Lipoarabinomannan and related glycoconjugates: structure, biogenesis and role in Mycobacterium tuberculosis physiology and host–pathogen interaction

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Received 22 April 2010; revised 28 March 2011; accepted 19 April 2011. Final version published online 31 May 2011.

DOI:10.1111/j.1574-6976.2011.00276.x

Editor: Michael Bott

Keywords
bacterial; polysaccharides; cell wall; biosynthesis; glycosyltransferases; drug-targets.

Abstract
Approximately one third of the world’s population is infected with Mycobacterium tuberculosis, the causative agent of tuberculosis. This bacterium has an unusual lipid-rich cell wall containing a vast repertoire of antigens, providing a hydrophobic impermeable barrier against chemical drugs, thus representing an attractive target for vaccine and drug development. Apart from the mycolyl–arabinogalactan–peptidoglycan complex, mycobacteria possess several immunomodulatory constituents, notably lipomannan and lipoarabinomannan. The availability of whole-genome sequences of M. tuberculosis and related bacilli over the past decade has led to the identification and functional characterization of various enzymes and the potential drug targets involved in the biosynthesis of these glycoconjugates. Both lipomannan and lipoarabinomannan possess highly variable chemical structures, which interact with different receptors of the immune system during host–pathogen interactions, such as Toll-like receptors-2 and C-type lectins. Recently, the availability of mutants defective in the synthesis of these glycoconjugates in mycobacteria and the closely related bacterium, Corynebacterium glutamicum, has paved the way for host–pathogen interaction studies, as well as, providing attenuated strains of mycobacteria for the development of new vaccine candidates. This review provides a comprehensive account of the structure, biosynthesis and immunomodulatory properties of these important glycoconjugates.

Introduction
Tuberculosis (TB) is a major cause of death worldwide, with approximately 9 million cases and 1.7 million deaths registered in 2008 (WHO, 2009). To compound this situation, 50 000 cases were reported as multidrug-resistant tuberculosis (MDR-TB) and 55 countries globally had reported at least one case of extensively drug-resistant tuberculosis (XDR-TB) (WHO, 2009). Mycobacterium tuberculosis is the causative agent of tuberculosis. It has an unusual lipid-rich cell wall that is unique to the order Actinomycetes, including the genera Mycobacterium, Rhodococcus, Corynebacterium and Nocardia (Brennan & Nikaido, 1995). The mycobacterial cell wall is composed of a mycolyl–arabinogalactan–peptidoglycan (mAGP) complex (Daffé et al., 1990; McNeil et al., 1990, 1991; Besra et al., 1995; Brennan, 2003; Dover et al., 2004), of which the mycolic acids and extractable lipids form the mycobacterial outer membrane (Hoffmann et al., 2008). The mycolic acid layer provides a hydrophobic mesh for intercalating additional complex lipids, resulting in a highly impermeable barrier for the penetration of antimicrobial drugs, such as penicillins (Amberson et al., 1931; Minnikin et al., 2002). Other cell wall-associated lipids, such as phosphatidyl-myoinositol mannosides (PIMs) and lipoglycans, termed lipomannan (LM) and lipoarabinomannan (LAM), are also found in the cell wall (Hill & Ballou, 1966; Brennan & Ballou, 1967, 1968a; Brennan & Nikaido, 1995; Besra et al., 1997; Morita et al., 2004). In addition to their physiological function, these complex glycoconjugates play a key role in modulating the host response during infection. PIMs, lipomannan and lipoarabinomannan all display several immunomodulatory properties by interaction with different receptors of the immune system. While lipomannan is mainly associated with Toll-like
receptors (TLR) signaling, the higher-order PIMs and mannose-capped lipoarabinomannan (Man-LAM) are recognized by the C-type lectins, such as dendritic cell-specific intercellular adhesion molecule-3 (ICAM-3) grabbing non-integrin (DC-SIGN) and the macrophage mannose receptor (MMR) (Schlesinger et al., 1994; Chatterjee & Khoo, 1998; Nigou et al., 2002; Geijtenbeek et al., 2003; Maeda et al., 2003).

Because of the advent of MDR and XDR strains of M. tuberculosis (Sreevatsan et al., 1997; Telenti et al., 1997; Heymann et al., 1998; Chan & Iseman, 2008; Wright et al., 2009), there is an urgent need to identify novel drug targets and the development of active compounds. In this respect, the biosynthetic machinery of the mycobacterial cell wall, which is the site of action of many front-line tuberculosis drugs, represents an attractive drug target (Bhatt et al., 2007; Bhowruth et al., 2007; Brennan & Crick, 2007; Dover et al., 2008). Furthermore, a complete investigation of the roles of PIMs, lipomannan and lipoarabinomannan in mycobacterial pathogenicity requires mutants defective in their respective biosynthetic pathways. The availability of complete genome sequences of several mycobacteria and related actinomycetes and the development of novel tools for genetic manipulation have opened up the possibilities to achieve this (Cole et al., 1998).

Herein, we report recent advances in the biogenesis of lipoarabinomannan and related glycoconjugates, followed by a comprehensive analysis of their role in host–pathogen interactions. Furthermore, we review the localization and trafficking of these immunomodulatory lipoglycans and discuss recent findings concerning the role of CD1, TLR, DC-SIGN and MMR in M. tuberculosis infection.

**Part I – Structure and biogenesis of PIMs, lipomannan and lipoarabinomannan**

**Structural features of PIMs, lipomannan and lipoarabinomannan**

The majority of bacteria from suborder Corynebacterineae, including Corynebacterium diphtheriae, Corynebacterium glutamicum, pathogenic M. tuberculosis complex and nonpathogenic Mycobacterium smegmatis, possess the amphipathic lipoglycans, lipoarabinomannan and other related glycoconjugates, lipomannan and PIMs (Fig. 1). All Mycobacterium species possess two forms of acylated PIMs, tri- and tetra-acylated (Ac1- and Ac2-) phospho-myoinositol-dimannoside (PIM2) and tri- and tetra-acylated phospho-myoinositol-hexamannoside (Ac1/Ac2PIM6) (we have used Ac1/Ac2PIMx for two different acylated versions of PIMs throughout the text, and PIM as a synonym where the acylation state is not clear), and different acylated versions of lipomannan and lipoarabinomannan (Khoo et al., 1995a), which are believed to be noncovalently attached to the cell membrane via a lipid anchor (Fig. 2) (Hunter & Brennan, 1990).

