyclohexane). In mycobacteria, the stereochemical form is *myo*-D-inositol. As early as 1930's, it was recognized by Anderson that the phospholipidic fraction extracted from *M.tb* and related mycobacteria contained inositol and mannose (Anderson, 1938). Besides phosphatidylethanolamide, PIMs are the major phospholipid components of the *M.tb* cell wall (Brennan, 2003). PIMs are found as a mixture of compounds differing one from the other by the number of mannosyl residues and fatty acids. Their structures consist of a mannosyl unit attached to position C-2 of the *myo*-inositol of a PI anchor. Position C-6 of *myo*-inositol is further substituted by an α-D-

mannosyl or a linked trimannosyl unit, giving PIM_2 and PIM_4, respectively. PIM_4 may be further substituted at position C-2 by a α-D-mannosyl leading PIM_5, which can also be further substituted at the same position leading PIM_6 [α-D-Manp(1→2)-α-D-Manp(1→2)-α-D-Manp(1→6)-α-D-Manp(1→6)-α-D-Manp(1→6)-*myo*-inositol], the higher PIM encountered in mycobacteria (Torrelles & Schlesinger, 2010). Studies have shown that PIMs, which are known to interact with the plasma membrane, are also present on the *M.tb* cell wall surface (Ortalo-Magné *et al*., 1996). The intrinsic heterogeneity of PIMs is evident looking at their carbohydrate constitution (PIM to PIM_6). A difficulty is added, however, when we look at the acylation sites of the PIMs. Many studies have shown evidence for multiacylated forms of PIMs in *M.tb* [reviewed in (Torrelles & Schlesinger, 2010)]. Differences in the degree of acylation and the kind of fatty acid linked was studied in detail by Khoo *et al*. (Khoo *et al*., 1995), who confirm the existence of triacylated PIMs (Ac_1PIM_x) esterified by palmitic (16:0) and tuberculostearic (TBST or 10-methyl-octadecanoic) acids, and discussed the presence of tetracylated PIMs (Ac_2PIM_x), where an additional fatty acyl could be carried in the *myo*-inositol ring. This fact was later corroborated by Gilleron *et al.* (Gilleron *et al*., 1999) showing an unambiguous localization of a fourth fatty acid on the C-3 of the *myo*-inositol beside the fatty acids on C-1 and C-2 position of the glycerol and on the C-6 position of the (1→2) linked mannose. PIMs can be grouped in lower- and higher-order depending of the number of mannoses, where lower-order PIMs contain 1 to 4 mannoses and higher-order PIMs contain 5 to 6 mannoses (Torrelles *et al*., 2006). The most common PIMs found are $AcPIM_2$ and Ac_1PIM_2 (di- and triacylated PIM_2) and $AcPIM_6$ and Ac_1PIM_6 (di-and triacylated PIM_6) (Khoo *et al*., 1995). Lower-order PIMs have a terminal α(1→6)-mannose and are shown to participate in the phagocytosis process through association with the non-opsonic domain of complement receptor-3 (Villeneuve *et al*., 2005), and also participate in trafficking processes within the phagocyte by facilitating early endomal fusion with phagosomes (Vergne *et al*., 2004). Higher-order PIMs have a terminal α(1→2)-mono- or di- mannoside (Ac_xPIM_5 or Ac_xPIM_6, respectively) similar to the mannose caps of mannose-capped lipoarabinomannan [see section 2.2.2.1 for details]. Only triacylated forms of higher-order PIMs are shown to interact with the mannose receptor (MR) and interfering with trafficking pathways by limiting phagosome-lysosome fusion (Torrelles *et al*., 2006). Moreover, several studies have also shown that all *M.tb* PIMs interact with dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) (Torrelles *et al*., 2006), although differences in DC-SIGN PIM recognition specificity may be species dependent (Driessen *et al*., 2009). Additional studies have also shown that cytosolic soluble CD1e is involved in PIM_6 processing and presentation via CD1 with subsequent T cell activation (de la Salle *et al*., 2005) (Table 1).

Apart from the mannosides of phosphatidylinositol, there are other mycobacterial phosphodiacylglycerol, whose origins are based on phosphatidic acid. These are phosphatidylglycerol, diphosphatidylglycerol (DPG) and phosphatidylethanolamine. Although the role of these *M.tb* cell wall phospholipids during infection is uncertain, *M.tb* cardiolipin (a DPG molecule) is shown to be processed into lysocardiolipin by the lysosomal phospholipase A_2 during *M.tb* infection (Fischer *et al*., 2001). Antibodies against *M.tb* cardiolipin are also found in sera from TB patients (Santiago *et al*., 1989), and their production is shown to be strictly related to IL-4 and T cells (Fischer *et al*., 2002). Anti-

cardiolipin antibodies are also capable of activating complement (Santiago *et al.*, 1991). Small quantities of glycosylphosphopolyisoprenols, which are involved in *M.tb* cell wall biosynthesis, have also been isolated from the cytoplasmic membranes of *M.tb*, where ribosyl-, mannosyl- and arabinosyl-phosphopolyprenols have been characterized (Takayama *et al.*, 1973; Takayama & Goldman, 1970; Wolucka & De Hoffmann, 1995).

In summary, *M.tb* elaborates a great variety of glycolipids of rather unusual structure. Some of these lipids are abundant in the inner cell wall and others are exposed on the bacillus surface. These include acylglucosides, sulfatides, lipooligosaccharides, phenolic glycolipids, dimycocerosates, and the ubiquitous phosphatidyl-*myo*-inositol-mannosides. Some of these glycolipids are described as virulence factors helping *M.tb* to survive as intracellular 'parasites' that infect and reside in the host cell. The biological activities attributed to these surface-exposed glycolipids may derive, at least in part, from the modulation of cell functions through the interactions between host membranes and them, whose structures are different from those of mammalian cell membrane components. Biologically active glycolipids have been shown to profoundly affect the physical and functional properties of biologic membranes (Brandley & Schnaar, 1986) as well as inhibit both macrophage antimicrobial activities and lymphocyte proliferation (Vergne & Daffe, 1998). Therefore, the enzymes involved in their biosynthesis may represent potential drug targets (Kaur *et al.*, 2009). Nevertheless, for some of these lipids confirmation of their role in *M.tb* pathogenicity is still lacking, opening the necessity to genetically manipulate *M.tb* to obtain glycolipid deficient mutants. These mutants may be unable to elaborate determined glycolipid thought to be involved in *M.tb* pathogenesis. Thus, the lack of specific glycolipids in virulent strains of *M.tb* may help us to understand their real implication in pathogenesis.

2.2.3 Lipoglycoconjugates of the *M. tuberculosis* cell wall

An extensive study of the mannose-capped lipoarabinomannan (ManLAM) has been performed in *M.tb* [reviewed in (Torrelles & Schlesinger, 2010)]. *M.tb* ManLAM is an extremely heterogeneous lipoglycan with a defined tripartite structure that possesses a carbohydrate core, a mannosyl-phophatidyl-*myo*-inositol anchor (MPI) and various capping motifs. Following the earlier work performed by Chatterjee and co-workers (Chatterjee & Khoo, 1998), a series of detailed structural analyses have produced evidence of this tripartite structure, in which ManLAM was distinguished from the related lipomannan (LM) by virtue of having an additional immunodominant arabinan domain that extends from a common phosphatidyl-*myo*-inositol mannan core in an as yet undefined manner. The polysaccharide core of *M.tb* ManLAM consists of two very well differentiated polymers, a D-mannan and a D-arabinan. The D-mannan structure consists of a linear α(1→6) linked mannopyranosyl backbone that is linked to a phosphatidyl-*myo*-inositol anchor, and presents substitutions/ branches on their C-2 with another single mannose (α-D-Man*p*(2→1)t-α-D-Man*p*). The D-mannan size and the degree of branching can vary among *M.tb* strains. This mannosyl backbone carries an unknown number of branched arabinosyl side chains, which form the D-arabinan. To date, the linkage between the arabinan polymer and the D-mannan core is still not determined. The arabinan is based on the rare α-D-arabinofuranose (Ara*f*), and consists of a branched linear α(1→5) linked Ara*f* backbone. Branching residues carry an additional α(1→3) linked Ara*f*. At its non-reducing end we can

find two types of arrangements or motifs, a linear tetraarabinofuranoside (Ara_4) defined as β-D-Ara*f*-(1→2)-α-D-Ara*f*-(1→5)-α-D-Ara*f*-(1→5)-α-D-Ara*f* and a branched hexaarabinofuranoside (Ara_6) defined as [β-D-Ara*f*-(1→2)-α-D-Ara*f*-(1→]$_2$-3 and 5-α-D-Ara*f*-(1→5)-α-D-Ara*f*. In the case of slow-growing mycobacteria like *M.tb*, *M. leprae* and *M. bovis* BCG, some of the terminal arabinan motifs are extensively capped at C-5 with one or more α-mannoses attached to these β-Ara*f* termini. The mannooligosaccharides linked to the terminal β-Ara*f* thus define ManLAM. The mannose caps are defined as a single Man*p*, a dimannoside (α-D-Man*p*-(1→2)-α-D-Man*p*) or a trimannoside (α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*). These units are located in both tetra- and hexaarafuranosyl motifs of the arabinan non-reducing terminal (Chatterjee *et al.*, 1993). Data reported from different studies shown that the disaccharide unit is the cap most frequently found in both linear (Ara_4) and branched (Ara_6) termini. Man_2Ara_4 and Man_4Ara_6 are then the most frequent motifs in all ManLAM studied (Chatterjee *et al.*, 1993), however, trimannoside caps in the linear Ara_4 have also been found (*i.e.* Man_3Ara_4), and in the branched Ara_6, all three combinations of mannose caps have been found, *i.e.* ($Man_{[(1\ to\ 3)x2]}$ Ara_6). The degree of ManLAM capping varies according to the *M.tb* strain studied, where *M.tb* Erdman is the most capped when compared to *M.tb* $H_{37}R_v$ and $H_{37}R_a$ strains [reviewed in (Torrelles & Schlesinger, 2010)]. The anchor structure in *M.tb* ManLAM is similar to the one in PIMs, and consists in an *ns*-glycerol 3-phospho-(1-D-myo-inositol) unit with a α-D-mannopyranosyl residue at C-2 of the *myo*-inositol (MPI). In the C-6 position of this *myo*-inositol there is *O*-linked the mannan polymer described previously (Chatterjee & Khoo, 1998). Some of the heterogeneity that characterizes ManLAM occurs through the number, the location, and the nature of the fatty acids esterifying the PI anchor. The characteristic fatty acids described in the ManLAM anchor are 16:0 and TBST (Hunter *et al.*, 1986). However, traces of stearic (18:1), myristic (14:0), heptadecanoic (17:0), 10-methyl-heptadecanoic, 12-O-(methoxypropioryl)-12-hydroxy-stearic and 12-hydroxy-tuberculostearic acids have also been described (Leopold & Fischer, 1993;Nigou *et al.*, 1997). The average number of fatty acids per ManLAM molecule cannot be generalized. Some studies confirmed an average of 3 fatty acids per molecule of ManLAM in positions 1 and 2 of the *ns*-glycerol and position 6 of the Man*p* unit linked to C-2 of the *myo*-inositol (Khoo *et al.*, 1995). Studies by Chatterjee and colleagues performed in an ethambutol resistant strain of *M.tb* supported the existence of tetraacylated ManLAM as the most common molecular form (Torrelles *et al.*, 2004). Thus, the only fact that can be generalized is that, with the exception of the lyso-forms of ManLAM (only one fatty acid in the ManLAM anchor), ManLAM at least has two fatty acids, with both fatty acids in the *ns*-glycerol unit, where 16:0 and TBST are at position 1 and at position 2, respectively.

The presence of additional acyl groups on ManLAM has been reported by several authors. Hunter *et al.* reported the existence of succinates and lactates (Hunter *et al.*, 1986). Delmas *et al.* used nuclear magnetic resonance spectroscopy to locate the succinic groups (1 to 4 per molecule) in the C-2 of the 3,5-α-D-Ara*f* and/or 5-α-D-Ara*f* residues in ManLAM from different *Mycobacterium bovis* BCG strains (Delmas *et al.*, 1997). Later, studies performed by Chatterjee and colleagues analyzing the content of succinates in ManLAMs from different mycobacterial species and strains showed that *Mycobacterium leprae*, the *M.tb* laboratory strain $H_{37}R_v$, and a *M.tb* clinical isolate (CSU 20) had also succinates (Torrelles *et al.*, 2004); where ManLAM from *M. leprae*, the laboratory strain $H_{37}R_v$, and CSU20 had an average

number of 7, 2 and 4 succinates, respectively. The succinates biological function in ManLAM is still a question to be resolve. Our recent studies show that a biosynthetically related lipoglycan to ManLAM, the lipomannan, also contains succinates, where succinates seem to influence CD1-Ag presentation to T cells and subsequent T cell activation (Torrelles *et al.*, 2011). Recently, Treumann *et al.* using nuclear magnetic resonance spectrometry defined a new terminal sugar located in the caps of *M.tb* ManLAM (Treumann *et al.*, 2002). This sugar consisted in a 5-deoxy-5-methylthio-α-xylofuranosyl (MTX), and may be involved in *M.tb*-host interactions battling the effects of reactive oxygen species by adding to the antioxidant properties of ManLAM (Turnbull *et al.*, 2004). The orientation of LAM in the *M.tb* cell wall is still unresolved. There are many hypotheses, but the most accepted is that ManLAM is anchored by its lipidic anchor into the plasma membrane, and projects through the thickness of the wall so that its terminal arabinose or mannose-capped arabinose units are accessible to the outside (McNeil & Brennan, 1991). Other possibilities are that ManLAM is interacting by its lipid anchor with the mycolic acid layer and with other polar wall associated lipids (Rastogi, 1991), or that ManLAM has a non-permanent location in the cell wall, and is essentially a secreted molecule in transit through the envelope. The many studies carried out on ManLAM have led to data that supports each of these hypotheses. For example, Lemassu and Daffe demonstrate the existence of non-PI containing mannose-capped arabinomannan in the so called capsular/outer material polysaccharide associated with *M.tb* (Lemassu & Daffe, 1994). Other studies, subdivided ManLAM into two different kinds, the parietal ManLAM and the cellular ManLAM. Both had similar core structure presenting remarkable differences in the degree of mannose-capping and the acylation of the PI-anchor (Gilleron *et al.*, 2000). The fact that the parietal LAM is obtained without cell disruption reinforces the hypothesis of two different locations for ManLAM. Thus, ManLAM may be firmly, but not covalently, attached to the *M.tb* cell wall and it may also be anchored to the plasma membrane. The biological function of *M.tb* ManLAM is discussed later in this chapter (see also Table 1), and information about its biosynthesis pathway(s) can be found elsewhere (Kaur *et al.*, 2009).

Another remarkable lipoglycan in the *M.tb* cell wall is lipomannan (LM). The α (1→6) mannose polymer of LM presents identical characteristics to the mannan backbone of ManLAM. The mannan of LM is directly attached to position C-6 of the *myo*-inositol of its MPI anchor. The *M.tb* ManLAM and LM MPI anchor is indistinguishable from the *M.tb* dimannosylated phophatidyl-*myo*-inositol (Ac_xPIM_2), the structure of which was established by Lee and Ballou (Lee and Ballou, 1965). LMs are considered multimannosylated forms of PIMs by the fact that both types of molecules have an elaborated anchor in common (Gilleron *et al.*, 1999). Their common structure with ManLAM also enforces the hypothesis that LM is a precursor of ManLAM (Besra *et al.*, 1997). However, it seems that LM could also be a co-lateral final product in the biosynthetic pathway of ManLAM (Besra *et al.*, 1997). Few biological properties of *M.tb* LM have been described, mainly because this molecule is still understudied (Table 1).

Mycobacterial LM is shown to regulate cytokine, oxidant and T cell responses (Barnes *et al.*, 1992; Chan *et al.*, 2001;Gilleron *et al.*, 2001). *M.tb* LM is shown to associate with DC-SIGN and not with the MR (Torrelles *et al.*, 2006), and to induce apoptosis and a pro-inflammatory response through TLR2 (Dao *et al.*, 2004;Nigou *et al.*, 2008). However, recent studies showed that although *M.tb* LM is capable of activating macrophages via TLR2 inducing

signaling cascades required for TNF mRNA expression, the TNF mRNA produced is poor translated and faster degraded (Rajaram *et al*., 2011).

***M.tb* cell wall location**	***M.tb* cell wall component**	**Host Cell Receptor(s)**	**Phagosome maturation blockade**	**Host Immune Response**	**Sero-activity**	**Cyto-toxicity**
Outer Material	α-Glucan	DC-SIGN, CR3?	No	Anti-inflammatory	Unknown	ND
Cell wall Core	Peptidoglycan (PG)-MDP	Nod2	ND	Pro-inflammatory	Unknown	ND
	Arabinogalactan (AG)	ND	ND	ND	Yes	ND
	Mycolic Acids	CD1 (in Ag-presentation	ND	Pro-inflammatory	Yes	Yes
Peripheral lipid layer	Trehalose dimycolate (TDM)	Mincle-FcγR TLRs	Yes	Pro-inflammatory	Yes	Yes
	Trehalose monomycolate (TMM)	ND	ND	Pro-inflammatory	Yes	Yes
	Sulfolipid-1 (SL-1)	ND	Yes	Pro-inflammatory	Yes	Yes
	Diacyl- and Triacyl-trehalose (DAT & TAT)	ND	Yes	Pro-inflammatory	Yes	ND
	Lipooligosaccharides (LOSs)	ND	ND	Pro-inflammatory	Yes	ND
	Phenolic glycolipid (PGL-TB)	CR3?	ND	Pro-inflammatory	Yes	Yes
	Triglycerides	TLRs	ND	Pro-inflammatory	Unknown	Yes
	Phthiocerol dimycocerosate (PDIM)	Direct insertion into host Mbrs	Yes	Pro-inflammatory	Unknown	Yes
	Lower-order phosphatidyl-*myo*-inositol mannosides (PIMs)	CR3, TLRs, DC-SIGN	No	Pro-inflammatory	Yes	ND
	Higher-order phosphatidyl-*myo*-inositol mannosides (PIMs)	MR, DC-SIGN	Yes (through the MR only)	Anti-inflammatory	Yes	ND
	Lipomannan (LM)	TLRs, DC-SIGN	No	Pro-inflammatory	Yes	ND
	Mannose-capped lipoarabinomannan (ManLAM)	MR, DC-SIGN	Yes (through the MR only)	Anti-inflammatory	Yes	ND

Mbrs: Membranes.

Table 1. *M.tb* cell wall components and their interaction with the host outcome.

Due to the complexity of the cell wall of *M.tb,* we may need to be careful when assessing the essentiality of a specific cell wall component thought to be a virulence factor. Current strategies are directed in creating isogenic strains of *M.tb* deficient in the production of a specific virulent factor. We may not obtain the real answer by just depleting the presence of a potential virulent factor in the cell wall of *M.tb*. In this context, efforts to discern the enzymes involved in the biosynthetic pathways of the *M.tb* lipids are critical to address their essentiality in *M.tb* survival. These will allow us to uncover novel drug targets. However, when evaluating the role of the omitted/mutated lipid in *M.tb* pathogenesis, we need to be careful in considering the rearrangement that the *M.tb* cell wall may suffer upon the lack of a specific lipid. Depending of the structural nature of the lipid depleted from the *M.tb* cell wall (*i.e.* size, charge, hydrophobicity, etc.), we may find that the bacterial cell wall is altered in a way that the absence of the lipid is unexpectedly compensated. This is the case for SL-1, where the lack of this lipid in *M.tb* isolates is being linked to TB pathogenesis, however, studies performed by Jackson and colleagues clearly showed that when using an isogenic SL-1 mutant, SL-1 deficiency did not affect *M.tb* virulence (Rousseau *et al.*, 2003). As Jackson and collaborators stated in this study, there are several explanations behind this observed discrepancy, but one of them is related to the presence of a potent attenuator lipid in the clinical isolates lacking SL-1 that compensated the SL-1 phenotype (Goren *et al.*, 1982). Other factor to account for is the synergy between *M.tb* lipids; this is also observed for SL-1 and TDM, where purified SL-1 alone at high doses was innocuous, but when administered simultaneously with TDM, a synergistic increase in the TDM cytotoxicity was observed (Kato & Goren, 1974a, 1974b). Thus, indicating that the lack of a specific lipid may also significantly alter the cytotoxic properties of other *M.tb* cell wall components. Finally, we will need to consider evaluating the constitution of the *M.tb* cell wall during infection. Are the properties of the *M.tb* cell wall altered during infection? Some studies indicate that this may happen in the case for TDM, which is overproduced during *M.tb* infection (Backus *et al.*, 2011;Fischer *et al.*, 2001). Why do clinical isolates of *M.tb* present different cell wall rearrangements than the widely studied *M.tb* laboratory strains Erdman, $H_{37}R_v$, and $H_{37}R_a$? In this context, hypervirulent *M.tb* strains of the Beijing family (Tsenova *et al.*, 2005) are shown to contain large amounts of triglycerides (Reed *et al.*, 2007), and some of them also contain the PGL-TB (Reed *et al.*, 2004). *M.tb* clinical isolates deficient in ManLAM and PIMs surface exposure, but presenting in their cell wall large quantities of triglycerides, PGL-TB, and dimycocerosates, are also shown to have reduced phagocytosis but faster intracellular growth rate in human macrophages (Torrelles *et al.*, 2008b). These studies performed by Schlesinger and colleagues concluded that the clinical spectrum of TB is not only dictated by the host but also it may be related to the amounts and ratios of specific surface-exposed *M.tb* adherence factors defined by *M.tb* strain genotype (Torrelles *et al.*, 2008b;Torrelles & Schlesinger, 2010). Is this *M.tb* genotypic/phenotypic adaptation due to their multiple passages through the host? Triglycerides and DIMs are a major part of the peripheral lipid layer in the *M.tb* cell wall; however, their role in pathogenesis has been until recently overlooked due the presence of other hydrophilic and hydrophobic cell wall components more attractive to TB researchers due to their potential (or already established) role in dictating *M.tb*-host cell interactions. Other questions demanding answers refer to the structural properties of the cell wall of MDR-, XDR-, XXDR, and TDR-*M.tb* strains. What is the cell wall constitution of these strains? Studies performed using transmission electron

and atomic force microscopy techniques started to dig into this question showing that MDR-, XDR-, XXDR- and TDR- strains have thicker cell wall and rougher cell surface (supposedly produced by the progressive erosion of their cell wall by the action of the drugs) with tubular extensions than susceptible strains (Velayati *et al.*, 2009a, 2010). Because some XDR- and TDR-*M.tb* strains are related to the Beijing family (Velayati *et al.*, 2009b), which are shown to have their cell wall overpopulated with triglycerides, it is plausible to question any relationship between the abundance of a specific hydrophobic lipid on the *M.tb* cell wall and drug resistance. Many of these questions remain unanswered.

3. *M.tb*-Host interface

The initial recognition of *M.tb* by the host is quite complex and involves alveolar resident cells and many of their surface receptors. The concept of studying the contribution of a specific receptor(s) in the *M.tb* recognition and/or uptake is critical to our understanding of the pathway(s) that the bacillus exploits to gain entrance into the host cell minimizing or triggering the immune response. However, it is important to link the results obtained studying a specific receptor to the existence of other receptors that may also participate at the same time in recognizing *M.tb* generating a completely different outcome. Normally the outcome of *M. tuberculosis*-host recognition is beneficial for the host, triggering the innate immune response; however, engagement of *M.tb* with specific phagocytic receptors is shown to be beneficial for *M.tb* leading to a pathway of survival and subversion of the immune response. Here we will describe the host phagocytic and signaling receptors [some of them known as a pattern recognition receptors, PRRs, for their unique capability to recognize specific motifs on the *M.tb* cell surface (these motifs are also known as pathogen/microbial-associated molecular patterns, PAMPs/MAMPs)] involved in *M.tb* recognition and the subsequent inflammatory response attending in our discussion to the fact that *M.tb* recognition simultaneously includes multiple receptors.

3.1 Phagocytic receptors

The encounter of *M.tb* with the host triggers the phagocytosis process. This process depends of two important factors; one is the constitution of the cell wall of *M.tb* (which is strain dependent) and the surface receptor repertoire present on the phagocyte (which is host cell dependent). Mainly *M.tb* infections occur by airborne transmission of droplet nuclei containing few viable bacilli. The first contact between *M.tb* and the human host cell is within the alveolar space of the lung. When *M.tb* reaches the alveolar space, resident alveolar macrophages (AMs), and alveolar epithelial cells together with recruited monocytes, neutrophils, lymphocytes and fibroblast represent the array of immune cells that participate in host defense. Phagocytic receptors involved in *M.tb* recognition by the host mainly are: the mannose receptor (MR), DC-SIGN, and complement receptors (CRs). *M.tb* uptake by these receptors leads to the formation of an *M.tb* containing phagosome with different outcomes as noted below (Table 1).

3.1.1 The mannose receptor

The cell wall of certain *M.tb* strains has been characterized to be heavily mannosylated with molecules exposing their α(1→2)-Man*p* termini on the bacterial surface acting as ligands for

host cell receptors contributing to *M.tb* pathogenesis (Torrelles & Schlesinger, 2010). These molecules are ManLAM, LM, PIMs, arabinomannan, mannan, and mannosylated glycoproteins. The MR is conceived as a homeostatic receptor, whose main function is the recycling of endogenous highly *N*-mannosylated glycoproteins normally generated during inflammation (Martinez-Pomares *et al.*, 2001). Studies by Schlesinger and colleagues have suggested that *M.tb* may be capable of using its surface mannose coating to gain entrance and survive within the host cell by associating with the MR. In this context, we demonstrated that *M.tb* can use two of its mannosylated cell wall components, ManLAM and higher-order PIMs, to associate with the MR, leading to a pathway of intracellular survival within the host by blocking phagosome acidification (Kang *et al.*, 2005;Torrelles *et al.*, 2006). Association with the MR has also been shown to reduce microbicidal activities by down-regulating the generation of pro-inflammatory cytokines, nitric oxide, oxygen radicals and by blocking *M.tb*-induced Ca^{2+}-depending apoptosis[reviewed in (Torrelles *et al.*, 2008a)]. In particular, *M.tb* ManLAM has been shown to interact with the MR triggering an anti-inflammatory response by blocking the production of inflammatory cytokines such as TNF and IL-12, and inducing the generation of IL-10 and TGF-β (Astarie-Dequeker *et al.*, 1999;Chieppa *et al.*, 2003;Nigou *et al.*, 2001)(Table 1). Recently, studies by Schlesinger and colleagues showed that engaging of the MR by ManLAM and/or virulent *M.tb* upregulates the peroxisome proliferator-activated receptor-gamma (PPAR-γ, a transcription factor showed to be important in regulating the inflammatory response) leading to a simultaneous increase in the generation of CXCL-8 (or IL-8), expression of cycloxygenase 2 (COX_2), and production of prostaglandin 2 (PGE_2) (Rajaram *et al.*, 2010). Moreover, this study depicts how *M.tb* negatively regulates protective inflammatory modulators through the MR, where engaging of the MR down-regulates TNF levels via PPAR-γ (Rajaram *et al.*, 2010). In addition to *M.tb* ManLAM blocking the generation of TNF via the MR, Schlesinger and colleagues also identified a novel molecular and cellular mechanism underlying the ability of another major *M.tb* cell wall component, the LM, to block TLR2 induced biosynthesis of TNF in human macrophages, thereby allowing *M.tb* to subvert the host immune response and potentially increase its virulence (Rajaram *et al.*, 2011).

3.1.2 DC-SIGN

M.tb is shown to associate with dendritic cell–specific ICAM-3-grabbing non-integrin (or DC-SIGN) (Geijtenbeek *et al.*, 2000) through is cell surface cell wall components ManLAM, LM and PIMs [reviewed in (Ehlers, 2009)](Table 1). Recently, α-glucan was also described as another *M.tb* cell wall ligand for DC-SIGN (Geurtsen *et al.*, 2009). Binding of *M.tb* to DC-SIGN in DCs leads to bacterial killing by acidification of the *M.tb* phagosome (Geijtenbeek *et al.*, 2003). However, the implication of DC-SIGN in triggering the immune response is still controversial. On one hand, engaging of *M.tb*, mannosylated cell wall components, or α-glucan has been shown to induce generation of anti-inflammatory modulators such as IL-10 (Ehlers, 2009;Geurtsen *et al.*, 2009). These findings were supported by *in vivo* studies using mice expressing human DC-SIGN homologues (McGreal *et al.*, 2005;Park *et al.*, 2001;Powlesland *et al.*, 2006) or transgenic mice expressing human DC-SIGN, showing that DC-SIGN may act damping the immune response, and thus, promote host protection by limiting tissue damage (Schaefer *et al.*, 2008;Tanne *et al.*, 2009;Wieland *et al.*, 2007). On the other hand, another study concluded that the ManLAM-PIM/DC-SIGN pathway may not

be significantly involved in regulating cytokine secretion using an engineered *M. marinum* strain lacking essential mannosylated components (Appelmelk *et al.*, 2008).

3.1.3 Complement receptors

Complement receptors are described on the surface of all mononuclear phagocytes. In *M.tb* phagocytosis CR1, CR3 and CR4 have been implicated (Fenton *et al.*, 2005). Several studies have established the role of the complement component 3 (C3) in *M.tb* opsonization. C3 deposition in the form of C3b and C3bi happens quickly via covalent linkages with cell wall components located on the *M.tb* surface (Ferguson *et al.*, 2004). As C3 opsonization depends of serum levels in the tissue, it is still unknown how C3 opsonization varies in form (classical and/or the alternative pathways) and amount among different stages of *M.tb* infection or tissue sites. *M.tb* surface-exposed lower-order PIMs (*i.e.* PIM_2) and specific polysaccharides have been shown to directly interact with the lectin domain of CR3 (Cywes *et al.*, 1997;Hoppe *et al.*, 1997;Villeneuve *et al.*, 2005) and thus, presumably mediate *M.tb* uptake by macrophages (Table 1). PGL-TB from *M.tb* may also interact with CR3, as this is the case for the structurally related PGL-1 from *M. leprae* (Tabouret *et al.*, 2010). Although CR3 seems to drive *M.tb* uptake under opsonic and non-opsonic conditions, *in vitro* and *in vivo* studies using wild type and CR3-deficient mice did not show differences in lung pathology and bacterial burden (Hu *et al.*, 2000), and thus CR3 role in *in vivo* infections remains unanswered. Less attention has been put into CR4, which together with the MR, is highly expressed on AMs and other cells involved in *M.tb* uptake (Hirsch *et al.*, 1994;Zaffran *et al.*, 1998;Schlesinger *et al.*, 2008), and thus may be playing a major role in the uptake of *M.tb* by the naïve host in early stages of infection.

3.2 Signaling receptors

Apart from the phagocytosis process, *M.tb* also is shown to signal through specific signaling receptors located on the host cell surface and/or cytosol. The main signaling receptors for *M.tb* are Toll-like receptors (TLRs), and nucleotide-binding oligomerization domain-like (NODs) receptors.

3.2.1 Toll-like receptors

TLRs are a set of PPRs expressed on many cell types but their function on phagocytes is particularly important [reviewed in (Kawai & Akira, 2010)]. On macrophages TLRs are either expressed on the surface (like TLR2 and 4) or inside cell compartments (like TLR8 and 9) (Kawai & Akira, 2010). TLRs detect a wide range of PAMPs on *M.tb*, which activates the innate immune response and enhance adaptive immunity by mediating the secretion of various pro-inflammatory cytokines along with other anti-bacterial modulators (Table 1). TLRs shown to be key players in triggering immunity against *M.tb* infection are TLR2 (alone or as a heterodimer with TLR1 or TLR6), TLR9, and probably TLR4 (Harding & Boom, 2010). TLR2 alone or dimerized with TLR1 or TLR6, is shown to trigger a strong pro-inflammatory response by recognizing *M.tb* 19 KDa lipoglycoprotein, lower- and higher-order PIMs, LM and TDM [reviewed in (Jo *et al.*, 2007)]. This pro-inflammatory response via TLR2 is shown to be mediated through its adaptor protein myeloid differentiation primary-response protein 88 (MyD88)(Quesniaux *et al.*, 2004), triggering a nuclear factor kappa-light chain-

enhancer of activated B cell (NFκB) signaling cascade through the recruitment of MyD88 and TIRAP (toll-interleukin 1 receptor [TIR] domain containing adaptor protein) (Kawai & Akira, 2010). Of no surprise the intensity of the immune response observed via TLR2 depends on the *M.tb* ligand and the nature of the host cell studied (Thoma-Uszynski *et al.*, 2001;Underhill *et al.*, 1999). Surprisingly, prolonged TLR2 signaling can also benefit *M.tb*. Studies implicating a prolonged stimulation of TLR2 showed an inhibition of antigen presentation due to the down-regulation in the expression of the major histocompatibility complex (MHC) class II in macrophages infected with *M.tb* (Harding & Boom, 2010). Other studies also show that *M.tb* is capable of inhibit MHC-I antigen cross processing and presentation to $CD8^+$ T cells via TLR2 signaling (Harding & Boom, 2010). TLR2-dependent inhibition of TLR9-dependent IFN-α/β expression, thus leading to a decrease of IFN-α/β-dependent MHC-I cross processing is also shown in DCs (Simmons *et al.*, 2010). In this regard, it is unknown if these described mechanisms of MHC-I and -II inhibition via TLR2 will be beneficial for *M.tb* by passing the host immune response, or will be beneficial for the host by limiting the harmful effects of excessive inflammation. Thus, it is not unreasonable to search for a regulatory mechanism(s) among TLRs signaling networks necessary to control inflammation during *M.tb* chronic infection (Drennan *et al.*, 2004;Simmons *et al.*, 2010). TLR9 recognizes unmethylated CpG (cytosine phosphate guanosine motif) found in *M.tb* DNA (Kawai & Akira, 2010). Activation of TLR9 induces IFNα/β and MHC-I antigen cross processing (Simmons *et al.*, 2010). The role of TLR4 in *M.tb* infection is unclear as only a few *M.tb* ligands for TLR4 have been described. Recently, recombinant *M.tb* heat shock protein (hsp) 65 was shown to induce the generation of TLR4-dependent NFκB via MyD88-, TIRAP-, TRIF- (TIR-domain-containing adapter-inducing interferon-β) and TRAM- (TRIF-related adaptor molecule)-dependent signaling pathways (Bulut *et al.*, 2005).

3.2.2 Cytosolic receptor: NOD2

Cytosolic regulators known as NODs receptors (Franchi *et al.*, 2008) are known to participate in the induction of pro-inflammation during *M.tb* infection. Specifically Nod2, which is found in epithelial cells and antigen presenting cells (Gutierrez *et al.*, 2002;Inohara and Nunez, 2003;Ogura *et al.*, 2001), is shown to regulate the production of inflammatory mediators in response to *M.tb* PG components such as muramyl dipeptide (MDP) (Brooks *et al.*, 2011;Franchi *et al.*, 2008;Sirard *et al.*, 2007) (Table 1). Nod2 polymorphism studies in humans are linked to susceptibility to mycobacterial infection (Austin *et al.*, 2008;F.R. Zhang *et al.*, 2009). Studies done *in vitro* using different models and *in vivo* using the mouse model dispute the significance of Nod2 in controlling *M.tb* growth during infection (Divangahi *et al.*, 2008;Gandotra *et al.*, 2007). However, recent studies using human macrophages align with the human polymorphism studies showing that Nod2 plays a role in controlling pro-inflammation and *M.tb* intracellular growth (Brooks *et al.*, 2011). How Nod2 intersects with signaling/trafficking networks starts to be uncovered (Pandey *et al.*, 2009). Although, Nod2 can synergize with TLR-signaling pathways enhancing pro-inflammation (Ferwerda *et al.*, 2005), its capacity to interfere/associate with phagocytic receptor trafficking networks is not well established. As cytosolic Nod2 appears to be associated with intracellular vesicles (Brooks *et al.*, 2011), its role in triggering pro-inflammation may depend on vesicular fusion events controlled during *M.tb* phagocytosis and phagosomal maturation (Sasindran & Torrelles, 2011).