**Structure of PIMs**

PIMs are categorized as glycolipids composed of fatty acids attached to a glycerol unit, linked by a phosphodiester

![Fig. 1. Lipoarabinomannan and related glycoconjugates found on the cell wall of Mycobacterium tuberculosis, Mycobacterium smegmatis and Corynebacterium glutamicum. Biochemical analysis of the mycobacterial cell wall suggests that different acylated variants of di- and hexa-mannosylated PIMs, Ac1/Ac2PIM2 and PIM6, and the higher glycosylated polymers lipomannan and lipoarabinomannan accumulate in the cell wall. However, in C. glutamicum, only PIM1, two types of lipomannan (LM-A and LM-B, Tatituri et al., 2007a; Mishra et al., 2008b) and singular Araf capped lipoarabinomannan are present on the cell wall. For the purpose of simplicity, only diacylated forms of these glycoconjugates and LM-A, i.e. MPI anchored lipomannan, are shown. In these glycoconjugates, phosphatidyl-myoinositol (phosphate in gray and inositol in blue) acts as an anchor to the plasma membrane and further glycosylated by Manp (green) and Araf (pink) sugars yielding different forms of PIMs, lipomannan and lipoarabinomannan that are species specific. In M. tuberculosis and other pathogenic mycobacteria, lipoarabinomannan is terminated by phospho inositol, yielding PI-LAM.](image-url)
moiety to myo-inositol (Vilkas & Lederer, 1956; Ballou et al., 1963) (see Fig. 2 for Ac₂PIM₂ and Ac₂PIM₆). This phosphatidylinositol (PI) is based on an sn-glycero-3-phospho-(1-d-myoinositol) unit and is further substituted at the O-2 and O-6 positions of myo-inositol with α-d-mannopyranosyl (Manₚ) units in case of PIM₂, resulting in a mannosyl phosphate inositol (MPI) anchor, a derivative of the typical glycosyl phosphate inositol anchor, found in Eukaryotes (Lee & Ballou, 1964; Chatterjee et al., 1992a; Severn et al., 1998).

The MPI anchor is heterogeneous, with variations occurring within the number, location and nature of the fatty acids. There are four potential sites of acylation within the MPI anchor, with different fatty acids at 1-OH and 2-OH of the Manₚ unit in the anchor, 3-OH of myo-inositol and position 6 of the Manₚ unit linked at O-2 of myo-inositol in Ac₂PIM₆ (See the inset in light blue color). Manₚ at the O-6 position of myo-inositol is linked to further three and two residues of myo-inositol in Ac₂PIM₂ (see the inset in light indigo color). In lipomannan and the mannan backbone of lipoarabinomannan, PIM₂ is linked to another 17–19 residues of Manₚ in the 6→1 direction and 7–9 singular branched α(1→2)-Manₚ units. Mature lipomannan is further linked via an unknown linkage to an arabinous domain made up of approximately 70 Ara residues. The majority of the arabin domain consists of a linear α(1→5)-Araf polymer branched at certain positions, with α(3→5)-Araf residues towards its nonreducing end resulting in a linear tetra-arabinoside or branched hexa-arabinoside domain, which in turn is terminated by β(1→2)-Araf and capped by α(1→2)-Manₚ units. Here R₁, R₂, R₃ and R₄ show different acyl groups found at different locations in the MPI anchor, and n, m, o, p and q represent different degrees of species-specific glycosylation in lipomannan and lipoarabinomannan.

Acylated forms of PIM₂ serve as substrates for the synthesis of higher-order PIMs, such as Ac₁/Ac₂PIM₆ (Figs 2 and 4). Studies with a crude cell extract of M. tuberculosis and Mycobacterium phlei identified PIM₆, which is a pentamannose side attached to the position O-6 of the myo-inositol of PI of PIM₁. Manₚ-α(1→2)-Manₚ-α(1→2)-Manₚ-α(1→2)-Manₚ-α(1→6)-Manₚ-α(1→2)-Manₚ-α(1→6) (Lee & Ballou, 1965), which was later verified by others (Chatterjee et al., 1992a; Severn et al., 1998) (Fig. 2). A biosynthetic relationship between PIM₁ and PIM₂ was also suggested, which involves a stepwise glycosylation of PI, first at the O-2 position and then at the O-6 position of the inositol ring (Ballou & Lee, 1964; Chatterjee et al., 1992a). It was also suggested that this acylated version of PIM₂ i.e. Ac₁PIM₂ is both a metabolic end-product

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**Fig. 2.** Schematic structures of lipoarabinomannan and related glycoconjugates. As described in the text, PI acts as an anchor around which PIMs, lipomannan and lipoarabinomannan are built. PI is glycosylated at the 2-OH and 6-OH positions of inositol by Manₚ residues, and acylated at position 3 of myo-inositol and position 6 of the Manₚ unit linked at O-2 of myo-inositol in Ac₂PIM₆. There are four potential sites of acylation within the ring within the number, location and nature of the fatty acids. There are four potential sites of acylation within the ring within the number, location and nature of the fatty acids.
and an intermediate in Ac₃/PIM₆, lipomannan and lipoarabinomannan biosynthesis (Chatterjee et al., 1992a; Khoo et al., 1995a; Besra et al., 1997).

More recently, the presence of a glucuronic acid diacyl-glycerol-based glycolipid, Manp-\(\alpha(1 \rightarrow 4)\)-d-glucopyranosyluronic acid-diacyl glycerol, ManGlcAGroAc₂, and a novel lipomannan variant (Cg-LM-B) were reported in C. glutamicum, in addition to the MPI anchored-LM (Cg-LM-A) and lipoprotein variants, and to date, these glycoconjugate variants have not been identified in mycobacteria (Tatituri et al., 2007a; Lea-Smith et al., 2008; Mishra et al., 2008b, 2009). However, Rv0557 [MgtA] of M. tuberculosis was shown to have the ability to synthesize these novel lipids and lipoglycans in vitro and in vivo (Tatituri et al., 2007a; Mishra et al., 2009), and the majority of the genus Mycobacterium possesses an ortholog of MgtA. Therefore, theoretically, the possibilities remain for the identification of glucuronic acid-anchor-based glycolipids in mycobacteria in addition to MPI anchor-based ones.

**Structure of lipomannan and lipoarabinomannan**

In 1930, Masucci and colleagues isolated a polysaccharide containing D-arabinofuranoside (Araf) and Manp with high serological activity from mycobacteria (Masucci et al., 1930), which was also identified in Mycobacterium bovis bacilli Calmette-Guérin (BCG) (Chargaff & Schaefer, 1935), and separated using electrophoresis (Seibert & Watson, 1941). Later, one of the polysaccharides was identified as arabinogalactan (AG), the basic constituent of the mAGP complex (Misaki & Yukawa, 1966), and the other as a pool of immunologically active lipoarabinomannan and inactive lipomannan (Misaki et al., 1977), both sharing a similar mannan core (Hunter et al., 1986; Hunter & Brennan, 1990; Chatterjee et al., 1992a) (Fig. 2). Further studies identified Ac₁/Ac₂/PIM₃ as the attachment point (Hunter & Brennan, 1990) for the synthesis of the \(\alpha(1 \rightarrow 6)\)-mannan backbone of lipomannan and lipoarabinomannan, which is composed of around 21–34 residues of \(\alpha(1 \rightarrow 6)\)-Manp and decorated by singular 5–10 units of \(\alpha(1 \rightarrow 2)\)-Manp, resulting in the formation of lipomannan (Chatterjee et al., 1992a; Kaur et al., 2008). The mannan core is further elaborated by the addition of an arabinan domain consisting of approximately 55–70 Araf residues in a linear \(\alpha(1 \rightarrow 5)\)-d-Araf fashion with 3,5-\(\alpha\)-d-Araf branches (Kaur et al., 2008; Birch et al., 2010). The arabinan domain is highly branched and conserved with two types of chain arrangements. Firstly, linear tetra-arabinoside (Ara-4) of the structure \(\beta\)-d-Araf\((1 \rightarrow 2)\)-\(\alpha\)-d-Araf\((1 \rightarrow 5)\)-\(\alpha\)-d-Araf\((1 \rightarrow 5)\)-\(\alpha\)-d-Araf, and secondly, branched hexa-arabinoside (Ara-6) motifs with the structure [\(\beta\)-d-Araf\((1 \rightarrow 2)\)-\(\alpha\)-d-Araf\][\(\alpha\)-d-Ara\((1 \rightarrow 5)\)-\(\alpha\)-d-Araf](Chatterjee et al., 1991; Chatterjee et al., 1993). In both cases, the nonreducing end is characterized by the disaccharide unit, Araf\((1 \rightarrow 2)\)-Araf\((1 \rightarrow .\) (Fig. 2) (Chatterjee et al., 1991, 1993; McNeil et al., 1994).

The arabinan termini of lipoarabinomannan from the Erdman strain of M. tuberculosis were shown to be capped with Manp residues and it was established that the tetra-/hexa-arabinofuranoside unit was further extended by mono, di- and tri-\(\alpha(1 \rightarrow 2)\)-d-Manp saccharide units (Fig. 2) (Chatterjee et al., 1992b, 1993; Venisse et al., 1993). The number of mannose caps is species specific, with M. tuberculosis H37Rv and M. bovis BCG Man-LAM equally capped with around seven residues per molecule of lipoarabinomannan (Khoo et al., 1995b; Nigou et al., 2003). Surprisingly, lipoarabinomannan from a fast-growing Mycobacterium species is devoid of any mannose cap (Chatterjee et al., 1992b) and, in turn, a novel phosphoinositol capping motif was identified from *M. smegmatis* strains ATCC 14468 and mc²155 (PI-LAM) (Fig. 1) (Khoo et al., 1995b) and the
absence of a capping motif in lipoarabinomannan from *Mycobacterium chelonae*, AraLAM (Guerardel et al., 2002).

**Further chemical modifications of lipoarabinomannan**

In its physiological form, Man-LAM is found in two different fractions: parietal and cellular (Vercellone et al., 1998; Gilleron et al., 2000; Hoffmann et al., 2008). These fractions differ in terms of the percentage of mannose caps and acylation groups of the MPI anchor. Parietal Man-LAM possess a novel fatty acid assigned as 12-O-(methoxypropa-noyl)-12-hydroxystearic acid, esterified at C-1 of the glycerol residue of PI, while cellular Man-LAMs are largely heterogeneous with palmitic and tuberculostearic acid (Nigou et al., 1997). More likely, cellular lipoarabinomannan is more strongly attached to the cell wall due to higher acylation as compared with parietal lipoarabinomannan (Pitarque et al., 2008). Furthermore, in different *M. bovis* BCG strains (Pasteur, Glaxo, Copenhagen and Japanese strains), the presence of succinyl groups on O-2 of the 3,5-di-α-D-Araf residue of Man-LAM was also reported (Delmas et al., 1997). Recently, Treumann et al. (2002) identified a 5-methylthiopentose substituent on the terminal Manp in the cap structure of Man-LAM in several strains of *M. tuberculosis*, which was later characterized as 5-deoxy-5-methylthio-xylofuranose (Turnbull et al., 2004) with a D-configuration (Joe et al., 2006) and linked by an α(1 → 2) linkage to a Manp residue in the mannan portion of the glycan (Guerardel et al., 2003).

**Biogenesis of PIMs, lipomannan and lipoarabinomannan**

**Biosynthesis of substrates**

**GDP-Manp biosynthesis**

Besides being part of glycolipids and lipoglycans, mannose is also involved in the synthesis of a number of glycosylated proteins (VanderVen et al., 2005) and a few other key components, such as polymethylated polysaccharides in mycobacteria (Jackson & Brennan, 2009). These molecules are synthesized by both pathogenic and nonpathogenic species, raising the possibility of as yet undefined ‘housekeeping’ functions in these organisms. The mannose metabolism is essential for growth in *M. smegmatis* and it was suggested that apart from glycolipid and lipoglycan biosynthesis, mannose-containing molecules may also play a role in regulating septation and cell division (Patterson et al., 2003).

In mycobacteria, mannose is probably obtained by two distinct pathways: firstly, by transport of extracellular mannose from the medium or the extracellular environment with the activity of a hexokinase (Kowalska et al., 1980). The phosphorylated mannose, mannose-1-phosphate, is then converted into GDP-Manp by GDP-mannose pyrophosphorylase, ManC [Rv3264c] (Ning & Elbein, 1999; Ma et al., 2001) (Fig. 3). Secondly, in the absence of extracellular mannose, it can be derived from glucose and other sugars via the glycolytic pathway, where fructose-6-phosphate is converted to mannose-6-phosphate by an essential enzyme, phosphomannose isomerase, encoded by manA [Rv3255c] (Patterson et al., 2003). Mannose-6-phosphate is then converted to mannose-1-phosphate by a phosphomannomutase, ManB [Rv3257c] (McCarthy et al., 2005), followed by conversion into GDP-Manp by ManC (Ning & Elbein, 1999; Ma et al., 2001) (Fig. 3).