3.3 Other phagocyte receptors

Collectins such as surfactant protein –A and –D, and mannose binding protein, and their specific receptors have been shown to be important in *M.tb* recognition by the host; and their contribution in *M.tb* pathogenesis is discussed elsewhere (Sasindran & Torrelles, 2011;Torrelles & Schlesinger, 2010;Torrelles *et al.*, 2008a). Other receptors involved in the recognition of *M.tb* and inflammation are CD14, scavenger receptor-A, Fcγ-receptor, Mincle, and Dectin-1 (Sasindran & Torrelles, 2011). CD14 (Khanna *et al.*, 1996) and the scavenger receptor SR-A (Zimmerli *et al.*, 1996), are shown to participate in the uptake of non-opsonized bacilli by tissue-specific macrophages; where their role in inflammation varies depending on the species-specific cell type used. Dectin-1 (dendritic cell-associated C-type lectin 1), a β-glucan receptor, in combination with TLR2 has also been shown involved in the immune response against *M.tb* (Yadav & Schorey, 2006). Recently, Mincle (macrophage-inducible C-type lectin) (Yamasaki *et al.*, 2008) on the macrophage surface, has been shown to specifically recognize *M.tb* TDM, inducing a pro-inflammatory response by working together with the Fcγ receptor transmembrane segment (Ishikawa *et al.*, 2009;Schoenen *et al.*, 2010)(Table 1). Conversely, Fcγ receptors do not play a role in the phagocytosis of *M.tb* in the absence of specific antibody (Schlesinger *et al.*, 1990).

In this type of studies we should carefully consider differences between model systems used. There are multiple examples of contradictions when comparing studies performed *in vivo vs. in vitro* and/or when comparing cells from an animal model *vs.* human primary cells. In this context, it is plausible that depending of the model used (*i.e.* primary alveolar macrophage *vs.* THP-1 cell; or human *vs.* another mammalian host cell), a host cell may differentially express the targeted receptor on its surface, or this targeted receptor may be involved in triggering additional or different signaling and/or trafficking network(s). The same concept can be attributed when studying different strains of *M.tb*. A clear example is the variable degree of mannosylation observed on the *M.tb* surface among different strains (*i.e.* less ManLAM and PIMs and more triglycerides, PGL-TB and PDIM on the cell wall of *M.tb* clinical isolates *vs. M.tb* laboratory strains $H_{37}R_v$ and Erdman) (Torrelles *et al.*, 2008b), and how this may impact the infection outcome (Torrelles & Schlesinger, 2010). In light of these findings, we need to be careful in considering which cell wall components are heavily present on the surface of the *M.tb* strain(s) studied and their implications in the host cell phenotype observed.

4. *M. tuberculosis*-Host relationship with the alveolar environment(s) found during infection

It is thought that initial interaction between *M.tb* and the host dictates the pathway and outcome of infection. When *M.tb* infection occurs by airborne transmission, bacilli are deposited in the alveolar spaces of the lungs. The traditional view is that *M.tb* is somewhat "static" during initial infection, does not induce an immune response, and it is taken up by non-activated AMs that serve as an important reservoir for infection. However, we envision that upon deposition in the alveolar space *M.tb* may enter a dynamic phase where it encounters pulmonary surfactant that contains homeostatic and antimicrobial enzymes (Hawgood & Poulain, 2001;van Golde, 1985) (called hydrolases) which alter the *M.tb* cell wall. Due to the dynamics of *M.tb* infection (Chroneos *et al.*, 2009), when *M.tb* is initially

deposited in the terminal bronchioles and alveoli, as well as, following release from lysed macrophages and in cavities in reactivated TB, *M.tb* bacilli are in intimate contact with lung surfactant hydrolases. We recently demonstrated that hydrolases present in the human lung surfactant (Mason, 2006;Williams, 2003), at their relevant concentrations *in vivo*, dramatically alter the cell wall of *M.tb* during infection (Arcos *et al.*, 2011). As a result of these cell wall modifications, a significant decrease in association of *M.tb* with human macrophages was observed followed by an increase in phagosome-lysosome fusion (35%), which translated to a significant decrease in *M.tb* intracellular survival within these cells and an increase in inflammatory cytokine production leading to better control of infection (Arcos *et al.*, 2011). Importantly, we demonstrated that minimal contact time (15 min) with human lung surfactant hydrolases significantly reduced the cell surface exposure of two major *M.tb* virulence factors, ManLAM and TDM (Arcos *et al.*, 2011). As mentioned above, both, ManLAM and TDM have been shown to play important roles in the intracellular survival of *M.tb* in the host by blocking the phagosome maturation process (Axelrod *et al.*, 2008;Kang *et al.*, 2005). Thus, below we will address which are the sources of these hydrolases in the alveolar space.

4.1 The *M. tuberculosis* infection pathway and the alveolar environment: The potential role of human lung surfactant hydrolases

The first interaction between *M.tb* and the human host takes place in the lung. The respiratory epithelium is actively involved in inflammation and host defense in multiple ways: providing a physical barrier, constituting the structural basis of mucociliary clearance aimed at the physical removal of inhaled bacteria; recognizing PAMPs/MAMPs by PPRs expressed on epithelial and myeloid cells, and secreting a variety of pro- and anti-inflammatory mediators, including a large variety of hydrolases (Nicod, 2005). When *M.tb* bacilli reach the alveolar space, AMs, monocytes, and neutrophils represent the array of innate immune myeloid cells that will participate in host defense.

4.1.1 The alveolar macrophage

The AM is the first professional phagocyte to encounter inhaled *M.tb* bacilli. AMs are placed in a unique location within the alveolar surfactant film, the latter of which is produced by type II alveolar epithelial cells and is composed of phospholipids and proteins (Jonsson *et al.*, 1986). AMs are at the interface between air and lung tissue, and represent the first line of defense against inhaled *M.tb* found in the air (Lohmann-Matthes *et al.*, 1994). AMs possess a high phagocytic and clearance potential. In a normal healthy individual, they represent more than 90% of the cells in bronchoalveolar lavage fluid (Reynolds, 1987).

Many studies have demonstrated that resident AMs can phagocytose large numbers of microbes through both opsonic and non-opsonic receptors (Fels & Cohn, 1986;Lohmann-Matthes *et al.*, 1994;Palecanda & Kobzik, 2001;Serrano-Gomez *et al.*, 2004;Stephenson and Shepherd, 1987;Tailleux *et al.*, 2005;Taylor *et al.*, 2002;F.X. Zhang *et al.*, 1999). Though AMs have high phagocytic activity, their microbicidal capacity is less well-defined. Efficient microbial phagocytosis followed by slow intracellular killing may be sufficient to control infection with many routinely encountered extracellular pathogens. Intracellular pathogens like *M.tb*, however, may take advantage of the reduced microbial activity of the AM by residing and multiplying within these cells (Ferguson & Schlesinger, 2000). The

participation of AMs in host defense, inflammatory processes and immune mechanisms has been amply documented (Schlesinger, 1997). In general, their primary function is the intracellular breakdown and disposal of particulate elements. In this regard, they contain a wide variety of hydrolases such as abundant lipase, acid phosphatase, cathepsin, lysozyme, esterase, acid ribonuclease, and β-glucuronidase activities [on a specific activity basis] (Cohn & Wiener, 1963;Sorber *et al.*, 1973). Interestingly, dead BCG-stimulated AMs exhibited up to a 4-fold increase in the activities for lipases, acid phosphatases and lysozyme compared to control (Cohn & Wiener, 1963) indicating an up-regulation of these hydrolases within the AMs in the presence of antigen. This observation was corroborated in AMs obtained from live BCG-vaccinated rabbits, where acid phosphatase, lipase and lysozyme activities increased up to 40-fold when compared to AMs from control rabbits (Sorber *et al.*, 1973). How these hydrolases are regulated during *M.tb* infection and their role in redecorating the cell envelope of *M.tb* are currently unknown.

4.1.2 Monocytes and neutrophils in the alveolar space

Mononuclear phagocytes enter the lung both constitutively to maintain AM and dendritic cell populations, and during lung inflammation (Srivastava *et al.*, 2005). The role of monocyte accumulation in the lung in acute and chronic pulmonary inflammation is largely unknown, although these cells are accessible by bronchoaleolar lavage (Maus *et al.*, 2001). In the mouse, alveolar deposition of a stimulus provoked a significant influx of monocytes into the interstitium of the alveolar compartment along with a characteristic recruitment of neutrophils (Gunn *et al.*, 1997;Li *et al.*, 1998;Ulich *et al.*, 1991). This was confirmed by studies showing that circulating leukocytes could be recruited across the endothelial and epithelial barriers into the alveolar space under both non-inflammatory and highly inflammatory conditions (Li *et al.*, 1998;Maus *et al.*, 2001). Given the capacity of monocytes to produce hydrolases, reactive oxygen species, or inflammatory cytokines (Van Furth, 1988), their accumulation has been implicated in several inflammatory diseases in the pulmonary system (Antoniades *et al.*, 1992). As is the case for AMs, how monocytes and their secreted products affect the cell wall of *M.tb* within the alveolar space remains unknown.

Neutrophils may play an important role in controlling *M.tb* infection (Pedrosa *et al.*, 2000;Seiler *et al.*, 2003). In the bronchoalveolar lavage fluid, they normally represent less than 2% of all cells, however, during inflammation a massive influx of neutrophils occurs (Mizgerd, 2002;P. Zhang *et al.*, 2000). Neutrophils eliminate microbes by a number of oxidative and non-oxidative mechanisms (P. Zhang *et al.*, 2000) including secreted hydrolases such as *N*-acetyl-β-glucosaminidase, β-glucuronidase, α-mannosidases, and lysozyme. Neutrophils can kill *M.tb* through both oxidative and non-oxidative processes (Brown *et al.*, 1987;Jones *et al.*, 1990). Although neutrophils may interact with *M.tb* cell wall components during alveolar deposition [such as the 19-KDa lipoglycoprotein, SL-1 and PGLs (Faldt *et al.*, 1999;Neufert *et al.*, 2001;L. Zhang *et al.*, 1991)], there is no information regarding how alveolar neutrophil-derived hydrolases affect the integrity of the cell wall of *M.tb* during infection.

4.1.3 Alveolar epithelial cells

Alveolar epithelium lines the alveoli air sacs of the lung and is comprised predominantly of two specialized cell types (type I and type II). Alveolar type I cells function in gas exchange.

These cells have an extremely thin cytoplasm extending away from the nuclear body and contain a large number of plasmalemmal invaginations termed caveolae (Gil *et al.*, 1981; Williams, 2003). Caveolae regulate removal of endogenous and exogenous particulates from the alveolar space by regulating activities of receptors, hydrolase secretion to the alveolar lumen and signaling molecules (Gumbleton, 2001;Marx, 2001;Razani & Lisanti, 2001). Importantly, these caveolae contain lipid phosphate phosphohydrolase, a critical enzyme in hydrolyzing a variety of phospholipids to produce diacylglycerol (Nanjundan & Possmayer, 2003). Type I cells (as well as AMs) also produce carboxypeptidase which increases during bacterial deposition in the alveolar space and functions in the processing of many peptides (Skidgel & Erdos, 1998). Other epithelial cell enzymes are related to the regulation of ion transport to the alveolar space (Johnson *et al.*, 2002).

Alveolar type II cells are considerably smaller than the type I cells and are richly endowed with organelles and microvilli on their apical membrane. These cells are located in the corners of the alveolus where their physiological functions include surfactant production, secretion and recycling (Fehrenbach, 2001). Surfactant is released to the alveolar space by exocytosis from intracellular storage organelles termed lamellar bodies which contain the majority of the components of the surfactant. During active secretion of contents from the lamellar bodies to the alveoli and during the surfactant recycling process, a variety of hydrolases have been related to these organelles (de Vries *et al.*, 1985; DiAugustine, 1974; Edelson *et al.*, 1988; Gilder *et al.*, 1981; Hook & Gilmore, 1982; S.L. Young *et al.*, 1993), many of which have lysosomal-type degradative functions. The presence of hydrolases within the lamellar bodies implies that if the contents of these structures are secreted, then hydrolases should also be secreted with surfactant. It has been shown that surfactant contains substantial quantities of hydrolases (Hook, 1978), where some hydrolases are highly active (*i.e.* α-mannosidase and β-*N*-acetylglucosaminidase) while others much less so (*i.e.* β-glucuronidase and arylsulfatase). Thus, the lamellar bodies provide a vehicle for the release of hydrolases into the alveoli, and their influence on host defense is being to be elucidated by our laboratory. In general, studies on epithelial cells have focused on phospholipases as second messengers in signaling, however, these same phospholipases are also degradative hydrolases with great potential to redecorate any microbial cell wall. For example, phospholipase A_2 can release the fatty acids from phospholipids (Dennis, 2000). The action of phophatidylinositol phospholipase C provides both diacylglycerol and inositol trisphosphate. Other hydrolases include secreted and extracellular membrane-associated phosphatidic acid phosphatases which act on 1,2-diacyl,*sn*-glycerol phosphate to produce diacylglycerol and inorganic phosphate (Brindley & Waggoner, 1998), however, the membrane-associated hydrolases also act on a variety of phospholipids to generate anionic and/or neutral lipids (Nanjundan & Possmayer, 2003). These hydrolases may also be active against the rich phospholipid content of the cell wall of *M.tb.* (*i.e.* PIMs, cardiolipid, phosphatidylethanolamine, etc.) (Arcos *et al.*, 2011). To what extent *M.tb* bacilli directly interact with epithelial cells and their secreted products remains unknown. However, the location of the alveolar epithelium, as well as, the relatively large alveolar epithelial surface area estimated at 100 to 140 m^2, makes it likely that *M.tb* will interact with components of the alveolar space prior to and following its residence in the AM. The low alveolar fluid volume relative to the alveolar epithelial surface area (7- 20 ml per 100 m^2) likely increases the local concentration of released hydrolases when compared to other tissue compartments.

This, in turn, increases the probability that secreted hydrolases will impact the *M.tb* bacillus in the alveoli, altering its cell envelope and metabolism.

4.1.4 Pulmonary surfactant

Pulmonary surfactant prevents alveoli from collapsing at low lung volumes by reducing the surface tension in the alveolar space. Dipalmitoylphosphatidylcholine comprises almost 50% of total surfactant and is its major surface-active component (van Golde, 1985). The exact function of the remaining lipid components, such as unsaturated phosphatidyl-cholines, phosphatidylglycerols, phosphatidylethanolamines, phosphatidyl-inositols, and cholesterol are still uncertain (King, 1982;van Golde, 1985). The surfactant protein fraction comprises a highly variable amount of serum proteins, a wide variety of specific hydrolases (Griese, 1999) and four apoproteins (the surfactant proteins termed SP-A, -B, -C, and –D) that contribute to its specific function (Weaver & Whitsett, 1991). As mentioned above, surfactant contains several products secreted by alveolar myeloid and epithelial cells, some of them already defined in host defense, such as the bacteriolytic lysozyme (Haller *et al.*, 1992;Singh *et al.*, 1988). The wide variety of hydrolases secreted into surfactant resemble but are distinct from lysosomal enzymes (Hook and Gilmore, 1982). The surfactant resident hydrolases alter the *M.tb* cell wall (Arcos *et al.*, 2011).

There remain many fundamental questions about how the alveolar environment influences *M.tb* pathogenesis. We recently showed that secreted hydrolases involved in surfactant homeostasis affect the *M.tb* cell wall (Arcos *et al.*, 2011) and/or trigger *M.tb* endogenous hydrolases to modify its own cell wall prior to contacting its natural niche, the AM, or after its lysis from the cell. Exposure of '*de novo*' motifs on the surface of *M.tb* after alveolar enzymatic processing will provide insight into the real nature of the *M.tb* cell envelope during infection. Since components of the *M.tb* cell envelope dictate the innate immune response against the bacillus via their interaction with surface receptors on myeloid cells, the identification of hydrolases that shape the surface of the *M.tb* cell envelope will enable more predictive *in vitro* models to be developed and novel drug targets to be identified.

5. Conclusions

The interface between *M.tb* and the host depends of many factors. *M.tb* strains differ in their cell wall components exposed on their surface. Even within the same strain, it is likely that some bacilli differ, and thus bacilli may interact different with the host. Thus, at a given infection we may find a mixture of events. On one hand, and depending on the cell wall components exposed on their surface, we may find bacilli that interact with a receptor inducing pro-inflammation and promoting *M.tb* killing, and on the other hand, we may find bacilli interacting with another receptor inducing anti-inflammation and *M.tb* intracellular survival. From the host perspective, genetic predisposition and living conditions dictate the predilection for *M.tb* infection. Even in the context of the same infected person and within the same host cell population, differences amongst cells in the expression of cell surface receptors, signaling, trafficking and innate and adaptive function exist. These differences in the host get enhanced even more when we compare host cells from different model systems. Our current studies on the impact of the alveolar space in the *M.tb* infection outcome also

indicate that environmental host factors, such as alveolar hydrolases, play important roles in the establishment of the infection. With this in mind, what are the necessary elements that result in a succesful *M.tb* infection? Is *M.tb* infection just chance or a perfect combination of bacterial and host elements? Upon the successful establishment of *M.tb* infection, the ultimate goal of the host is to reduce inflammation and tissue destruction and in this scenario *M.tb* has learned to evolve, adapt, and survive.