**Synthesis of β-D-mannosyl-1-monophosphoryldecaprenol**

GDP-Manp serves as an intracellular nucleotide-derived mannose donor for the synthesis of several glycolipids and mannosylated proteins by the GT-A/B superfamily of glycosyltransferases (Liu & Mushegian, 2003). However, for periplasmic biosynthetic events, a polyprenyl-phosphate-based mannose donor is required, which acts as a mannose donor for the GT-C superfamily of glycosyltransferases for the synthesis of higher PIMs, lipomannan and lipomannan...
lipoarabinomannan. Takayama & Goldman (1970) were the first to report the presence of a C_{50}-polyprenol-based manno-lipid, C_{35}-decaprenyl-phospho-mannose (C_{35}-P-Manp, PPM), in *M. tuberculosis* (Takayama & Goldman, 1970). Later on, another alkali-stable, C_{35}-octahydrohepta-prenyl-phospho-mannose, C_{35}-P-Manp, was identified in *M. smegmatis* (Wolucka & Hoffmann, 1998). Based on similarities to the known eukaryotic dolichol monophosphomannose synthases, Rv2051c [Ppm1] from *M. tuberculosis* was identified as a polyprenyl monophosphomannose synthase, PPM synthase (Gurcha et al. 2002; Gibson et al. 2002). Surprisingly, Ppm1 possesses an unusual two-domain architecture in *M. tuberculosis*, of which the second domain, Mt-Ppm1/D2, is sufficient for PPM synthesis (Gurcha et al., 2002; Gibson et al., 2003). However, *M. smegmatis*, *Mycobacterium avium* and *M. leprae* produce two distinct proteins, one for each of the two domains found in Mt-Ppm1, with Ms-Ppm2 and Ma-Ppm2 having a catalytic activity similar to that of domain 2 of Mt-Ppm1. Recently, a transmembrane glycosyltransferase, Rv3779, was identified and suggested to be involved in the synthesis of C_{35}-decaprenyl-phospho-mannose as a second PPM synthase (Scherman et al., 2009). However, Skovierova et al. (2010) recently described the function of Rv3779 as the glycosyltransferase involved in transferring galactosamine from a polyprenyl-phospho-\(N\)-acytylgalactosamine in *M. tuberculosis*.

**Orin and synthesis of decaprenyl-phospho-arabinose**

Generally, in nature, \(d\)-arabinose exists in two cyclic forms: a rare pyranose-ring (Arap) and the furanose-ring (Araf) (Wolucka, 2008). Araf forms a key component of both arabinogalactan and lipoarabinomannan in mycobacteria and the only known Araf sugar donor is a lipid-linked decaprenyl-phospho-arabinose (DPA) and also termed C_{35}-P-Araf (Wolucka et al., 1994). However, a putative role of a nucleotide-based Araf donor was also suggested in the addition of the single terminal \(d\)-Araf residues of lipoarabinomannan in *C. glutamicum* (Tatituri et al., 2007b). The majority of DPA synthesized in mycobacteria comes from the pentose phosphate pathway (Marks, 1956). A transketolase, Rv4494, links the glycolytic and pentose phosphate pathway to produce ribose-5-phosphate (Wolucka, 2008). Alternatively, ribose-5-phosphate isomerase [Rv2465] also synthesizes \(d\)-ribulose-5-phosphate into ribose-5-phosphate (Roos et al., 2004; Roos et al., 2005). Furthermore, Rv1017c, a ribose-5-phosphate diphosphokinase (PrsA), converts ribose 5-phosphate into 5-phosphoribosyl-\(\alpha\)-1-pyrophosphate (pRpp) (Alderwick et al., 2010), which is dephosphorylated by a phosphatase as the first committed step in decapolyenphosphoribose (DPR) and also termed C_{35}-P-Rib) and DPA biosynthesis (Mikusová et al., 2005) (Fig. 3). In the genome of *M. tuberculosis*, an unknown poly-(A)-polymerase2 (PAP2)-superfamily phospholipid phosphatase [Rv3807c] exists that is present in the arabinogalactan biosynthetic cluster (Rv3779-Rv3809c) and next to Rv3806c, UbiA (Huang et al., 2005), which may be responsible for pRpp phosphatase activity. Furthermore, the deletion of the Rv3807c homolog in *C. glutamicum* remains unsuccessful, suggesting it as a prime candidate (L. Eggeling & G.S. Besra, unpublished data).

The synthesis of DPA and DPR from pRpp was shown experimentally and it was concluded that DPA is formed from pRpp via a two-step pathway, with an additional epimerization step that converts DPR to DPA (Scherman et al., 1996; Mikusová et al., 2005) (Fig. 3). Recently, a 5-phospho-\(\alpha\)-\(\alpha\)-ribose-1-diphosphatedecaprenyl-phosphate 5-phospho-ribosyltransferase, UbiA [Rv3806c], was identified in *M. tuberculosis* (Huang et al., 2005). The deletion of *ubiA* in *C. glutamicum* produced a mutant that possessed a galactan core consisting of alternating \(\beta(1 \rightarrow 5)\)-galactofuranose (Galf) and \(\beta(1 \rightarrow 6)\)-Galf residues and completely devoid of arabinan and cell-wall-bound corynomycolic acids, confirming its role in the synthesis of DPR and DPA biosynthesis in *Corynebacteriaceae* (Alderwick et al., 2005; Alderwick et al., 2006a). More recently, Mikusová et al. (2005) identified an epimerase, which is involved in the epimerization of DPR to DPA. It was established that the 2-OH of ribose is oxidized to decaprenylphosphoryl-2-keto-\(d\)-erythro-pentofuranose, which is then reduced to form DPA. These activities are encoded by Rv3790 and Rv3791, respectively, and the simultaneous expression of both is required for complete activity of the epimerase reaction (Mikusová et al., 2005) (Fig. 3). Interestingly, Rv3790 has been shown to be a target of benzothiazinones, potential tuberculosis drugs (Christophe et al., 2009; Makarov et al., 2009).

**Synthesis of phosphatidyl-mylo-inositol**

Inositol is an essential metabolite in *Mycobacterium* (Kataoka & Nojima, 1967), *Corynebacterium* (Brennan & Lehane, 1971), *Nocardia* (Yano et al., 1969), *Micromonospora* (Taubaud et al., 1971) and *Propionibacterium* (Brennan & Ballou, 1968b). In mycobacteria, inositol is essential for growth and derived directly via glycolysis (Jackson et al., 2000). Glucose-6-phosphate is cyclized by an inositol-1-phosphate synthase, Ino1 [Rv0046c] (Bachhawat & Mande, 1999; Movahedzadeh et al., 2004), into myo-inositol-1-phosphate, followed by its dephosphorylation utilizing an inositol monophosphatase (IMP) (Fig. 3). On the basis of homology, the *M. tuberculosis* genome shows four ORFs encoding putative proteins with an IMP domain: Rv1604 (ImpA), Rv2701c (SuhB), Rv2131c (CysQ) and Rv3137 (ImpC). Out of these, *impC* was found to be essential for mycobacterial growth, and it was suggested that *impA*, *suhB* and *cysQ* may make a minor contribution towards inositol biosynthesis (Movahedzadeh et al., 2009).
et al. 2010) as suggested by the in vitro IMP activity of SuhB (Fig. 3) (Parish et al., 1997; Nigou & Besra, 2002a; Brown et al., 2007).