What do we know about the *M.tb* cell wall adaptation to the host? The most revealing attribute of the *M.tb* cell wall is its complexity. Studies have been focused on depicting the composition, structure, biosynthesis, and the spatial conformation of the *M.tb* cell wall and its components for decades. Currently, researchers are focusing to reveal how the cell wall of *M.tb* is during infection (*in vivo* and *in vitro*). The development of new technologies and/or the use of known technologies already successfully applied in other fields (such as cancer research) are moving fast into the field of TB. Thus, studies using scanning electron and atomic force microscopy revealed that the *M.tb* cell wall from MDR- and XDR-strains differs from susceptible strains (Velayati *et al.*, 2009a, 2009b, 2010). Experiments analyzing infected granulomas by using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS-NMR) showed that we are capable of analyzing the cell wall of *M.tb* when inside of the granuloma without further manipulation (Somashekar *et al.*, 2011). The introduction of novel reporters that can be used for selective labeling of the cell wall of *M.tb* during infection *in vitro* and *in vivo* is already allowing us to see how the cell wall of *M.tb* is remodeling during infection (Backus *et al.*, 2011). Efforts in improving purification techniques are also allowing us to be able to purify *M.tb* directly from infected tissues. The use of novel *state-of-the art* mass spectrometry techniques such as LC/MS/MS (Sartain *et al.*, 2006, 2011), ESI/MS (Barry *et al.*, 2011) and MALDI-Tissue Imaging (Prideaux *et al.*, 2011) in drug discovery will allow us to obtain new information about the cell wall composition from a single *M.tb* bacillus isolated from tissue and also to see how *M.tb* cell wall components, and other biomarkers and drugs are distributed within the infected tissue. These are few of many other novel biotechniques that are starting to be applied in the field of TB. But this is only the beginning, more efforts improving the protocols and the development of new technology will allow us to move quickly to solve the "mystery" involving how *M.tb* adapted to the host and became such a successful infection currently affecting one third of the world population and taking away ~2 million lives every year.

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7. References

Andersen, A. B. & Brennan P.J. (1994) Proteins and antigens of *M. tuberculosis*. In *Tuberculosis: Pathogenesis, Protection, and Control*. Bloom BR (ed.), pp. 307-332, American Society for Microbiology, ISBN 1555810721, Washington, DC.

Anderson, R. J. (1938) The chemistry of the lipoids of the tubercle bacillus and certain other microorganisms. *Prog Chem Organ Natur Products*, Vol. 3, pp 145-202. ISSN 0071-7886

Andrews, J. R., Shah, N. S., Weissman, D., Moll, A. P., Friedland, G., & Gandhi, N. R. (2010) Predictors of multidrug- and extensively drug-resistant tuberculosis in a high HIV prevalence community. *PLoS One*, Vol. 5, No. 12, (December 2010), pp e15735. ISSN 1932-6203

Antoniades, H. N., Neville-Golden, J., Galanopoulos, T., Kradin, R. L., Valente, A. J., & Graves, D. T. (1992) Expression of monocyte chemoattractant protein 1 mRNA in human idiopathic pulmonary fibrosis. *Proc Natl Acad Sci USA*, Vol. 89, No. 12, (June 1992), pp 5371-5375. ISSN 0027-8424

Appelmelk, B. J., den, D. J., Driessen, N. N., Ummels, R., Pak, M., Nigou, J., Larrouy-Maumus, G., Gurcha, S. S., Movahedzadeh, F., Geurtsen, J., Brown, E. J., Eysink Smeets, M. M., Besra, G. S., Willemsen, P. T., Lowary, T. L., van, K. Y., Maaskant, J. J., Stoker, N. G., van der, L. P., Puzo, G., Vandenbroucke-Grauls, C. M., Wieland, C. W., Van Der, P. T., Geijtenbeek, T. B., van der Sar, A. M., & Bitter, W. (2008) The mannose cap of mycobacterial lipoarabinomannan does not dominate the M.-host interaction. *Cell Microbiol*, Vol. 10, No. 4, (April 2008), pp 930-944. ISSN 1462-5814

Arcos, J., Sasindran, S. J., Fujiwara, N., Turner, J., Schlesinger, L. S., & Torrelles, J. B. (2011) Human lung hydrolases delineate *M. tuberculosis*-macrophage interactions and the capacity to control infection. *J Immunol*, Vol. 187, No. 2, (July 2011), pp. 372-381. ISSN 0022-1767

Artman, M., Bekierkunst, A., & Goldenberg, I. (1964) Tissue metabolism in infection: Biochemical changes in mice treated with cord factor. *Arch Biochem Biophys*, Vol. 105, (April 1964), pp 80-85. ISSN 0003-9861

Asselineau, J. & Lederer, E. (1950) Structure of the mycolic acids of Mycobacteria. *Nature,* Vol. 166, NO. 4427, (November 1950), pp 782-783. ISSN 0028-0836

Astarie-Dequeker, C., N'Diaye, E. N., Le Cabec, V., Rittig, M. G., Prandi, J., & Maridonneau-Parini, I. (1999) The mannose receptor mediates uptake of pathogenic and nonpathogenic mycobacteria and bypasses bactericidal responses in human macrophages. *Infect Immun,* Vol. 67, No. 2, (February 1999), pp 469-477. ISSN 0019-9567

Astarie-Dequeker, C., Le, G. L., Malaga, W., Seaphanh, F. K., Chalut, C., Lopez, A., & Guilhot, C. (2009) Phthiocerol dimycocerosates of *M. tuberculosis* participate in macrophage invasion by inducing changes in the organization of plasma membrane lipids. *PLoS Pathog*, Vol. 5, No. 2, (February 2009), pp e1000289. ISSN 1553-7366

Austin, C. M., Ma, X., and Graviss, E. A. (2008) Common nonsynonymous polymorphisms in the NOD2 gene are associated with resistance or susceptibility to tuberculosis disease in African Americans. *J Infect Dis*, Vol. 197, No. 12, (June 2008), pp 1713-1716. ISSN 0022-1899

Axelrod, S., Oschkinat, H., Enders, J., Schlegel, B., Brinkmann, V., Kaufmann, S. H., Haas, A., & Schaible, U. E. (2008) Delay of phagosome maturation by a mycobacterial lipid is reversed by nitric oxide. *Cell Microbiol*, Vol. 10, No. 7, (July 2008), pp. 1530-1545. ISSN 1462-5814

Backus, K. M., Boshoff, H. I., Barry, C. S., Boutureira, O., Patel, M. K., D'Hooge, F., Lee, S. S., Via, L. E., Tahlan, K., Barry, C. E., III, & Davis, B. G. (2011) Uptake of unnatural trehalose analogs as a reporter for *M. tuberculosis*. *Nat Chem Biol,* Vol. 7, No. 4, (April 2011), pp 228-235. ISSN: 1552-4450

Barnes, P. F., Chatterjee, D., Abrams, J. S., Lu, S., Wang, E., Yamamura, M., Brennan, P. J., & Modlin, R. L. (1992) Cytokine production induced by *M. tuberculosis* lipoarabinomannan. Relationship to chemical structure. *J Immunol,* Vol. 149, No. 2, (July 1992), pp 541-547. ISSN 0022-1767

Barry, C. E., III & Mdluli, K. (1996) Drug sensitivity and environmental adaptation of mycobacterial cell wall components. *Trends Microbiol,* Vol. 4, No. 7, (July 1996), pp 275-281. ISSN: 0966-842X

Barry, C. E., III, Lee, R. E., Mdluli, K., Sampson, A. E., Schroeder, B. G., Slayden, R. A., & Yuan, Y. (1998) Mycolic acids: structure, biosynthesis and physiological functions. *Prog Lipid Res,* Vol. 37, No. 2-3, (July 1998), pp 143-179. ISSN: 0163-7827

Barry, C. S., Backus, K. M., Barry, C. E., & Davis, B. G. (2011) Precise ESI-MS Assay of *M. tuberculosis* cell wall Ag85 enzymes permits substrate profiling and design of a mechanism-based Inhibitor. *J Am Chem Soc*. [Epub ahead of print] ISSN 0002-7863

Basu, S., Friedland, G. H., Medlock, J., Andrews, J. R., Shah, N. S., Gandhi, N. R., Moll, A., Moodley, P., Sturm, A. W., & Galvani, A. P. (2009) Averting epidemics of extensively drug-resistant tuberculosis. *Proc Natl Acad Sci USA*, Vol. 106, No. 18, (May 2009), pp 7672-7677 ISSN 0027-8424.

Baulard, A. R., Besra, G. S., & Brennan P.J. (1998) The cell wall core of mycobacteria: Structure, biogenesis and genetics. In *Mycobacteria: molecular biology and virulence*. Ratledge, C. and Dale, J. (ed.), pp. 240-259, ISBN 0632053046, Blackwell Science, London.

Beckman, E. M., Porcelli, S. A., Morita, C. T., Behar, S. M., Furlong, S. T., & Brenner, M. B. (1994) Recognition of a lipid antigen by CD1-restricted alpha beta+ T cells. *Nature,* Vol. 372, No. 6507, (December 1994), pp 691-694. ISSN 0028-0836

Bekierkunst, A. (1968) Acute granulomatous response produced in mice by trehalose-6,6-dimycolate. *J Bacteriol,* Vol. 96, NO. 4, (October 1968), pp. 958-961. ISSN 0021-9193

Bekierkunst, A., Levij, I. S., & Yarkoni, E. (1971a) Suppression of urethan-induced lung adenomas in mice treated with trehalose-6,6-dimycolate (cord factor) and living bacillus Calmette Guerin. *Science,* Vol. 174, No. 15, (December 1971), pp 1240-1242. ISSN 0036-8075

Bekierkunst, A., Levij, I. S., Yarkoni, E., Vilkas, E., & Lederer, E. (1971b) Cellular reaction in the footpad and draining lymph nodes of mice induced by mycobacterial fractions and BCG bacilli. *Infect Immun,* Vol. 4, No. 3, (September 1971), pp 245-255. ISSN 0019-9567

Belisle, J. T., Vissa, V. D., Sievert, T., Takayama, K., Brennan, P. J., & Besra, G. (1997) Role of the major antigen of *M. tuberculosis* in cell wall biogenesis. *Science,* Vol. 276, No. 5317, (May 1997), pp 1420-1422. ISSN 0036-8075

Besra, G. & Chatterjee, D. (1994) Lipids and Carbohydrates of *M. tuberculosis*. In *Tuberculosis: Pathogenesis, Protection and Control*. Bloom, B. R. (ed.), pp. 285-306, ASM Press, ISBN 1555810721, Washington, DC.

Besra, G. S., Khoo, K.-H., McNeil, M. R., Dell, A., Morris, H. R., & Brennan, P. J. (1995) A new interpretation of the structure of the mycolyl-arabinogalactan complex of *M. tuberculosis* as revealed through characterization of oligoglycosylalditol fragments by fast- atom bombardment mass spectrometry and ^{1}H nuclear magnetic resonance spectroscopy. *Biochemistry,* Vol. 34, No. 13, (April 1995), pp 4257-4266. ISSN 0006-2960

Besra, G. S., Morehouse, C. B., Rittner, C. M., Waechter, C. J., & Brennan, P. J. (1997) Biosynthesis of mycobacterial lipoarabinomannan. *J Biol Chem,* Vol. 272, No. 29, (July 1997), pp 18460-18466. ISSN 0021-9258

Bhamidi, S., Scherman, M.S., Rithner, C.D., Prenni, J.E., Chatterjee, D., Khoo. K.H., & McNeil, M.R. (2008). The identification and location of succinyl residues and the characterization of the interior arabinan region allow for a model of the complete primary structure of *Mycobacterium tuberculosis* mycolyl arabinogalactan. *J Biol Chem*, Vol. 283, No. 19, (May 9), pp 12992-13000. ISSN 0021-9258

Bhamidi, S., Scherman, M. S., Jones, V., Crick, D. C., Belisle, J. T., Brennan, P. J., & McNeil, M. R. (2011) Detailed structural and quantitative analysis reveals the spatial organization of the cell walls of *in vivo* grown *M. leprae* and in vitro Grown *M. tuberculosis*. *J Biol Chem,* Vol. 286, No. 26, (July 2011), pp 23168-23177. ISSN 0021-9258

Bloch, H. (1950) Studies on the virulence of tubercle bacilli: isolation and biological properties of a constituent of virulent organisms. *J Exp Med,* Vol. 91, pp 197-219. ISSN 0022-1007

Boneca, I. G. (2005) The role of peptidoglycan in pathogenesis. *Curr Opin Microbiol,* Vol. 8, NO. 1, (February 2005), pp 46-53. ISSN 1369-5274

Brandley, B. K. & Schnaar, R. L. (1986) Cell-surface carbohydrates in cell recognition and response. *J Leukoc Biol,* Vol. 40, No. 1, (July 1986), pp 97-111. ISSN 0741-5400

Brennan, P. J. (1988) *Mycobacterium* and other actinomycetes. In *Microbial Lipids*. Ratledge, C. and Wilkinson, V. L. (ed.), pp. 203-298, Acad. Press, ISBN 0125823053, London.

Brennan, P. & Draper, P. (1994) Ultrastructure of *M. tuberculosis*. In *Tuberculosis: Pathogenesis, Protection, and Control*. Bloom BR (ed.), pp. 271-284, ASM Press, ISBN 1555810721, Washington DC.

Brennan, P. J. & Nikaido, H. (1995) The envelope of mycobacteria. *Annu Rev Biochem,* Vol. 64, pp 29-63. ISSN 0066-4154

Brennan, P. J. & Besra, G. S. (1997) Structure, function and biogenesis of the mycobacterial cell wall. *Biochem Soc Trans,* Vol. 25, No. 1, (February 1997), pp 188-194. ISSN 0300-5127

Brennan, P. J. (2003) Structure, function, and biogenesis of the cell wall of *M. tuberculosis*. *Tuberculosis (Edinb),* Vol. 83, No. 1-3, pp 91-97. ISSN: 1472-9792

Brindley, D. N. & Waggoner, D. W. (1998) Mammalian lipid phosphate phosphohydrolases. *J Biol Chem,* Vol. 273, No. 38, (September 1998), pp 24281-24284. ISSN 0021-9258

Brodin, P., Poquet, Y., Levillain, F., Peguillet, I., Larrouy-Maumus, G., Gilleron, M., Ewann, F., Christophe, T., Fenistein, D., Jang, J., Jang, M. S., Park, S. J., Rauzier, J., Carralot, J. P., Shrimpton, R., Genovesio, A., Gonzalo-Asensio, J. A., Puzo, G., Martin, C., Brosch, R., Stewart, G. R., Gicquel, B., & Neyrolles, O. (2010) High content phenotypic cell-based visual screen identifies *M. tuberculosis* acyltrehalose-

containing glycolipids involved in phagosome remodeling. *PLoS Pathog,* Vol. 6, No. 9, (September 2010), pp ppi e1001100. ISSN 1553-7366

Brooks, M. N., Rajaram, M. V., Azad, A. K., Amer, A. O., Valdivia-Arenas, M. A., Park, J. H., Nunez, G., & Schlesinger, L. S. (2011) NOD2 controls the nature of the inflammatory response and subsequent fate of *M. tuberculosis* and *M. bovis* BCG in human macrophages. *Cell Microbio,* Vol. 13, No. 3, (March 2011), pp 402-418. ISSN 1462-5814

Brown, A. E., Holzer, T. J., & Andersen, B. R. (1987) Capacity of human neutrophils to kill *M. tuberculosis*. *J Infect Dis,* Vol. 156, No. 6, (December 1987), pp 985-989. ISSN 0022-1899

Brozna, J. P., Horan, M., Rademacher, J. M., Pabst, K. M., & Pabst, M. J. (1991) Monocyte responses to sulfatide from *M. tuberculosis*: Inhibition of priming for enhanced release of superoxide, associated with increased secretion of IL-1 and TNF-a, and altered protein phosphorylation. *Infect Immun,* Vol. 59, No. 8, (August 1991), pp 2542-2548. ISSN 0019-9567

Bulut, Y., Michelsen, K. S., Hayrapetian, L., Naiki, Y., Spallek, R., Singh, M., & Arditi, M. (2005) *M. tuberculosis* heat shock proteins use diverse Toll-like receptor pathways to activate pro-inflammatory signals. *J Biol Chem,* Vol. 280, No. 22, (June 2005), pp 20961-20967. ISSN 0021-9258

Centers for Disease Control and Prevention (2006). Emergence of *M. tuberculosis* with extensive resistance to second-line drugs - Worldwide, 2000-2004. *Centers for Disease Control and Prevention-Morbidity and Mortality Weekly Report,* Vol. 55, No. 11, (March 2006), pp 301-305.

Chan, E. D., Morris, K. R., Belisle, J. T., Hill, P., Remigio, L. K., Brennan, P. J., & Riches, D. W. H. (2001) Induction of inducible nitric oxide synthase-NO by lipoarabinomannan of *M. tuberculosis* is mediated by MEK1-ERK, MKK7-JNK, and NF-kB signaling pathways. *Infect Immun,* Vol. 69, No. 4, (April 2001), pp 2001-2010. ISSN 0019-9567

Chatterjee, D., Khoo, K.-H., McNeil, M. R., Dell, A., Morris, H. R., & Brennan, P. J. (1993) Structural definition of the non-reducing termini of mannose-capped LAM from *M. tuberculosis* through selective enzymatic degradation and fast atom bombardment-mass spectrometry. *Glycobiology,* Vol. 3, No. 5, (October 1993), pp 497-506. ISSN 0959-6658

Chatterjee, D. & Khoo, K.-H. (1998) Mycobacterial lipoarabinomannan: An extraordinary lipoheteroglycan with profound physiological effects. *Glycobiology,* Vol. 8, No. 2, (February 1998), pp 113-120. ISSN 0959-6658

Chieppa, M., Bianchi, G., Doni, A., Del Prete, A., Sironi, M., Laskarin, G., Monti, P., Piemonti, L., Biondi, A., Mantovani, A., Introna, M., & Allavena, P. (2003) Cross-linking of the mannose receptor on monocyte-derived dendritic cells activates an anti-inflammatory immunosuppressive program. *J Immunol,* Vol. 171, No. 9, (November 2003), pp 4552-4560. ISSN 0022-1767

Cho, S.-N., Yanagihara, D. L., Hunter, S. W., Gelber, R. H., & Brennan, P. J. (1983) Serological specificity of phenolic glycolipid 1 from *M. leprae* and use in serodiagnosis of leprosy. *Infect Immun*, Vol. 41, No. 3, (September 1983), pp 1077-1083. ISSN 0019-9567

Cho, S.-N., Shin, J.-S., Daffe, M., Chong, Y., Kim, S.-K., & Kim, J.-D. (1992) Production of monoclonal antibody to a phenolic glycolipid of *M. tuberculosis* and its use in detection of the antigen in clinical isolates. *J Clin Microbiol,* Vol. 30, pp 3065-3069. ISSN 0095-1137

Chroneos, Z. C., Midde, K., Sever-Chroneos, Z., & Jagannath, C. (2009) Pulmonary surfactant and tuberculosis. *Tuberculosis (Edinb)* , Vol. 89, No. Suppl 1, (December 2009), pp S10-S14. ISSN 1472-9792

Cohn, Z. A. & Wiener, E. (1963) The particulate hydrolases on macrophages. I. Comparative enzymology, isolation, and properties. *J Exp Med,* Vol. 118, (December 1963), pp 991-1008. ISSN 0022-1007

Coulombe, F., Divangahi, M., Veyrier, F., de, L. L., Gleason, J. L., Yang, Y., Kelliher, M. A., Pandey, A. K., Sassetti, C. M., Reed, M. B., & Behr, M. A. (2009) Increased NOD2-mediated recognition of *N*-glycolyl muramyl dipeptide. *J Exp Med,* Vol. 206, No. 8, (August 2009), pp 1709-1716. ISSN 0022-1007

Cox, J. S., Chen, B., McNeil, M., & Jacobs, W. R., Jr. (1999) Complex lipid determines tissue-specific replication of *M. tuberculosis* in mice. *Nature*, Vol. 402, No. 6757, (November 1999), pp 79-83. ISSN 0028-0836

Crick, D. C., Mahapatra, S., & Brennan, P. J. (2001) Biosynthesis of the arabinogalactan-peptidoglycan complex of *M. tuberculosis*. *Glycobiology,* Vol. 11, No. 9, (September 2001), pp 107R-118R. ISSN 0959-6658

Crick, D. C., Brennan, P. J., & McNeil, M. R. (2003) The cell wall of *M. tuberculosis*. In *Tuberculosis*. Rom, W. M. and Garay, S. M. (ed.) pp 115-134, Lippincott Williams and Wilkins, ISBN 0-7817-3678-1, Philadelphia.

Cywes, C., Hoppe, H. C., Daffe, M., and Ehlers, M. R. W. (1997). Nonopsonic binding of *M. tuberculosis* to complement receptor type 3 is mediated by capsular polysaccharides and is strain dependent. *Infect Immun,* Vol. 65, No. 10, (October 1997), pp 4258-4266. ISSN 0019-9567

Daffe, M., Lacave, C., Laneelle, M., & Laneelle, G. (1987) Structure of the major triglycosyl phenol-phthiocerol of *M. tuberculosis* (strain Canetti). *Eur J Biochem,* Vol. 167, No. 1, (August 1987), pp 155-160.

Daffe, M., Brennan, P. J., & McNeil, M. (1990) Predominant structural features of the cell wall arabinogalactan of *M. tuberculosis* as revealed through characterization of oligoglycosyl alditol fragments by gas chromatography/mass spectrometry and by ^{1}H and ^{13}C NMR analyses. *J Biol Chem,* Vol. 265, No. 12, (April 1990), pp 6734-6743. ISSN 0021-9258

Daffe, M., Cho, S. N., Chatterjee, D., & Brennan, P. J. (1991) Chemical synthesis and seroreactivity of a neoantigen containing the oligosaccharide hapten of the *M. tuberculosis*-specific phenolic glycolipid. *J Infect Dis,* Vol. 163, No. 1, (January 1991), pp 161-168. ISSN 0022-1899

Daffe, M., McNeil, M., & Brennan, P. J. (1993) Major structural features of the cell wall arabinogalactans of *Mycobacterium, Rhodococcus*, and *Nocardia* spp. *Carbohydr Res* , Vol. 249, No. 2, (November 1993), pp 383-398. ISSN 0008-6215

Dao, D. N., Kremer, L., Guerardel, Y., Molano, A., Jacobs, W. R., Jr., Porcelli, S. A., & Briken, V. (2004) *M. tuberculosis* lipomannan induces apoptosis and interleukin-12

production in macrophages. *Infect Immun,* Vol. 72, No. 4, (April 2004), pp 2067-2074. ISSN 0019-9567

de la Salle H., Mariotti, S., Angenieux, C., Gilleron, M., Garcia-Alles, L. F., Malm, D., Berg, T., Paoletti, S., Maitre, B., Mourey, L., Salamero, J., Cazenave, J. P., Hanau, D., Mori, L., Puzo, G., & De Libero, G. (2005) Assistance of microbial glycolipid antigen processing by CD1e. *Science,* Vol. 310, No. 5752, (November 2005), pp 1321-1324. ISSN 0036-8075

de Vries, A. C., Schram, A. W., Tager, J. M., Batenburg, J. J., & van Golde, L. M. (1985) A specific acid alpha-glucosidase in lamellar bodies of the human lung. *Biochim Biophys Acta,* Vol. 837, No. 3, (December 1985), pp 230-238. ISSN 0005-2736

Delmas, C., Gilleron, M., Brando, T., Vercellone, A., Gheorghui, M., Riviere, M., &Puzo, G. (1997) Comparative structural study of the mannosylated-lipoarabinomannans from *M. bovis* BCG vaccine strains: Characterization and localization of succinates. *Glycobiology,* Vol. 7, No. 6, (September 1997), pp 811-817. ISSN 0959-6658

Dennis, E. A. (2000) Phospholipase A2 in eicosanoid generation. *Am J Respir Crit Care Med,* Vol. 161, No. 2Pt2, (February 2000), pp S32-S35. ISSN 1073-449X

DiAugustine, R. P. (1974) Lung concentric laminar organelle. Hydrolase activity and compositional analysis. *J Biol Chem,* Vol. 249, No. 2, (January 1974), pp 584-593. ISSN 0021-9258

Divangahi, M., Mostowy, S., Coulombe, F., Kozak, R., Guillot, L., Veyrier, F., Kobayashi, K. S., Flavell, R. A., Gros, P., & Behr, M. A. (2008) NOD2-deficient mice have impaired resistance to *M. tuberculosis* infection through defective innate and adaptive immunity. *J Immunol,* Vol. 181, No. 10, (November 2008), pp 7157-7165. ISSN 0022-1767

Dmitriev, B. A., Ehlers, S., Rietschel, E. T., & Brennan, P. J. (2000) Molecular mechanics of the mycobacterial cell wall: From horizontal layers to vertical scaffolds. *Int J Med Microbiol,* Vol. 290, No. 3, (July 2000), pp 251-258. ISSN: 1438-4221

Domenech, P., Barry, C. E., III, & Cole, S. T. (2001) *M. tuberculosis* in the post-genomic age. *Curr Opin Microbiol,* Vol. 4, No. 1, (February 2001), pp 28-34. ISSN 1369-5274

Draper, P. (1971) The walls of *M. lepraemurium*: Chemistry and ultrastructure. *J Gen Microbiol* , Vol. 69, No. 3, (December 1971), pp 313-324. ISSN 0022-1287

Drennan, M. B., Nicolle, D., Quesniaux, V. J., Jacobs, M., Allie, N., Mpagi, J., Fremond, C., Wagner, H., Kirschning, C., & Ryffel, B. (2004) Toll-like receptor 2-deficient mice succumb to *M. tuberculosis* infection. *Am J Pathol,* Vol. 164, No. 1, (January 2004), pp 49-57. ISSN 0002-9440

Driessen, N. N., Ummels, R., Maaskant, J. J., Gurcha, S. S., Besra, G. S., Ainge, G. D., Larsen, D. S., Painter, G. F., Vandenbroucke-Grauls, C. M., Geurtsen, J., & Appelmelk, B. J. (2009) Role of phosphatidylinositol mannosides in the interaction between mycobacteria and DC-SIGN. *Infect Immun,* Vol. 77, No. 10, (October 2009), pp 4538-4547. ISSN 0019-9567

Edelson, J. D., Shannon, J. M., & Mason, R. J. (1988) Alkaline phosphatase: A marker of alveolar type II cell differentiation. *Am Rev Respir Dis,* Vol. 138, No. 5, (November 1988), pp 1268-1275. ISSN 0003-0805

Ehlers, S. (2009) DC-SIGN and mannosylated surface structures of *M. tuberculosis*: A deceptive liaison. *Eur J Cell Biol,* Vol. 89, No. 1, (January 2009), pp 95-101. ISSN 0171-9335

Faldt, J., Dahlgren, C., Karlsson, A., Ahmed, A. M., Minnikin, D. E., & Ridell, M. (1999) Activation of human neutrophils by mycobacterial phenolic glycolipids. *Clin Exp Immunol,* Vol. 118, No. 2, (November 1999), pp 253-260. ISSN 0009-9104

Fehrenbach, H. (2001) Alveolar epithelial type II cell: Defender of the alveolus revisited. *Respir Res,* Vol. 2, pp 33-46. ISSN 1465-9921

Fels, A. & Cohn, Z. A. (1986) The alveolar macrophage. *J Appl Physiol,* Vol. 60, No. 1, (January 1986), pp 353-369. ISSN 8750-7587

Fenton, M. J., Riley, L. W., & Schlesinger, L. S. (2005) Receptor-mediated recognition of *M. tuberculosis* by host cells. In *Tuberculosis and the Tubercle Bacillus*. Cole, S. T., Eisenach, K. D., McMurray, D. N., and Jacobs, W. R., Jr. (ed.), pp. 405-426, ASM Press, ISBN 1555812953, New York.

Ferguson, J. S. & Schlesinger, L. S. (2000) Pulmonary surfactant in innate immunity and the pathogenesis of tuberculosis. *Tubercle Lung Dis*, Vol. 80, No. 4-5, pp 173-184. ISSN 0962-8479

Ferguson, J. S., Weis, J. J., Martin, J. L., & Schlesinger, L. S. (2004) Complement protein C3 binding to *M. tuberculosis* is initiated by the classical pathway in human bronchoalveolar lavage fluid. *Infect Immun,* Vol. 72, No. 5, (May 2004), pp 2564-2573. ISSN 0019-9567

Ferwerda, G., Girardin, S. E., Kullberg, B. J., Le Bourhis, L., de Jong, D. J., Langenberg, D. M., van Crevel, R., Adema, G. J., Ottenhoff, T. H., Van der Meer, J. W., and Netea, M. G. (2005) NOD2 and toll-like receptors are nonredundant recognition systems of *M. tuberculosis*. *PLoS Pathog,* Vol. 1, No. 3, (November 2005), pp 279-285. ISSN 1553-7366

Ferwerda, B., Kibiki, G. S., Netea, M. G., Dolmans, W. M., & van der Ven, AJ (2007) The toll-like receptor 4 Asp299Gly variant and tuberculosis susceptibility in HIV-infected patients in Tanzania. *AIDS,* Vol. 21, No. 10, (June 2007), pp 1375-1377. ISSN 0269-9370

Fischer, K., Chatterjee, D., Torrelles, J., Brennan, P. J., Kaufmann, S. H. E., & Schaible, U. E. (2001) Mycobacterial lysocardiolipin is exported from phagosomes upon cleavage of cardiolipin by a macrophage-derived lysosomal phospholipase A_2. *J Immunol,* Vol. 167, No. 4, (August 2001), pp 2187-2192. ISSN 0022-1767

Fischer, K., Collins, H., Taniguchi, M., Kaufmann, S. H., & Schaible, U. E. (2002) IL-4 and T cells are required for the generation of IgG1 isotype antibodies against cardiolipin. *J Immunol,* Vol. 168, No. 6, (March 2002), pp 2689-2694. ISSN 0022-1767

Franchi, L., Park, J. H., Shaw, M. H., Marina-Garcia, N., Chen, G., Kim, Y. G., & Nunez, G. (2008) Intracellular NOD-like receptors in innate immunity, infection and disease. *Cell Microbiol,* Vol. 10, No. 1, (Janaury 2008), pp 1-8. ISSN 1462-5814

Gandhi, N. R., Moll, A., Sturm, A. W., Pawinski, R., Govender, T., Lalloo, U., Zeller, K., Andrews, J., & Friedland, G. (2006) Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet,* Vol. 368, No. 9547, (November 2006), pp 1575-1580. ISSN 0140-6736

Gandhi, N. R., Shah, N. S., Andrews, J. R., Vella, V., Moll, A. P., Scott, M., Weissman, D., Marra, C., Lalloo, U. G., & Friedland, G. H. (2010) HIV coinfection in multidrug- and extensively drug-resistant tuberculosis results in high early mortality. *Am J Respir Crit Care Med,* Vol. 181, No. 1, (Janaury 2010), pp 80-86. ISSN 1073-449X

Gandotra, S., Jang, S., Murray, P. J., Salgame, P., & Ehrt, S. (2007) Nucleotide-binding oligomerization domain protein 2-deficient mice control infection with *M. tuberculosis*. *Infect Immun,* Vol. 75, No. 11, (November 2007), pp 5127-5134. ISSN 0019-9567

Gangadharam, P., Cohn, M. L., & Middlebrook, G. (1963) Infectivity, Pathogenicity and sulpholipid fraction of some Indian and British strains of tubercle bacilli. *Tubercle,* Vol. 44, (December 1963), pp 452-455. ISSN 0041-3879

Gautier, N., López Marín, L. M., Lanéelle, M.-A., & Daffé, M. (1992) Structure of mycoside F, a family of trehalose-containing glycolipids of *M. fortuitum*. *FEMS Microbiol Lett,* Vol. 98, No. 1-3, (November 1992), pp 81-88. ISSN 0378-1097

Geijtenbeek, T. B., Torensma, R., Van Vliet, S. J., van Duijnhoven, G. C., Adema, G. J., Van Kooyk, Y., & Figdor, C. G. (2000) Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell,* Vol. 100, No. 5, (March 2000), pp 575-585. ISSN 0092-8674

Geijtenbeek, T. B., Van Vliet, S. J., Koppel, E. A., Sanchez-Hernandez, M., Vandenbroucke-Grauls, C. M., Appelmelk, B., & Van Kooyk, Y. (2003) Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med,* Vol. 197, No. 1, (January 2003), pp 7-17. ISSN 0022-1007

Geurtsen, J., Chedammi, S., Mesters, J., Cot, M., Driessen, N. N., Sambou, T., Kakutani, R., Ummels, R., Maaskant, J., Takata, H., Baba, O., Terashima, T., Bovin, N., Vandenbroucke-Grauls, C. M., Nigou, J., Puzo, G., Lemassu, A., Daffe, M., & Appelmelk, B. J. (2009) Identification of mycobacterial alpha-glucan as a novel ligand for DC-SIGN: Involvement of mycobacterial capsular polysaccharides in host immune modulation. *J Immunol,* Vol. 183, No. 8, (October 2009), pp 5221-5231. ISSN 0022-1767

Gil, J., Silage, D. A., & McNiff, J. M. (1981) Distribution of vesicles in cells of air-blood barrier in the rabbit. *J Appl Physiol,* Vol. 50, No. 2, (February 1981), pp 334-340. ISSN 8750-7587

Gilder, H., Haschemeyer, R. H., Fairclough, G. F., Jr., & Mynarcik, D. C. (1981) Isolation and characterization of lamellar body material from rat lung homogenates by continuous linear sucrose gradients. *J Lipid Res,* Vol. 22, No. 8, (November 1981), pp 1277-1285. ISSN 0022-2275

Gilleron, M., Vercauteren, J., &Puzo, G. (1994) Lipo-oligosaccharidic antigen from *M. gastri*. Complete structure of a novel C4-branched 3,6-dideoxy-a- *xylo*-hexopyranose. *Biochemistry,* Vol. 33, No. 7, (February 1994), pp 1930-1937. ISSN 0006-2960

Gilleron, M., Nigou, J., Cahuzac, B., & Puzo, G. (1999) Structural study of the lipomannans from *M. bovis* BCG: Characterisation of multiacylated forms of the phosphatidyl-*myo*-inositol anchor. *J Mol Biol,* Vol. 285, No. 5, (February 1999), pp 2147-2160. ISSN 0022-2836

Gilleron, M., Bala, L., Brando, T., Vercellone, A., & Puzo, G. (2000) *M. tuberculosis* $H_{37}R_v$ parietal and cellular lipoarabinomannans. Characterization of the acyl- and glyco-forms. *J Biol Chem,* Vol. 275, No. 1, (Janaury 2000), pp 677-684. ISSN 0021-9258

Gilleron, M., Ronet, C., Mempel, M., Monsarrat, B., Gachelin, G., &Puzo, G. (2001) Acylation state of the phosphatidylinositol mannosides from *Mycobcterium bovus* bacillus calmette guerin and ability to induce granuloma and recruit natural killer T cells. *J Biol Chem,* Vol. 276, No. 37, (September 2001), pp 34896-34904. ISSN 0021-9258

Goren, M. B. (1970a) Sulfolipid I of *M. tuberculosis*, strain $H_{37}R_v$. I. Purification and properties. *Biochim Biophys Acta,* Vol. 210, No. 1, (June 1970), pp 116-126. ISSN 0005-2736

Goren, M. B. (1970b) Sulfolipid I of *M. tuberculosis*, strain $H_{37}R_v$. II. Structural studies. *Biochim Biophys Acta,* Vol. 210, No. 1, (June 1970), pp 127-138. ISSN 0005-2736

Goren, M. B., Brokl, O., & Schaefer, W. B. (1974) Lipids of putative relevance to virulence in *M. tuberculosis*: Correlation of virulence with elaboration of sulfatides and strongly acidic lipids. *Infect Immun,* Vol. 9, No. 1, (January 1974), pp 142-149. ISSN 0019-9567

Goren, M. B., Hart, P. D., Young, M. R., & Armstrong, J. A. (1976) Prevention of phagosome-lysosome fusion in cultured macrophages by sulfatides of *M. tuberculosis*. *Proc Natl Acad Sci USA,* Vol. 73, No. 7, (July 1976), pp 2510-2514. ISSN 0027-8424

Goren, M. B. & Brennan P.J. (1980) Mycobacterial lipids: Chemistry and biological activities. In *Tuberculosis*. Youmans GP (ed.), pp. 63-193, W. B. Saunders Co., Philadelphia.