The first step in the production of many phospholipids, including PI, is the phosphorylation of diacylglycerol (DAG) by a diacylglycerol kinase [Rv2252] to form phosphatidic acid (Owens et al., 2006). Phosphatidic acid is then activated by CTP to form CDP-DAG by a CDP-DAG synthase [Rv2881c], a homolog of which has been characterized in M. smegmatis [Rv2252] (Nigou & Besra, 2002b). Furthermore, it was shown that a cell wall fraction (Percoll-60, P 60) from M. smegmatis [Rv2881c], a homolog of which has been characterized in M. smegmatis [Rv2252] (Nigou & Besra, 2002b). Furthermore, it was shown that a cell wall fraction (Percoll-60, P 60) from M. smegmatis [Rv2881c], a homolog of which has been characterized in M. smegmatis (Nigou & Besra, 2002b). Furthermore, it was shown that a cell wall fraction (Percoll-60, P 60) from M. smegmatis (Nigou & Besra, 2002b). Furthermore, it was shown that a cell wall fraction (Percoll-60, P 60) from M. smegmatis (Nigou & Besra, 2002b).

Overview of PIM biosynthesis

The current model of mycobacterial PIM biosynthesis supported by biochemical and genetic studies follows a linear pathway from PI → PIM2 → PIM4 → PIM6 (Fig. 4) (Chatterjee et al., 1992a; Besra & Brennan, 1999; Morita et al., 2004, 2006). Glycosylation of PI by different α-mannopyranosyltransferases, PimA, PimB', PimC, unidentifie d PimD?, PimE, unidentified PimF? and acylation by acyltransferase(s), results in the synthesis of Ac1/Ac2PIMs (Kordulakova et al., 2002, 2003; Kremer et al., 2006; Lea-Smith et al., 2008; Guerin et al., 2009; Mishra et al., 2009), out of which Ac1/Ac2PIM2 and Ac1/Ac2PIM6 accumulate onto the mycobacterial cell wall (Figs 1 and 4).

Conversion of PI into Ac1PIM1

The enzymes involved in the synthesis of early PIMs are encoded by a conserved cluster of six ORFs in an operon, which is found in all members of Corynebacterinae (Cole & Barrell, 1998; Cole et al., 1998). The first ORF of this cluster, Rv2614c, encodes a protein with an aminoacyl-tRNA synthase class-II motif and is similar to Escherichia coli threonyl-t-RNA synthases. The second ORF, Rv2613c, has similarity to the proteins involved in nucleotide biosynthesis, while the third ORF, Rv2612c, encodes for PgsA and the fourth ORF, Rv2611c, encodes an acyltransferase. An M. smegmatis Rv2611c mutant exhibited severe growth defects and accumulated nonacylated PIM1 and PIM2, suggesting its role in acylation of PIMs. Further biochemical analysis suggested that Rv2611c acylates the 6-position of Manp residue linked to the 2-OH position of myo-inositol (Kordulakova et al., 2003). Very recently, the identification of an α-d-mannose-α(1 → 6)-phosphatidyl- myo-inositol-mannopyranosyltransferase, PimB', involved in the biosynthesis of Ac1/Ac2PIM2, shed further light on the acylation step in PIM biosynthesis. The deletion of pimB' in C. glutamicum resulted in the abrogation of Ac1/Ac2PIM2 and the accumulation of Ac1PIM1 (Lea-Smith et al., 2008; Mishra et al., 2008b), suggesting that the first acylation step, i.e. acylation of PIM1 (Kordulakova et al., 2003), precedes the second mannosylation step, resulting in the formation of Ac1PIM2 (Schaeffer et al., 1999).

PimA [Rv2610c] is the fifth ORF of the operon and is essential in M. smegmatis (Kordulakova et al., 2002). In cell-free assays with partially purified Rv2610c and/or membranes from M. smegmatis overexpressing PimA and GDP-[14C]-Manp, Kordulakova et al. (2002) identified the incorporation of radioactivity into PIM1 and Ac1PIM1. They deduced that Rv2610c encodes for an α-mannopyranosyltransferase and that PimA is responsible for the formation of PIM1 from PI and GDP-Manp (Kordulakova et al., 2002). The crystal structure of PimA in complex with GDP-Manp from M. smegmatis shows a two-domain organization with the catalytic machinery typical of GT-B glycosyltransferases (Guerin et al., 2007). The sixth ORF, Rv2609c, encodes for a putative GDP-Manp hydrolase containing a mutT domain (see below for further discussion).

Synthesis of Ac1PIM2, an important step in higher PIMs, lipomannan and lipoarabinomannan biosynthesis

Recently, Rv2188c and its homologs in C. glutamicum (Lea-Smith et al., 2008; Mishra et al., 2008b) and M. smegmatis (Guerin et al., 2009) were identified as PimB'. This identification augmented the confusion in the field, as another gene, Rv0557, had already been assigned the function of PimB as an α-d-mannose-α(1 → 6)-phosphatidyl-myoo-inositol-mannopyranosyltransferase (Schaeffer et al., 1999). This study was based on the utilization of cell-free assays using GDP-[14C]-Manp, Ac1PIM6, M. smegmatis membranes and/or partially purified recombinant Rv0557 (Schaeffer et al., 1999). Furthermore, the disruption of Rv0557 in M. tuberculosis did not affect the biosynthesis of Ac1PIM2 (Torrelles et al., 2009), suggesting either gene duplication or that Rv0557 performed another function in M. tuberculosis. Recently, Rv0557 was shown to be involved in the biosynthesis of ManGlcAGroAc2 and a Cg-LM-B (also see Structure of PIMs) in C. glutamicum, and has been suggested to have an α-mannosyl-glucopyranosyluronic acid transferase, MgtA, activity (Tatituri et al., 2007a).