Goren, M. B., Grange, J. M., Aber, V. R., Allen, B. W., & Mitchison, D. A. (1982) Role of lipid content and hydrogen peroxide susceptibility in determining the Guinea-pig virulence of *M. tuberculosis*. *Br J Exp Pathol,* Vol. 63, No. 6, (December 1982), pp 693-700. ISSN 0007-1021

Goren, M. B. & Mor, N. (1990) Influence of phagosomal contents on the apparent inhibition of phagosome-lysosome fusion mediated by polyanionic substances in mouse peritoneal macrophages. *Biochem Cell Biol,* Vol. 68, No. 1, (Janaury 1990), pp 24-32. ISSN 0829-8211

Griese, M. (1999) Pulmonary surfactant in health and human lung diseases: State of the art. *Eur Respir J,* Vol. 13, No. 6, (June 1999), pp 1455-1476. ISSN 0903-1936

Gumbleton, M. (2001) Caveolae as potential macromolecule trafficking compartments within alveolar epithelium. *Adv Drug Deliv Rev,* Vol. 49, No. 3, (July 2001), pp 281-300. ISSN 0169-409X

Gunn, M. D., Nelken, N. A., Liao, X., & Williams, L. T. (1997) Monocyte chemoattractant protein-1 is sufficient for the chemotaxis of monocytes and lymphocytes in transgenic mice but requires an additional stimulus for inflammatory activation. *J Immunol,* Vol. 158, No. 1, (January 1997), pp 376-383. ISSN 0022-1767

Gutierrez, O., Pipaon, C., Inohara, N., Fontalba, A., Ogura, Y., Prosper, F., Nunez, G., & Fernandez-Luna, J. L. (2002) Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor-kappa B activation. *J Biol Chem,* Vol. 277, No. 44, (November 2002), pp 41701-41705. ISSN 0021-9258

Haller, E. M., Shelley, S. A., Montgomery, M. R., & Balis, J. U. (1992) Immunocytochemical localization of lysozyme and surfactant protein A in rat type II cells and extracellular surfactant forms. *J Histochem Cytochem,* Vol. 40, No. 10, (October 1992), pp 1491-1500. ISSN 0022-1554

Harding, C. V. & Boom, W. H. (2010) Regulation of antigen presentation by *M. tuberculosis*: A role for Toll-like receptors. *Nat Rev Microbiol,* Vol. 8, No. 4, (April 2010), pp 296-307. ISSN 1740-1526

Hawgood, S. & Poulain, F. R. (2001) The pulmonary collectins and surfactant metabolism. *Annu Rev Physiol,* Vol. 63, pp 495-519. ISSN 0066-4278

Hirsch, C. S., Ellner, J. J., Russell, D. G., & Rich, E. A. (1994) Complement receptor-mediated uptake and tumor necrosis factor- alpha-mediated growth inhibition of *M. tuberculosis* by human alveolar macrophages. *J Immunol,* Vol. 152, No. 2, (January 1994), pp 743-753. ISSN 0022-1767

Hook, G. E. (1978) Extracellular hydrolases of the lung. *Biochemistry,* Vol.17, No. 3, (February 1978), pp 520-528. ISSN 0006-2960

Hook, G. E. & Gilmore, L. B. (1982) Hydrolases of pulmonary lysosomes and lamellar bodies. *J Biol Chem,* Vol. 257, No. 15, (August, 1982), pp 9211-9220. ISSN 0021-9258

Hoppe, H. C., De Wet, J. M., Cywes, C., Daffe, M., and Ehlers, M. R. (1997) Identification of phosphatidylinositol mannoside as a mycobacterial adhesin mediating both direct and opsonic binding to nonphagocytic mammalian cells. *Infect Immun,* Vol. 65, No. 9, (September 1997),pp 3896-3905. ISSN 0019-9567

Hu, C., Mayadas-Norton, T., Tanaka, K., Chan, J., & Salgame, P. (2000) *M. tuberculosis* infection in complement receptor 3-deficient mice. *J Immunol,* Vol. 165, No. 5, (September 2000), pp 2596-2602. ISSN 0022-1767

Hunter, S. W. & Brennan, P. J. (1981) A novel phenolic glycolipid from *M. leprae* possibly involved in immunogenicity and pathogenicity. *J Bacteriol,* Vol. 147, No. 3, (September 1981), pp 728-735. ISSN 0021-9193

Hunter, S. W., Fujiwara, T., & Brennan, P. J. (1983) Structure and antigenicity of the major specific glycolipid antigen of *M. leprae*. *J Biol Chem,* Vol. 257, No. 24, (December 1983), pp 15072-15078. ISSN 0021-9258

Hunter, S. W., Jardine, I., Yanagihara, D. L., & Brennan, P. J. (1985) Trehalose-containing lipooligosaccharides from mycobacteria: Structures of the oligosaccharide segments and recognition of a unique *N*-acylkanosamine-containing epitope. *Biochemistry,* Vol. 24, No. 11, (May 1985), pp 2798-2805. ISSN 0006-2960

Hunter, S. W., Gaylord, H., & Brennan, P. J. (1986) Structure and antigenicity of the phosphorylated lipopolysaccharide antigens from the leprosy and tubercle bacilli. *J Biol Chem,* Vol. 261, No. 26, (September 1986), pp 12345-12351. ISSN 0021-9258

Indrigo, J., Hunter, R. L., Jr., & Actor, J. K. (2003) Cord factor trehalose 6,6'-dimycolate (TDM) mediates trafficking events during mycobacterial infection of murine macrophages. *Microbiology,* Vol. 149, No. Pt8, (August 2003), pp 2049-2059. ISSN: 1350-0872

Inohara, N. & Nunez, G. (2003) NODs: Intracellular proteins involved in inflammation and apoptosis. *Nat Rev Immunol,* Vol. 3, No. 5, (May 2003), pp 371-382. ISSN 1474-1733

Ishikawa, E., Ishikawa, T., Morita, Y. S., Toyonaga, K., Yamada, H., Takeuchi, O., Kinoshita, T., Akira, S., Yoshikai, Y., & Yamasaki, S. (2009) Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. *J Exp Med,* Vol. 206, No. 13, (December 2009), pp 2879-2888. ISSN 0022-1007

Jarlier, V. & Nikaido, H. (1994) Mycobacterial cell wall: Structure and role in natural resistance to antibiotics. *FEMS Microbiol Lett,* Vol. 123, No. 1-2, (October 1994), pp 11-18. ISSN 0378-1097

Jo, E. K., Yang, C. S., Choi, C. H., & Harding, C. V. (2007) Intracellular signaling cascades regulating innate immune responses to mycobacteria: Branching out from Toll-like receptors. *Cell Microbiol,* Vol. 9, No. 5, (May 2007), pp 1087-1098. ISSN 1462-5814

Johnson, M. D., Widdicombe, J. H., Allen, L., Barbry, P., & Dobbs, L. G. (2002) Alveolar epithelial type I cells contain transport proteins and transport sodium, supporting an active role for type I cells in regulation of lung liquid homeostasis. *Proc Natl Acad Sci USA,* Vol. 99, No. 4, (February 2002), pp 1966-1971. ISSN 0027-8424

Jones, G. S., Amirault, H. J., & Andersen, B. R. (1990) Killing of *M. tuberculosis* by neutrophils: A nonoxidative process. *J Infect Dis,* Vol. 162, No. 3, (September 1990), pp 700-704. ISSN 0022-1899

Jonsson, S., Musher, D. M., Goree, A., & Lawrence, E. C. (1986) Human alveolar lining material and antibacterial defenses. *Am Rev Respir Dis,* Vol. 133, No. 1, (January 1986), pp 136-140. ISSN 0003-0805

Kang, P. B., Azad, A. K., Torrelles, J. B., Kaufman, T. M., Beharka, A., Tibesar, E., Desjardin, L. E., & Schlesinger, L. S. (2005) The human macrophage mannose receptor directs *M. tuberculosis* lipoarabinomannan-mediated phagosome biogenesis. *J Exp Med,* Vol. 202, No. 7, (October 2005), pp 987-999. ISSN 0022-1007

Kato, M. & Goren, M. B. (1974a) Enhancement of cord factor-toxicity by sulfolipid. *Jpn J Med Sci Biol,* Vol. 27, No. 2, (April 1974), pp 120-124. ISSN 0021-5112

Kato, M. & Goren, M. B. (1974b) Synergistic action of cord factor and mycobacterial sulfatides on mitochondria. *Infect Immun,* Vol. 10, No. 4, (October 1974), pp 733-741. ISSN 0019-9567

Kaur, D., Guerin, M. E., Skovierova, H., Brennan, P. J., & Jackson, M. (2009) Chapter 2: Biogenesis of the cell wall and other glycoconjugates of *M. tuberculosis*. *Adv Appl Microbiol,* Vol.69, pp 23-78. ISSN 0019-9567

Kawai, T. & Akira, S. (2010) The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. *Nat Immunol,* Vol. 11, No. 5, (May 2010), pp 373-384. ISSN 1529-2908

Khanna, K. V., Choi, C. S., Gekker, G., Peterson, P. K., & Molitor, T. W. (1996) Differential infection of porcine alveolar macrophage subpopulations by nonopsonized *M. bovis* involves CD14 receptors. *J Leukoc Biol,* Vol. 60, No. 2, (August 1996), pp 214-220. ISSN 0741-5400

Khoo, K.-H., Dell, A., Morris, H. R., Brennan, P. J., & Chatterjee, D. (1995) Structural definition of acylated phosphatidylinositol mannosides from *M. tuberculosis*: Definition of a common anchor for lipomannan and lipoarabinomannan. *Glycobiology,* Vol. 5, No. 21, (May 1995),pp 117-127. ISSN 0959-6658

King, R. J. (1982) Pulmonary surfactant. *J Appl Physiol,* Vol. 53, No. 1, (July 1982), pp 1-8. ISSN 8750-7587

Kirksey, M. A., Tischler, A. D., Simeone, R., Hisert, K. B., Uplekar, S., Guilhot, C., & McKinney, J. D. (2011) Spontaneous phthiocerol dimycocerosate-deficient variants of *M. tuberculosis* are susceptible to IFNg-mediated immunity. *Infect Immun,* Vol. 79, No. 7, (July 2011), pp 2829-2838. ISSN 0019-9567

Kotani, S., Kato, T., Matsuda, T., Kato, K., & Misaki, A. (1971) Chemical structure of the antigenic determinants of cell wall polysaccharide of *M. tuberculosis* strain $H_{37}R_v$. *Biken J,* Vol. 14, No. 4, (December 1971), pp 379-387. ISSN 0006-2324

Lederer, E., Adam, A., Ciorbaru, R., Petit, J. F., & Wietzerbin, J. (1975) Cell walls of mycobacteria and related organisms; chemistry and immunostimulant properties. *Mol Cell Biochem,* Vol. 7, No. 2, (May 1975), pp 87-104. ISSN 0300-8177

Lee, B.-Y., Hefta, S. A., & Brennan, P. J. (1992) Characterization of the major membrane protein of virulent *M. tuberculosis*. *Infect Immun,* Vol. 60, No. 5, (May 1992), pp 2066-2074. ISSN 0019-9567

Lee, Y. C. & Ballou, C. E. (1965) Complete structures of the glycophospholipids of mycobacteria. *Biochemistry,* Vol. 4, No. 7, (July 1965), pp 1395-1404. ISSN 0006-2960

Leigh, C. D. & Bertozzi, C. R. (2008) Synthetic studies toward *M. tuberculosis* sulfolipid-I. *J Org Chem,* Vol. 73, No. 3, (February 2008), pp 1008-1017. ISSN 0022-3263

Lemassu, A. & Daffe, M. (1994) Structural features of the exocellular polysaccharides of *M. tuberculosis*. *Biochem J,* Vol. 297, No. Pt 2, (January 1994) , pp 351-357. ISSN 0264-6021

Leopold, K. & Fischer, W. (1993) Molecular analysis of the lipoglycans of *M. tuberculosis*. *Anal Biochem* , Vol. 208, No. 1, (January 1993), pp 57-64. ISSN 0003-2697

Li, X. C., Miyasaka, M., & Issekutz, T. B. (1998) Blood monocyte migration to acute lung inflammation involves both CD11/CD18 and very late activation antigen-4-dependent and independent pathways. *J Immunol,* Vol. 161, No. 11, (December 1998), pp 6258-6264. ISSN 0022-1767

Liu, J., Rosenberg, E. Y., & Nikaido, H. (1995) Fluidity of the lipid domain of cell wall from *M. chelonae*. *Proc Natl Acad Sci USA,* Vol. 92, No. 24, (November 1995), pp 11254-11258. ISSN 0027-8424

Liu, J., Barry, C. E. I., Besra, G. S., & Nikaido, H. (1996) Mycolic acid structure determines the fluidity of the mycobacterial cell wall. *J Biol Chem,* Vol. 271, No. 27, (November 1996), pp 29545-29551. ISSN 0021-9258

Lohmann-Matthes, M. L., Steinmuller, C., & Franke-Ullmann, G. (1994) Pulmonary macrophages. *Eur Respir J,* Vol. 7, No. 9, (September 1994), pp 1678-1689. ISSN 0903-1936

Martinez-Pomares, L., Linehan, S. A., Taylor, P. R., & Gordon, S. (2001) Binding properties of the mannose receptor. *Immunobiology,* Vol. 204, No. 5, (December 2001), pp 527-535. ISSN 0171-2985

Marx, J. (2001) Caveolae: A once-elusive structure gets some respect. *Science,* Vol. 294, No. 5548, (November 2001), pp 1862-1865. ISSN 0036-8075

Mason, R. J. (2006) Biology of alveolar type II cells. *Respirology,* Vol. 11, Suppl, pp S12-S15. ISSN 0036-8075

Maus, U., Herold, S., Muth, H., Maus, R., Ermert, L., Ermert, M., Weissmann, N., Rosseau, S., Seeger, W., Grimminger, F., & Lohmeyer, J. (2001) Monocytes recruited into the alveolar air space of mice show a monocytic phenotype but upregulate CD14. *Am J Physiol Lung Cell Mol Physiol,* Vol. 280, No. 1, (January 2001), pp L58-L68. ISSN 1040-0605

McGreal, E. P., Miller, J. L., & Gordon, S. (2005) Ligand recognition by antigen-presenting cell C-type lectin receptors. *Curr Opin Immunol,* Vol. 17, No. 1, (February 2005), pp 18-24. ISSN 0952-7915

McNeil, M., Wallner, S. J., Hunter, S. W., & Brennan, P. J. (1987) Demonstration that the galactosyl and arabinosyl residues in the cell-wall arabinogalactan of *M. leprae* and *M. tuberculosis* are furanoid. *Carbohydr Res,* Vol. 166, No. 2, (September 1987), pp 299-308. ISSN 0008-6215

McNeil, M., Daffe, M., & Brennan, P. J. (1990) Evidence for the nature of the link between the arabinogalactan and peptidoglycan of mycobacterial cell walls. *J Biol Chem,* Vol. 265, No. 30, (October 1990), pp 18200-18206. ISSN 0021-9258

McNeil, M. R. & Brennan, P. J. (1991) Structure, function and biogenesis of the cell envelope of mycobacteria in relation to bacterial physiology, pathogenesis and drug resistance; some thoughts and possibilities arising from recent structural information. *Res Microbio,* Vol. 142, No. 4, (May 1991), pp 451-463. ISSN 0923-2508

Middlebrook, G., Coleman, C. M., & Schaefer, W. B. (1959) Sulfolipid from virulent tubercle bacilli. *Proc Natl Acad Sci USA,* Vol. 45, No.12, (December 1959), pp 1801-1804. ISSN 0027-8424

Mikusova, K., Mikus, M., Besra, G. S., Hancock, I., & Brennan, P. J. (1996) Biosynthesis of the linkage region of the mycobacterial cell wall. *J Biol Chem,* Vol. 271, No. 13, (March 1996), pp 7820-7828. ISSN 0021-9258

Minnikin, D. E. (1982) Lipids: Complex lipids, their chemistry, biosynthesis and roles. In *The biology of the mycobacteria, vol. 1.* Ratledge, C. and Standford, J. (ed.), pp. 95-184, Academic Press, Inc., ISBN 0125823010, San Diego.

Misaki, A., Seto, N., & Azuma, I. (1974) Structure and immunological properties of D-arabino-D-galactans isolated from cell walls of *Mycobacterium* species. *J Biochem,* Vol. 76, No 1, (July 1974), pp 15-27. ISSN 0021-924X

Mizgerd, J. P. (2002) Molecular mechanisms of neutrophil recruitment elicited by bacteria in the lungs. *Semin Immunol,* Vol. 14, No. 2, (April 2002), pp 123-132. ISSN: 1044-5323.

Montamat-Sicotte, D. J., Millington, K. A., Willcox, C. R., Hingley-Wilson, S., Hackforth, S., Innes, J., Kon, O. M., Lammas, D. A., Minnikin, D. E., Besra, G. S., Willcox, B. E., & Lalvani, A. (2011) A mycolic acid-specific CD1-restricted T cell population contributes to acute and memory immune responses in human tuberculosis infection. *J Clin Invest,* Vol. 121, No. 6, (June 2011), pp 2493-2503. ISSN 0021-9738

Moody, D. B., Reinhold, B. B., Reinhold, V. N., Besra, G. S., & Porcelli, S. A. (1999) Uptake and processing of glycosylated mycolates for presentation to CD1b-restricted T cells. *Immunol Lett,* Vol. 65, No. 1-2, (January 1999), pp 85-91. ISSN 0165-2478

Muñoz, M., Laneelle, M. A., Luquin, M., Torrelles, J., Julian, E., Ausina, V., & Daffe, M. (1997a) Occurrence of an antigenic triacyl trehalose in clinical isolates and reference strains of *M. tuberculosis*. *FEMS Microbiol Lett,* Vol. 157, No. 2, (December 1997), pp 251-259. ISSN 0378-1097

Muñoz, M., Luquin, M., García-Barcelo, M., Julián, E., Ausina, V., & Lanéelle, M. A. (1997b) Distribution of surface-exposed antigenic glycolipids in recent clinical isolates of *M. tuberculosis*. *Res Microbiol,* Vol. 148, No. 5, (June 1997), pp 405-412. ISSN 0923-2508

Nanjundan, M. & Possmayer, F. (2003) Pulmonary phosphatidic acid phosphatase and lipid phosphate phosphohydrolase. *Am J Physiol Lung Cell Mol Physiol,* Vol. 284, No. 1, (January 2003), pp L1-23. ISSN 1040-0605

Neufert, C., Pai, R. K., Noss, E. H., Berger, M., Booom, W. H., & Hardin, C. V. (2001) *M. tuberculosis* 19-kDa lipoprotein promotes neutrophil activation. *J Immunol,* Vol. 167, No. 3, (August 2001), pp 1542-1549. ISSN 0022-1767

Nicod LP (2005) Lung defenses: An overview. *European Respiratory Reviews,* Vol. 14: 45-50.