To solve this puzzle, involving PimB, PimB' and MgtA, and to assign the correct function to each ORF, a double mutant deficient in orthologs of Rv0557 and Rv2188c was generated in C. glutamicum, and subsequently, Rv0557 and Rv2188c were overexpressed in the double mutant. Consequently, the in vivo complementation of α-d-mannose-
β(1 → 6)-phosphatidyl-myo-inositol-mannopyranosyltransferase activity was restored using plasmid-borne copies of Rv2188c resulting in the synthesis of Ac₆PIM, and the related lipoglycan in the C. glutamicum double mutant, while overexpression of Rv0557 resulted in the synthesis of ManGlcAGroAc₆, suggesting that Rv0557 has a β-mannosyl-glucopyranosyluronic acid transferase activity, and therefore, Rv2188c was suggested to be Mt-PimB, while Rv0557 was renamed as Mt-MgtA (Mishra et al., 2009). For consistency with the recent literature, we retain the designation PimB’ for Rv2188c (and its orthologs in M. smegmatis and C. glutamicum) (Lea-Smith et al., 2008; Mishra et al., 2008b, 2009; Guerin et al., 2009). The crystal structure of C. glutamicum PimB’ in complex with GDP vs. GDP-Manp shows the selectivity of PimB’ for 6-OH of the inositol moiety of PI (Batt et al., 2010). Rv0557 possesses relaxed substrate specificity towards Ac₆PIM₁ (Schaeffer et al., 1999; Mishra et al., 2009) and its deletion from M. tuberculosis resulted in a viable mutant with a subtle decrease in the lipomannan and lipoparabinomannan contents (Torrellas et al., 2009), indicating a superficial role of Rv0557 in the biosynthesis of PIMs, lipomannan and lipoparabinomannan. In contrast, Rv2188c is essential in M. smegmatis (Guerin et al., 2009), illustrating an example of a high-duplication event that lead to extensive functional redundancy in mycobacteria (Cole et al., 1998; Tekai et al., 1999).

More recently, the role of glycolipid hydrolases has been suggested to be regulated by glycolipid flux inside and outside the cell membrane and it was suggested that these glycolipid hydrolases work in close coordination with glycosyltransferases (Crespo et al., 2010). The presence of GDP-Manp hydrolases in the vicinity of pimA and pimB, suggests the metabolic role of these glycolipid hydrolases in the regulation of the sugar donors and glycolipids, such as GDP-Manp, PPM and PIMs. In addition, the presence of putative transporters Rv2190c in M. tuberculosis and NCgl2107 and NCgl2108 in C. glutamicum in the vicinity of pimB’ region suggests their role in PIM or PPM transport in Corynebacteriaceae. However, the deletion of the homolog of Rv2190c in C. glutamicum resulted in a viable mutant with no phenotype, suggesting gene redundancy, which is not surprising as these putative transporters are present in multicopies elsewhere in the genome (L. Eggeling & G.S. Besra, unpublished data). Future studies targeting the role of these glycosyl hydrolases and transporters may shed further light on the regulation of PIMs, lipomannan and lipoparabinomannan in mycobacteria.

**Synthesis of higher-order PIMs**

Bioinformatical analysis of the genome of M. tuberculosis CDC1551 has led to the identification of RvD2-ORF1 from M. tuberculosis CDC1551 as an Ac₆PIM₂:β-6-mannose-β(1 → 6)-phosphatidyl-myo-inositol-mannopyranosyltransferase, PimC, involved in the addition of Manp from GDP-Manp to the 6-OH of mannose at the nonreducing end of Ac₆/Ac₆PIM₂ (Kremer et al., 2002). The use of a cell-free assay containing GDP-Manp, amphomycin (an antibiotic that inhibits the synthesis of PPMs by inhibiting the PPM synthase) and membranes from M. smegmatis overexpressing PimC led to the synthesis of Ac₆/Ac₆PIM₃. However, the inactivation of pimC in M. bovis BCG did not affect the production of higher PIMs, lipomannan and lipoparabinomannan, and the fact that genes orthologous to pimC were found in only 22% of clinical isolates suggests the existence of redundant gene(s) or an alternate pathway that may compensate for PimC deficiency (Kremer et al., 2002).

Ac₆/Ac₆PIM₃ is further β(1 → 6) mannosylated at the nonreducing termini by an unidentified β(1 → 6)-mannopyranosyltransferase [PimD] or PimC itself, resulting in the formation of Ac₆/Ac₆PIM₄. This step in the biosynthesis of higher PIMs, lipomannan and lipoparabinomannan, has been suggested as a key branch point towards the synthesis of Ac₆/ Ac₆PIM₅o, lipomannan and lipoparabinomannan (Morita et al., 2004; Morita et al., 2006; Mishra et al., 2008a). It has been proposed that a transition occurs from glycosyltransferases, utilizing nucleotide-derived sugar substrates, characterized by the GT-A/B superfamily, to the glycosyltransferases utilizing polyprenyl-phosphate sugars, the GT-C superfamily (Liu & Mushegian, 2003), for the elongation and branching of lipomannan and lipoparabinomannan (Morita et al., 2006). Rv1159 [PimE] has been identified as an β(1 → 2)-mannopyranosyltransferase that utilizes PPM as a substrate and adds an β(1 → 2)-Manp to Ac₆/Ac₆PIM₃, resulting in the synthesis of Ac₆/Ac₆PIM₄ (Fig. 4) (Morita et al., 2006). However, it is not clear whether PimE is solely responsible for the synthesis of both Ac₆/Ac₆PIM₂ and Ac₆/ Ac₆PIM₄. So far, most of the putative glycosyltransferases belonging to the GT-C family (Liu & Mushegian, 2003) in M. tuberculosis have been functionally characterized. That leaves us with fewer possibilities, in which either PimE or one of the other uncharacterized GT-Cs (Rv0051 and Rv0541c) (Liu & Mushegian, 2003; Berg et al., 2007) adds the second Manp residue onto Ac₆/Ac₆PIM₃. However, an Rv0051 deletion mutant showed no phenotypic change in the cell wall glycolipid of M. tuberculosis (A.K. Mishra & G.S. Besra, unpublished data), leaving Rv0541c as a promising candidate.

Morita et al. (2005) suggested that enzymes involved in the biosynthesis of early PIM intermediates (PIM₁ and Ac₆PIM₁) are localized to a membrane subdomain termed PM₁ in the plasma membrane, while the majority of Ac₆/ Ac₆PIM₄ (and biosynthetic enzymes) involved in higher-order PIM (Ac₆/Ac₆PIM₄ and Ac₆/Ac₆PIM₅) biosynthesis are localized to a denser fraction that contains both plasma
membrane and cell wall markers (PM-CW) (Morita et al., 2005). On the basis of various cell-free assays, they concluded that higher PIM biosynthesis occurs in the plasma membrane rather than the PM-CW fraction, followed by their subsequent transport to the cell wall (Morita et al., 2005). The relative amount of higher PIMs and lipoglycans was suggested to be regulated by a recently identified lipoprotein [LpqW] in M. smegmatis (Kovacevic et al., 2006; Marland et al., 2006). However, the exact mechanism of PIM flux and its segregation for Ac1/ Ac2PIM6 or lipomannan biosynthesis is unknown. Furthermore, Ac1/ Ac2PIM4 was suggested to be a key regulatory product involved in the biosynthesis of Ac1/ Ac2PIM6 and/or lipomannan biosynthesis (Morita et al., 2004, 2006). PimE directs Ac1/ Ac2PIM4 towards Ac1/ Ac2PIM6 synthesis, while LpqW channels Ac1/ Ac2PIM4 for lipomannan synthesis (Crelin et al., 2008). It is speculated that Ac1/ Ac2PIM4 is transported by a flippase or a sugar transporter across the plasma membrane, where subsequent mannosylation occurs by distinct mannopyranosyltransferases belonging to the GT-C family (Liu & Mushegian, 2003; Mishra et al., 2008a).

Recently, the role of a putative acyl transferase, Rv1565c, was suggested in the acylation of higher-order PIMs, lipomannan and lipoarabinomannan. An Rv1565c deletion mutant in Mycobacterium marinum showed a reduced incorporation of 1,2-[14C]-acetate into the PIMs, lipomannan and lipoarabinomannan as compared with the wild type. Furthermore, lipoarabinomannan from the mutant lacks mannose caps and showed a higher degree of branching of both the arabinan domain and the mannan core, suggesting some important and unidentified role of Rv1565c in mycobacteria (Driessen et al., 2010).

**Overview of lipomannan and lipoarabinomannan biosynthesis**

**Synthesis of the mannan core**

Using mutant constructs in C. glutamicum, and cell-free assays, two 1(1 → 6)-mannopyranosyltransferase activities were reported from C. glutamicum, of which one enzyme (M. tuberculosis homolog, Rv2174) was characterized as MptA and shown to be involved in the synthesis of the distal end of the 1(1 → 6) mannan backbone of lipomannan (Kaur et al., 2007; Mishra et al., 2007), while a second 1(1 → 6)-mannopyranosyltransferase, Rv1459c (MptB), was shown to be involved in the synthesis of the proximal end of the mannan backbone and speculated to extend an Ac1/ Ac2PIM4 acceptor (Fig. 5) (Mishra et al., 2008a). The deletion of the MptB ortholog in C. glutamicum resulted in the absence of lipomannan and lipoarabinomannan and a reduction in 1(1 → 6)-mannopyranosyltransferase activity. Furthermore, cell-free assays involving C50-P-Man and heterologously expressed Rv1459c and/or its M. smegmatis homolog MSMEG_3120 in C. glutamicum showed that these enzymes possessed 1(1 → 6)-mannopyranosyltransferase activity.

![Fig. 5. Biogenesis of Man-LAM from Mycobacterium tuberculosis.](image)

**Cytoplasmic side**

Ac1PIM4 is a plausible acceptor and extended by MptB in the 1(1 → 6) direction, followed by MptA and further decorated by singular 1(1 → 2)-Manp units by Rv2181 (MptC), resulting in lipomannan. Mature lipomannan is subsequently primed by a singular 1-3-Manp at an unknown position, which is extended by EmbC and/or unidentified 1(1 → 5) arabinofuranosyltransferases. The linear 1(1 → 5)-o-Araf chain is further primed by AftC, which is subsequently extended by AftD and unknown arabinofuranosyltransferases and terminated by the action of AftB to form linear Ara-4 or branched Ara-6. The penultimate Araf of the arabinan domain is further capped by Manp residues by CapA and Rv2181 (MptC) to form Man-LAM. For simplicity, only triacylated versions of different lipoglycans are shown.
activity. On this basis, it was suggested that after the transport of Ac \(_1\)-PIM \(_4\) outside the plasma membrane by an unidentified flippase, Mt-MptB catalyzes the addition of further 12–15 Manp units (Mishra et al., 2008a). MptB is part of an operon that consists of four ORFs encoding ATP-binding cassette (ABC) transporters (Wang et al., 2006). This enhances a strong possibility for a functional coupling of the glycosyltransferase MptB with ABC transporters, Rv1458c, Rv1457c and Rv1456c. However, a \(C.\ glutamicum\) mutant deficient in these ABC transporters showed no difference in their PIM, lipomannan and lipoarabinomannan profiles (L. Eggeling & G.S. Besra, unpublished data), suggestive of gene redundancy, which is not surprising if these ABC transporters are found at multiple locations in the genome of \(Corynebacterineae\).

Interestingly, \(\alpha(1 \rightarrow 6)\) mannan extension is more complex in mycobacteria, based on the evidence that Mt-MptB and Ms-MptB fail to complement the \(C.\ glutamicum\) mutant, suggesting a slightly different substrate specificity of the MptB orthologs of \(M.\ tuberculosis\) and \(M.\ smegmatis\) as compared with Cg-MptB (Mishra et al., 2008a). Furthermore, the redundancy of Ms-MptB in \(M.\ smegmatis\) indicates that either another as yet unidentified mannonpyranosyltransferase is substituting for MptB in the mutant or the distal \(\alpha(1 \rightarrow 6)\)-mannoparanosyltransferase, MptA, is substituting for the deficiency of Ms-MptB. A mycobacterial strain devoid of MptA and MptB may shed further light on this aspect.

In order to identify the \(\alpha(1 \rightarrow 6)\)-mannopyranosyltransferase involved in the synthesis of the distal \(\alpha(1 \rightarrow 6)\) mannan backbone, the homologs of putative glycosyltransferase, Rv2174, were deleted from \(C.\ glutamicum\) (Mishra et al., 2007) and \(M.\ smegmatis\) (Kaur et al., 2007). The cell wall phenotype of the mutants suggested the accumulation of a truncated lipoglycan (t-LM), deficient in \(\alpha(1 \rightarrow 6)\)-Manp units. A cell-free assay involving Cg-P-Man\(_\beta\) and a synthetic disaccharide acceptor, Man-\(\alpha(1 \rightarrow 6)\)-Man-C\(_\alpha\), established that the mutant lacked \(\alpha(1 \rightarrow 6)\)-mannopyranosyltransferase activity and was termed MptA (Mishra et al., 2007). Pfam analysis (Bateman et al., 2004) of the ORF upstream of \(mptA\) revealed that Rv2173 (putative geranylgeranyl pyrophosphate synthetase, \(idsA2\)) bears structural similarities to polyisoprenyl synthetases, which could be functionally related to MptA, and both genes may form a transcriptional unit. Interestingly, mycobacterial MptA contains 13 transmembrane helices (TMH), of which TMH 3 and 4 are conserved and contain the catalytic DXD motif typified by glycosyltransferases (Liu & Mushegian, 2003), while the C-terminus extracellular loop is nonexistent, unlike other GT-C glycosyltransferases (Zhang et al., 2003; Alderwick et al., 2011), suggesting the existence of a different model for chain extension as reported already in the case of \(M.\ tuberculosis\) EmbC (Shi et al., 2006). Furthermore, the recent genome analysis of \(Actinobacterium\) and \(Micrococcus\ luteus\) identified the presence of homologs of MptA and MptB in a cluster with another gene encoding for a GT-C glycosyltransferase, which are cotranscribed and probably translationally coupled (Young et al., 2010). In contrast, MptA and MptB are dispersed in corynebacteria and mycobacteria.