Nigou, J., Gilleron, M., Cahuzac, B., Bounery, J. D., Herold, M., Thurnher, M., & Puzo, G. (1997) The phosphatidyl-*myo*-inositol anchor of the lipoarabinomannans from *M. bovis* bacillus Calmette Guerin. Heterogeneity, structure, and role in the regulation of cytokine secretion. *J Biol Chem,* Vol. 272, No. 37, (September 1997), pp 23094-23103. ISSN 0021-9258

Nigou, J., Zelle-Rieser, C., Gilleron, M., Thurnher, M., & Puzo, G. (2001) Mannosylated liparabinomannans inhibit IL-12 production by human dendritic cells: Evidence for a negative signal delivered through the mannose receptor. *J Immunol,* Vol. 166, No. 12, (June 2001), pp 7477-7485. I ISSN 0022-1767

Nigou, J., Vasselon, T., Ray, A., Constant, P., Gilleron, M., Besra, G. S., Sutcliffe, I., Tiraby, G., & Puzo, G. (2008) Mannan chain length controls lipoglycans signaling via and binding to TLR2. *J Immunol,* Vol. 180, No. 10, (May 2008), pp 6696-6702. ISSN 0022-1767

Noll, H. (1956) The chemistry of cord factor, a toxic glycolipid of *M. tuberculosis*. *Bibl Tuberc,* Vol. 10, pp 149-183. ISSN 0300-1121

Numata, F., Nishimura, K., Ishida, H., Ukei, S., Tone, Y., Ishihara, C., Saiki, I., Sekikawa, I., & Azuma, I. (1985) Lethal and adjuvant activities of cord factor (trehalose-6,6'-dimycolate) and synthetic analogs in mice. *Chem Pharm Bull (Tokyo),* Vol. 33, No. 10, (October 1985), pp 4544-4555. ISSN 0009-2363

Ogura, Y., Inohara, N., Benito, A., Chen, F. F., Yamaoka, S., & Nunez, G. (2001) Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J Biol Chem,* Vol. 276, No. 7, (February 2002), pp 4812-4818. ISSN 0021-9258

Ortalo-Magné, A., Andersen, A. B., & Daffé, M. (1996) The outermost capsular arabinomannans and other mannoconjugates of virulent and avirulent tubercle bacilli. *Microbiology,* Vol. 142, No. Pt 4, (April 1996), pp 927-935. ISSN: 1350-0872

Pabst, M. J., Gross, J. M., Brozna, J. P., & Goren, M. B. (1988) Inhibition of macrophage priming by sulfatide from *M. tuberculosis*. *J Immunol,* Vol. 140, No. 2, (January 1988), pp 634-640. ISSN 0022-1767

Palecanda, A. & Kobzik, L. (2001) Receptors for unopsonized particles: The role of alveolar macrophage scavenger receptors. *Curr Mol Med* Vol. 1, NO. 5, (November 2001), pp 589-595. ISSN 1566-5240

Pandey, A. K., Yang, Y., Jiang, Z., Fortune, S. M., Coulombe, F., Behr, M. A., Fitzgerald, K. A., Sassetti, C. M., & Kelliher, M. A. (2009) NOD2, RIP2 and IRF5 play a critical role in the type I interferon response to *M. tuberculosis*. *PLoS Pathog,* Vol. 5, No. 7, (July 2009), pp e1000500. ISSN 1553-7366

Parant, M., Parant, F., Chedid, L., Drapier, J. C., Petit, J. F., Wietzerbin, J., & Lederer (1977) Enhancement of nonspecific immunity to bacterial infection by cord factor (6,6'-

trehalose dimycolate). *J Infect Dis,* Vol. 135, No. 5, (May 1977), pp 771-777. ISSN 0022-1899

Parant, M., Audibert, F., Parant, F., Chedid, L., Soler, E., Polonsky, J., & Lederer, E. (1978) Nonspecific immunostimulant activities of synthetic trehalose-6,6'-diesters (lower homologs of cord factor). *Infect Immun,* Vol. 20, No. 1, (April 1978), pp 12-19. ISSN 0019-9567

Park, C. G., Takahara, K., Umemoto, E., Yashima, Y., Matsubara, K., Matsuda, Y., Clausen, B. E., Inaba, K., & Steinman, R. M. (2001) Five mouse homologues of the human dendritic cell C-type lectin, DC-SIGN. *Int Immunol,* Vol. 13, No. 10, (October 2001), pp 1283-1290. ISSN 0953-8178

Pedrosa, J., Saunders, B. M., Appelberg, R., Orme, I. M., Silva, M. T., & Cooper, A. M. (2000) Neutrophils play a protective nonphagocytic role in systemic *M. tuberculosis* infection of mice. *Infect Immun,* Vol. 68, No. 10, (February 2000), pp 577-583. ISSN 0019-9567

Powlesland, A. S., Ward, E. M., Sadhu, S. K., Guo, Y., Taylor, M. E., & Drickamer, K. (2006) Widely divergent biochemical properties of the complete set of mouse DC-SIGN-related proteins. *J Biol Chem,* Vol. 281, No. 29, (July 2006), pp 20440-20449. ISSN 0021-9258

Prideaux, B., Dartois, V., Staab, D., Weiner, D. M., Goh, A., Via, L. E., Barry, C. E., III, & Stoeckli, M. (2011) High-sensitivity MALDI-MRM-MS imaging of moxifloxacin distribution in tuberculosis-infected rabbit lungs and granulomatous lesions. *Anal Chem,* Vol. 83, No. 6, (March 2011), pp 2112-2118. ISSN 0003-2700

Quesniaux, V. J., Nicolle, D. M., Torres, D., Kremer, L., Guerardel, Y., Nigou, J., Puzo, G., Erard, F., & Ryffel, B. (2004) Toll-like receptor 2 (TLR2)-dependent-positive and TLR2-independent-negative regulation of proinflammatory cytokines by mycobacterial lipomannans. *J Immunol,* Vol. 172, No. 7, (April 2004), 4425-4434. ISSN 0022-1767

Qureshi, N., Takayama, K., Jordi, H. C., & Schnoes, H. K. (1978) Characterization of the purified components of a new homologous series of alpha-mycolic acids from *M. tuberculosis* $H_{37}R_a$. *J Biol Chem,* Vol. 253, No. 15, (August 1978), pp 5411-5417. ISSN 0021-9258

Rajaram, M. V., Brooks, M. N., Morris, J. D., Torrelles, J. B., Azad, A. K., & Schlesinger, L. S. (2010) *M. tuberculosis* activates human macrophage peroxisome proliferator-activated receptor gamma linking mannose receptor recognition to regulation of immune responses. *J Immunol,* Vol. 185, No. 2, (July 2010), pp 929-942. ISSN 0022-1767

Rajaram, M.V.S., Ni, B., Morris, J.D., Brooks, M.N., Carlson, T.K., Bakthavachalu, B., Schoenberg, D.R., Torrelles, J.B., & Schlesinger, L.S. (2011). *Mycobacterium tuberculosis* lipomannan blocks TNF biosynthesis by regulating macrophages MAPK-activated protein kinase (MK2) and microRNA miR-125b. *Proc Natl Acad Sci USA*, [In Press], (October 2011). ISSN 0027-8424

Rastogi, N. (1991) Recent observations concerning structure and function relationships in the mycobacterial cell envelope: Elaboration of a model in terms of mycobacterial pathogenicity, virulence and drug-resistance. *Res Microbiol,* Vol. 142, No. 4, (May 1991), pp 464-476. ISSN 0923-2508

Razani, B. & Lisanti, M. P. (2001) Caveolins and caveolae: Molecular and functional relationships. *Exp Cell Res,* Vol. 271, No. 1, (November 2001), pp 36-44. ISSN 0014-4827

Reed, M. B., Domenech, P., Manca, C., Su, H., Barczak, A. K., Kreiswirth, B. N., Kaplan, G., & Barry, C. E., III (2004) A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. *Nature,* Vol. 431, No. 7004, (September 2004), pp 84-87. ISSN 0028-0836

Reed, M. B., Gagneux, S., Deriemer, K., Small, P. M., & Barry, C. E., III (2007) The W-Beijing lineage of *M. tuberculosis* overproduces triglycerides and has the DosR dormancy regulon constitutively upregulated. *J Bacteriol,* Vol. 189, No. 7, (April 2007), pp 2583-2589. ISSN 0021-9193

Reynolds, H. Y. (1987) Bronchoalveolar lavage. *Am Rev Respir Dis ,* Vol.135, No. 1, (January 1987), pp 250-263. ISSN 0003-0805

Ribi, E., Milner, K. C., Granger, D. L., Kelly, M. T., Yamamoto, K., Brehmer, W., Parker, R., Smith, R. F., & Strain, S. M. (1976) Immunotherapy with nonviable microbial components. *Ann N Y Acad Sci,* Vol.277, No. 00, pp 228-238. ISSN 0077-8923

Rousseau, C., Turner, O. C., Rush, E., Bordat, Y., Sirakova, T. D., Kolattukudy, P. E., Ritter, S., Orme, I. M., Gicquel, B., & Jackson, M. (2003) Sulfolipid deficiency does not affect the virulence of *M. tuberculosis* $H_{37}R_v$ in mice and guinea pigs. *Infect Immun,* Vol. 71, No. 8, (August 2003), pp 4684-4690. ISSN 0019-9567

Santiago, M., Cossermelli, W., Tuma, M., Pinto, M., & Oliveira, R. M. (1989) Anticardiolipin antibodies in patients with infectious diseases. *Clin Rheumatol,* Vol. 8, No. 1, (March 1989), pp 23-28. ISSN 0770-3198

Santiago, M., Gaburo, N., Jr., de Oliveira, R. M., & Cossermelli, W. (1991) Complement activation by anticardiolipin antibodies. *Ann Rheum Dis,* Vol. 50, No. 4, (April 1991), pp 249-250. ISSN 0003-4967

Sartain, M. J., Slayden, R. A., Singh, K. K., Laal, S., & Belisle, J. T. (2006) Disease state differentiation and identification of tuberculosis biomarkers via native antigen array profiling. *Mol Cell Proteomics,* Vol. 5, No. 11, (November 2006), pp 2102-2113. ISSN 1535-9476

Sartain, M. J., Dick, D. L., Rithner, C. D., Crick, D. C., & Belisle, J. T. (2011) Lipidomic analyses of *M. tuberculosis* based on accurate mass measurements and the novel "Mtb LipidDB". *J Lipid Res,* Vol. 52, No. 5, (May 2011), pp 861-872. ISSN 0022-2275

Sasindran, J. & Torrelles JB (2011) *M. tuberculosis* infection and inflammation: What is beneficial for the host and for the bacterium? *Front Microbiol,* Vol. 2, No. 2, (January 2011), pp 1-16. ISSN 1664-302X

Sathyamoorthy, N. & Takayama, K. (1987) Purification and characterization of a novel mycolic acid exchange enzyme from *M. smegmatis*. *J Biol Chem,* Vol. 262, No. 28, (October 1987), pp 13417-13423. ISSN 0021-9258

Schaefer, M., Reiling, N., Fessler, C., Stephani, J., Taniuchi, I., Hatam, F., Yildirim, A. O., Fehrenbach, H., Walter, K., Ruland, J., Wagner, H., Ehlers, S., & Sparwasser, T. (2008) Decreased pathology and prolonged survival of human DC-SIGN transgenic mice during mycobacterial infection. *J Immunol,* Vol. 180, No. 10, (May 2010), pp 6836-6845. ISSN 0022-1767

Schleifer, K. H. & Kandler, O. (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev,* Vol. 36, No. 4, (December 1972), pp 407-477. ISSN 0005-3678

Schlesinger, L. S., Bellinger-Kawahara, C. G., Payne, N. R., & Horwitz, M. A. (1990) Phagocytosis of *M. tuberculosis* is mediated by human monocyte complement receptors and complement component C3. *J Immunol,* Vol. 144, No. 7, (April 1990), pp 2771-2780. ISSN 0022-1767

Schlesinger, L. S. (1997) The role of mononuclear phagocytes in tuberculosis. In *Lung macrophages and dendritic cells in health and disease*. Lipscomb, M. F. and Russell, S. W. (ed.), pp. 437-480, Marcel Dekker, Inc., ISBN 0824798171, New York.

Schlesinger LS, Azad AK, Torrelles JB, Roberts, E., Vergne, I., & Deretic, V. (2008) Determinants of phagocytosis, phagosome biogenesis and autophagy for *M. tuberculosis*. In *Handbook of Tuberculosis. Immunology and Cell Biology*. Kaufmann, S. H. E. and Britton, W. J. (ed.), pp. 1-22, Wiley-VCH Verlag GmbH&Co. KGaA, ISBN 3527318879, Weinheim, Germany.

Schoenen, H., Bodendorfer, B., Hitchens, K., Manzanero, S., Werninghaus, K., Nimmerjahn, F., Agger, E. M., Stenger, S., Andersen, P., Ruland, J., Brown, G. D., Wells, C., & Lang, R. (2010) Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. *J Immunol,* Vol. 184, No. 6, (March 2010), pp 2756-2760. ISSN 0022-1767

Seiler, P., Aichele, P., Bandermann, S., Hauser, A. E., Lu, B., Gerard, N. P., Gerard, C., Ehlers, S., Mollenkopf, H. J., & Kaufmann, S. H. (2003) Early granuloma formation after aerosol *M. tuberculosis* infection is regulated by neutrophils via CXCR3-signaling chemokines. *Eur J Immunol,* Vol. 33, No. 10, (October 2003), pp 2676-2686. ISSN 0014-2980

Senaratne, R. H., Mobasheri, H., Papavinasasundaram, K. G., Jenner, P., Lea, E. J., & Draper, P. (1998) Expression of a gene for a porin-like protein of the OmpA family from *M. tuberculosis* $H_{37}R_v$. *J Bacteriol,* Vol. 180, No. 14, (July 1998), pp 3541-3547. ISSN 0021-9193

Serrano-Gomez, D., Dominguez-Soto, A., Ancochea, J., Jimenez-Heffernan, J. A., Leal, J. A., & Corbi, A. L. (2004) Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of *Aspergillus fumigatus* conidia by dendritic cells and macrophages. *J Immunol,* Vol. 173, No. 9, (november 2004), pp 5635-5643. ISSN 0022-1767

Sigler, K. & Hofer, M. (1997) Biotechnological aspects of membrane function. *Crit Rev Biotechnol,* Vol. 17, No. 2, pp 69-86. ISSN 0738-8551

Silva, M. T. & Macedo, P. M. (1983) The interpretation of the ultrastructure of mycobacterial cells in transmission electron microscopy of ultrathin sections. *Int J Lepr Other Mycobact Dis,* Vol. 51, No. 2, (June 1983), pp 225-234. ISSN 0148-916X

Simmons, D. P., Canaday, D. H., Liu, Y., Li, Q., Huang, A., Boom, W. H., & Harding, C. V. (2010) M. tuberculosis and TLR2 agonists inhibit induction of type I IFN and class I MHC antigen cross processing by TLR9. *J Immunol,* Vol. 185, No. 4, (August 2010), pp 2405-2415. ISSN 0022-1767

Singh, G., Katyal, S. L., Brown, W. E., Collins, D. L., & Mason, R. J. (1988) Pulmonary lysozyme-a secretory protein of type II pneumocytes in the rat. *Am Rev Respir Dis,* Vol. 138, No. 5, (November 1988), pp 1261-1267. ISSN 0003-0805

Sinha, S., Arora, S., Kosalai, K., Namane, A., Pym, A. S., & Cole, S. T. (2002) Proteome analysis of the plasma membrane of *M. tuberculosis*. *Comp Funct Genomics,* Vol. 3, No. 6, pp 470-483. ISSN 1531-6912

Sirard, J. C., Vignal, C., Dessein, R., & Chamaillard, M. (2007) Nod-like receptors: cytosolic watchdogs for immunity against pathogens. *PLoS Pathog* Vol. 3, No. 12, (December 2007), pp e152. ISSN 1553-7366

Skidgel, R. A. & Erdos, E. G. (1998) Cellular carboxypeptidases. *Immunol Rev,* Vol. 161, (February 1998), pp 129-141. ISSN 0105-2896

Somashekar, B. S., Amin, A. G., Rithner, C. D., Troudt, J., Basaraba, R., Izzo, A., Crick, D. C., & Chatterjee, D. (2011) Metabolic profiling of lung granuloma in *M. tuberculosis* infected Guinea pigs: *Ex vivo* ^{1}H Magic Angle Spinning NMR Studies. *J Proteome Res*. [EPub ahead of Print]. ISSN 1535-3893

Sorber, W. A., Leake, E. S., & Myrvik, Q. N. (1973) Comparative densities of hydrolase-containing granules from normal and BCG-induced alveolar macrophages. *Infect Immun,* Vol. 7, No. 12, (January 1973), pp 86-92. ISSN 0019-9567

Srivastava, M., Jung, S., Wilhelm, J., Fink, L., Buhling, F., Welte, T., Bohle, R. M., Seeger, W., Lohmeyer, J., & Maus, U. A. (2005) The inflammatory versus constitutive trafficking of mononuclear phagocytes into the alveolar space of mice is associated with drastic changes in their gene expression profiles. *J Immunol,* Vol. 175, No. 3, (August 2005), pp 1884-1893. I ISSN 0022-1767

Stephenson, J. D. & Shepherd, V. L. (1987) Purification of the human alveolar macrophage mannose receptor. *Biochem Biophys Res Commun,* Vol. 148, No. 2, (October 1987), pp 883-889. ISSN 0006-291X

Tabouret, G., starie-Dequeker, C., Demangel, C., Malaga, W., Constant, P., Ray, A., Honore, N., Bello, N. F., Perez, E., Daffe, M., & Guilhot, C. (2010) *M. leprae* phenolglycolipid-1 expressed by engineered *M. bovis* BCG modulates early interaction with human phagocytes. *PLoS Pathog,* Vol. 6, No. 10, (October 2010), pp e1001159. ISSN 1553-7366

Tailleux, L., Pham-Thi, N., Bergeron-Lafaurie, A., Herrmann, J. L., Charles, P., Schwartz, O., Scheinmann, P., Lagrange, P. H., De Blic, J., Tazi, A., Gicquel, B., & Neyrolles, O. (2005) DC-SIGN induction in alveolar macrophages defines privileged target host cells for mycobacteria in patients with tuberculosis. *PLoS Med,* Vol. 2, No. 12, (December 2005), pp e381. ISSN 1549-1277

Takayama, K. & Goldman, D. S. (1970) Enzymatic synthesis of mannosyl-1-phosphoryl-decaprenol by a cell-free system of *M. tuberculosis*. *J Biol Chem,* Vol. 245, No. 23, (December 1970), pp 6251-6257. ISSN 0021-9258

Takayama, K., Schnoes, H. K., & Semmler, E. J. (1973) Characterization of the alkali-stable mannophospholipids of *M. smegmatis*. *Biochim Biophys Actav* Vol. 316, No. 2, (August 1973), pp 212-221. ISSN 0005-2736

Takayama, K., Wang C, & Besra, G. S. (2005) Pathway to synthesis and processing of mycolic acids in *M. tuberculosis*. *Clin Microbiol Rev,* Vol. 18, No. 1, (January 2005), pp 81-101. ISSN 0893-8512

Tanne, A., Ma, B., Boudou, F., Tailleux, L., Botella, H., Badell, E., Levillain, F., Taylor, M. E., Drickamer, K., Nigou, J., Dobos, K. M., Puzo, G., Vestweber, D., Wild, M. K., Marcinko, M., Sobieszczuk, P., Stewart, L., Lebus, D., Gicquel, B., & Neyrolles, O. (2009) A murine DC-SIGN homologue contributes to early host defense against *M. tuberculosis*. *J Exp Med,* Vol. 206, No. 10, (September 2009), pp 2205-2220. ISSN 0022-1007

Taylor, P. R., Brown, G. D., Reid, D. M., Willment, J. A., Martinez-Pomares, L., Gordon, S., & Wong, S. Y. (2002) The b-glucan receptor, Dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. *J Immunol,* Vol. 169, No. 7, (October 2002), pp 3876-3882. ISSN 0022-1767

Thoma-Uszynski, S., Stenger, S., Takeuchi, O., Ochoa, M., Engele, M., Sieling, P., Barnes, P., Rollinghoff, M., Bolcskei, P., Wagner, M., Akira, S., Norgard, M., Belisle, J., Godowski, P., Bloom, B., & Modlin, R. (2001) Induction of direct antimicrobial activity through mammalian Toll-like receptors. *Science,* Vol. 291, No. 5508, (February 2001), pp 1544-1547. ISSN 0036-8075

Torrelles, J. B., Khoo, K. H., Sieling, P. A., Modlin, R. L., Zhang, N., Marques, A. M., Treumann, A., Rithner, C. D., Brennan, P. J., & Chatterjee, D. (2004) Truncated structural variants of lipoarabinomannan in *M. leprae* and an ethambutol-resistant strain of *M. tuberculosis*. *J Biol Chem,* Vol. 279, No. 39, (September 2004), pp 41227-41239. ISSN 0021-9258

Torrelles J.B., Azad A.K., & Schlesinger L.S. (2006) Fine discrimination in the recognition of individual species of phosphatidyl-*myo*-inositol mannosides from *M. tuberculosis* by C-type lectin pattern recognition receptors. *J Immunol,* Vol. 177, No. 3, (August 2006), pp 1805-1816. ISSN 0022-1767

Torrelles, J. B., Azad, A. K., Henning, L. N., Carlson, T. K., & Schlesinger, L. S. (2008a) Role of C-type lectins in mycobacterial infections. *Curr Drug Targets,* Vol. 9, No. 2, (February 2008), pp 102-112. ISSN 1389-4501

Torrelles, J. B., Knaup, R., Kolareth, A., Slepushkina, T., Kaufman, T. M., Kang, P. B., Hill, P., Brennan, P. J., Chatterjee, D., Belisle, J. T., Musser, J. M., & Schlesinger, L. S. (2008b) Identification of *M. tuberculosis* clinical isolates with altered phagocytosis by human macrophages due to a truncated lipoarabinomannan. *J Biol Chem,* Vol. 283, No. 46, (November 2008), pp 31417-31428. ISSN 0021-9258

Torrelles, J. B. & Schlesinger, L. S. (2010) Diversity in *M. tuberculosis* mannosylated cell wall determinants impacts adaptation to the host. *Tuberculosis (Edinb),* Vol. 90, No. 2, (March 2010), pp 84-93. ISSN: 1472-9792

Torrelles, J.B., Sieling, P.A., Arcos, J., Knaup. R., Bartling, C., Rajaram, M.V.S., Stenger, S., Modlin, R.L., & Schlesinger, L.S. Structural differences in lipomannans from pathogenic and non-pathogenic mycobacteria that impact CD1b-restricted T cell responses. *J Biol Chem*, [Epub ahead of print] (August 2011) ISSN 0021-9258. PMID:21859718"

Treumann, A., Xidong, F., McDonnell, L., Derrick, P. J., Ashcroft, A. E., Chatterjee, D., & Homans, S. W. (2002) 5-Methylthiopentose: a new substituent on lipoarabinomannan in *M. tuberculosis*. *J Mol Biol*, Vol. 316, No. 1, (February 2002), pp 89-100. ISSN 0022-2836

Trias, J., Jarlier, V., & Benz, R. (1992) Porins in the cell wall of mycobacteria. *Science,* Vol. 258, No. 5087, (November 1992), pp 1479-1481. ISSN 0036-8075

Tsenova, L., Ellison, E., Harbacheuski, R., Moreira, A. L., Kurepina, N., Reed, M. B., Mathema, B., Barry, C. E., III, &Kaplan, G. (2005) Virulence of selected *M. tuberculosis* clinical isolates in the rabbit model of meningitis is dependent on phenolic glycolipid produced by the bacilli. *J Infect Dis,* Vol. 192, No. 1, (July 2005), pp 98-106. ISSN 0022-1899

Turnbull, W. B., Shimizu, K. H., Chatterjee, D., Homans, S. W., and Treumann, A. (2004) Identification of the 5-methylthiopentosyl substituent in *M. tuberculosis* lipoarabinomannan. *Angew Chem Int Ed Engl,* Vol. 43, No. 30, (July 2004), pp 3918-3922. ISSN 1433-7851

Ulich, T. R., Watson, L. R., Yin, S. M., Guo, K. Z., Wang, P., Thang, H., & del Castillo, J. (1991) The intratracheal administration of endotoxin and cytokines. I. Characterization of LPS-induced IL-1 and TNF mRNA expression and the LPS-, IL-1-, and TNF-induced inflammatory infiltrate. *Am J Pathol,* Vol. 138, No. 6, (June 1991), pp 1485-1496. ISSN 0002-9440

Underhill, D. M., Ozinsky, A., Smith, K. D., & Aderem, A. (1999) Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc Natl Acad Sci USA,* Vol. 96, No. 25, (December 1999), pp 14459-14463. ISSN 0027-8424

Van Furth R (1988) Phagocytic Cells: Development and distribution of mononuclear phagocytes in normal steady state and inflammation. In *Inflammation: Basic Principles and Clinical Correlates*. Gallin JI, Goldstein IM, and Snyderman R (ed.) pp. 281-295, Lippincott Williams & Wilkins, ISBN 0881673447, New York.

van Golde, L. M. (1985) Synthesis of surfactant lipids in the adult lung. *Annu Rev Physiol,* Vol. 47, pp 765-774. ISSN 0066-4278

Velayati, A. A., Farnia, P., Ibrahim, T. A., Haroun, R. Z., Kuan, H. O., Ghanavi, J., Farnia, P., Kabarei, A. N., Tabarsi, P., Omar, A. R., Varahram, M., & Masjedi, M. R. (2009a) Differences in cell wall thickness between resistant and nonresistant strains of *M. tuberculosis*: Using transmission electron microscopy. *Chemotherapy,* Vol. 55, No. 5, (June 2009), pp 303-307. ISSN 0009-3157

Velayati, A. A., Masjedi, M. R., Farnia, P., Tabarsi, P., Ghanavi, J., Ziazarifi, A. H., & Hoffner, S. E. (2009b) Emergence of new forms of totally drug-resistant tuberculosis bacilli: Super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. *Chest,* Vol. 136, No. 2, (August 2009), pp 420-425. ISSN 0012-3692

Velayati, A. A., Farnia, P., Merza, M. A., Zhavnerko, G. K., Tabarsi, P., Titov, L. P., Ghanavei, J., Farnia, P., Setare, M., Poleschuyk, N. N., Owlia, P., Sheikolslami, M., Ranjbar, R., & Masjedi, M. R. (2010) New insight into extremely drug-resistant tuberculosis: Using atomic force microscopy. *Eur Respir J,* Vol. 36, No. 6, (December 2010), pp 1490-1493. ISSN 0903-1936

Vergne, I. & Daffe, M. (1998) Interaction of mycobacterial glycolipids with host cells. *Front Biosci,* Vol. 3, (August 1998), pp d865-d876. ISSN 1093-9946

Vergne, I., Fratti, R. A., Hill, P. J., Chua, J., Belisle, J., & Deretic, V. (2004) *M. tuberculosis* phagosome maturation arrest: Mycobacterial phosphatidylinositol analog

phosphatidylinositol mannoside stimulates early endosomal fusion. *Mol Biol Cell,* Vol. 15, No. 2, (February 2004), pp 751-760. ISSN 1059-1524

Villeneuve, C., Gilleron, M., Maridonneau-Parini, I., Daffe, M., Astarie-Dequeker, C., & Etienne, G. (2005) Mycobacteria use their surface-exposed glycolipids to infect human macrophages through a receptor-dependent process. *J Lipid Res,* Vol. 46, No. 3, (March 1995), pp 475-483. ISSN 0022-2275

Weaver, T. E. & Whitsett, J. A. (1991) Function and regulation of expression of pulmonary surfactant-associated proteins. *Biochem J,* Vol. 273, No. Pt 2, (January 1991), pp 249-264. ISSN 0264-6021

WHO. (2007). Global Tuberculosis control surveillance, planning, and financing. *WHO Press*, Genova, Switzerland. Available from: *www.who.int/tb/publications/global_report/2007/pdf/full.pdf*

WHO (2010). Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. Zignol, M. (ed), *WHO Press*,WHO/HTM/TB/2010.3, Geneva, Switzerland. Available from: *whqlibdoc.who.int/publications/2010/9789241599191_eng.pdf*

Wieland, C. W., Koppel, E. A., den Dunnen, J., Florquin, S., McKenzie, A. N., van, K. Y., van der Poll, T., and Geijtenbeek, T. B. (2007) Mice lacking SIGNR1 have stronger T helper 1 responses to *M. tuberculosis*. *Microbes Infect,* Vol. 9, No. 2, (February 2007), pp 134-141. ISSN 1286-4579

Williams, M. C. (2003) Alveolar type I cells: Molecular phenotype and development. *Annu Rev Physiol,* Vol. 65, (May 2003), pp 669-695. ISSN 0066-4278

Winder, F. G. & Collins, P. B. (1970) Inhibition by isoniazid of synthesis of mycolic acids in *M. tuberculosis*. *J Gen Microbiol,* Vol. 63, No. 1, (September 1970), pp 41-48. ISSN 0022-1287

Wolucka, B. A. & De Hoffmann, E. (1995) The presence of b-D-ribosyl-1-monophosphodecaprenol in mycobacteria. *J Biol Chem,* Vol. 270, No. 34, (August 1995), pp 20151-20155. ISSN 0021-9258

Yadav, M. & Schorey, J. S. (2006) The b-glucan receptor Dectin-1 functions together with TLR2 to mediate macrophage activation by mycobacteria. *Blood,* Vol. 108, No. 9, (November 2006), pp 3168-3175. ISSN 0006-4971

Yamasaki, S., Ishikawa, E., Sakuma, M., Hara, H., Ogata, K., & Saito, T. (2008) Mincle is an ITAM-coupled activating receptor that senses damaged cells. *Nat Immunol*, Vol. 9, No. 10, (October 2008), pp 1179-1188. ISSN 1529-2908

Yarkoni, E. & Bekierkunst, A. (1976) Nonspecific resistance against infection with *Salmonella typhi* and *Salmonella typhimurium* induced in mice by cord factor (trehalose-6,6'-dimycolate) and its analogues. *Infect Immun,* Vol. 14, No. 5, (November 1976), pp 1125-1129. ISSN 0019-9567

Yokoyama, T. and Shimizu, T. (2002) Transmembrane-Protein-Gene Clusters in Prokaryotic Genomes. *Genome Informatics,* Vol. 13, pp 416-417. ISSN 0919-9454

Young, D. B. & Garbe, T. R. (1991) Lipoprotein antigens of *M. tuberculosis*. *Res Microbiol,* Vol. 142, No. 1, (January 1991), pp 55-65. ISSN 0923-2508

Young, S. L., Fram, E. K., Larson, E., and Wright, J. R. (1993) Recycling of surfactant lipid & apoprotein-A studied by electron microscopic autoradiography. *Am J Physiol Lung Cell Mol Physiol,* Vol. 265, No. 1Pt1, (July 1993), pp L19-L26. ISSN 1040-0605

Zaffran, Y., Zhang, L., & Ellner, J. J. (1998) Role of CR4 in *M. tuberculosis*-human macrophages binding and signal transduction in the absence of serum. *Infect Immun,* Vol. 66, No. 9, (September 1998), pp 4541-4544. ISSN 0019-9567

Zhang, F. R., Huang, W., Chen, S. M., Sun, L. D., Liu, H., Li, Y., Cui, Y., Yan, X. X., Yang, H. T., Yang, R. D., Chu, T. S., Zhang, C., Zhang, L., Han, J. W., Yu, G. Q., Quan, C., Yu, Y. X., Zhang, Z., Shi, B., Zhang, L. H., Cheng, H., Wang, C. Y., Lin, Y., Zheng, H. F., Fu, X. A., Zuo, X., Wang, Q., Long, H., Sun, Y. P., Cheng, Y. L., Tian, H. Q., Zhou, F., Liu, H. X., Lu, W. S., He, S. M., Du, W. L., Shen, M., Jin, Q. Y., Wang, Y., Low, H., Erwin, T., Yang, N., Li, J. Y., Zhao, X., Jiao, Y., Mao, L., Yin, G., Jiang, Z., Wang, X., Yu, J., Hu, Z., Gong, C., Liu, Y., Liu, R., Wang, D., Wei, D., Liu, J., Cao, W., Cao, H., Li, Y., Yan, W., Wei, S., Wang, K., Hibberd, M., Yang, S., Zhang, X., & Liu, J. J. (2009) Genomewide association study of leprosy. *N Engl J Med,* Vol. 361, No. 27, (December 2009), pp 2609-2618. ISSN 0028-4793

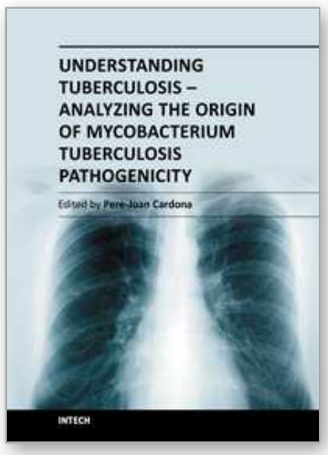
Zhang, F. X., Kirschning, C. J., Mancinelli, R., Xu, X. P., Jin, Y., Faure, E., Mantovani, A., Rothe, M., Muzio, M., & Arditi, M. (1999) Bacterial lipopolysaccharide activates nuclear factor-kappaB through interleukin-1 signaling mediators in cultured human dermal endothelial cells and mononuclear phagocytes. *J Biol Chem,* Vol. 274, No. 12, (march 1999), pp 7611-7614. ISSN 0021-9258

Zhang, L., Goren, M. B., Holzer, T. J., & Andersen, B. R. (1988) Effect of *M. tuberculosis*-derived sulfolipid I on human phagocytic cells. *Infect Immun,* Vol. 56, No. 11, (November 1988), pp 2876-2883. ISSN 0019-9567

Zhang, L., English, D., & Andersen, B. R. (1991) Activation of human neutrophils by *M. tuberculosis*-derived sulfolipid-1. *J Immunol,* Vol. 146, No. 8, (April 1991), pp 2730-2736. ISSN 0022-1767

Zhang, P., Summer, W. R., Bagby, G. J., & Nelson, S. (2000) Innate immunity and pulmonary host defense. *Immunol Rev,* Vol. 173, (February 2000), pp 39-51. ISSN 0105-2896

Zimmerli, S., Edwards, S., & Ernst, J. D. (1996) Selective receptor blockade during phagocytosis does not alter the survival and growth of *M. tuberculosis* in human macrophages. *Am J Respir Cell Mol Biol,* Vol. 15, No. 6, (December 1996), pp 760-770. ISSN 1044-1549



Understanding Tuberculosis - Analyzing the Origin of Mycobacterium Tuberculosis Pathogenicity

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Mycobacterium tuberculosis in an attempt to understand the extent to which the bacilli has adapted itself to the host and to its final target. On the other hand, there is a section in which other specialists discuss how to manipulate this immune response to obtain innovative prophylactic and therapeutic approaches to truncate the intimal co-evolution between Mycobacterium tuberculosis and the Homo sapiens.

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