The \(\alpha(1 \rightarrow 6)\) mannan core in lipomannan and lipoarabinomannan is further decorated by single \(\alpha(1 \rightarrow 2)\)-Manp branches (Hunter & Brennan, 1990; Chatterjee et al., 1992a). On the basis of known polyisoprenyl-dependent glycosyltransferases, Rv2181 (MptC), was identified from an 18-kb conserved region and suggested to be involved in the synthesis of \(\alpha(1 \rightarrow 2)\)-Manp side chains of lipomannan (Fig. 5) (Kaur et al., 2006; Kaur et al., 2008; Sena et al., 2010; Mishra et al., 2011). More recently, it was suggested that MptA and MptC may act in close coordination to synthesize mature lipomannan and lipoarabinomannan, and the length of the mannan core may be regulated by a branching-dependent chain termination mechanism (Sena et al., 2010).

Arabinan domain assembly of lipoarabinomannan

\(Ac_1/Ac_2\)-PIM \(_4\) is extended by MptB, MptA and MptC to yield a mature lipomannan that probably serves as an acceptor for an uncharacterized arabinofuranosyltransferase to initiate lipoarabinomannan synthesis (Besra et al., 1997). However, the number of arabinofuranosyltransferases required for arabinan domain biosynthesis will depend on the types of arabinan linkage present in mycobacterial lipoarabinomannan (Fig. 5). It is quite likely that mature lipomannan is primed by a few Araf units in a similar fashion as AftA primes the galactan of arabinogalactan in mycobacteria (Alderwick et al., 2006). However, the enzyme responsible for this activity is not known. The primed Araf-LM is then further extended by EmbC (Rv3793) (Zhang et al., 2003; Shi et al., 2006; Alderwick et al., 2011) for 12–16 \(\alpha(1 \rightarrow 5)\)-Araf residues (Birch et al., 2010). Recently, AftC (Rv2673) was shown to introduce the \(\alpha(1 \rightarrow 3)\)-Araf branch points in both arabinogalactan (Birch et al., 2008) and lipoarabinomannan (Birch et al., 2010). This \(\alpha(1 \rightarrow 3)\)-Araf branched product, \([Araf]_{12-16}\)-LM, is then further extended by an unidentified \(\alpha(1 \rightarrow 5)\)-arabinofuranosyltransferase.

More recently, Skovierova et al. (2009) proposed a second branching \(\alpha(1 \rightarrow 3)\)-arabinofuranosyltransferase, AftD (Rv0236c). However, unlike the role of AftC as an \(\alpha(1 \rightarrow 3)\)-arabinofuranosyltransferase, which was experimentally validated by creating a knockout in \(M.\ smegmatis\) defective in \(\alpha(1 \rightarrow 3)\)-arabinofuranosyltransferase activity (Birch et al., 2008), the role of AftD is debatable as Skovierova et al. (2009) were unable to create a viable mutant displaying a clear phenotype, and the study was
solely based on the usage of artificial chemically defined acceptors using crude *M. smegmatis* and *C. glutamicum* membranes (Skovierova et al., 2009). It is also interesting to note that the same authors also discussed the possibility of AfTD as an α(1 → 5)-arabinofuranosyltransferase involved in α(1 → 5)-Araf extension of the nonreducing termini of the arabinian domain of lipoarabinomannan and arabino-galactan (Skovierova et al., 2009) (Fig. 5).

The final enzyme involved in arabian domain biosynthesis is AfTB (Rv3805c), which results in a terminal tetra- and hexa-arabinofuranoside structure (Figs 2 and 5). The role of AfTB has been experimentally shown to be a β(1 → 2)-arabinofuranosyltransferase in the synthesis of arabinogalactan (Seidel et al., 2007). However, its role in the synthesis of similar Ara residues in lipoarabinomannan is highly likely after the discovery of a dual role of AftC in arabino-galactan (Birch et al., 2008) and lipoarabinomannan (Birch et al., 2010) biosynthesis.

**Mannan priming and Man-LAM synthesis**

All pathogenic species of the genus *Mycobacterium* are known to possess Man-LAM, which is responsible for some of the immunomodulatory properties of these strains (Briken et al., 2004). A close inspection of the *M. tuberculosis* genome in comparison with *M. smegmatis* and some of these genes are essential for the survival of lipoarabinomannan without mannose caps provided the first indication of the role of Rv1635c in Man-LAM biosynthesis. On this basis, the homolog of Rv1635c in *M. tuberculosis* CDC1551 was identified as a glycosyltransferase that could be involved in Man-LAM capping (Dinadayala et al., 2006). Simultaneously, mutants of Rv1635c homologs in *M. marinum* and *M. bovis* BCG showed that the gene encoded for an α(1 → 5)-mannopyranosyltransferase, CapA, was involved in the addition of the first Manp residue on the nonreducing arabinian termini of lipoarabino-mannan (Appelmelk et al., 2008). More recently, it was also shown that MptC (Rv2181), which adds α(1 → 2)-Manp residues onto the α(1 → 6) mannann backbone of lipomannan and lipoarabinomannan, also adds α(1 → 2)-Manp caps at the nonreducing end of lipoarabinomannan in combination with CapA (Kaur et al., 2008) (Fig. 5). Our recent studies with an *M. bovis* BCG mutant defective in *pimE* also suggested its tentative role in α(1 → 2)-Manp capping of Man-LAM (G.S. Besra & B.J. Appelmelk, unpublished data). However, more studies are needed to establish the exact interplay of these mannopyranosyltransferases involved in the mannan caps of Man-LAM.

Almost the entire repertoire of enzymes and genes involved in the biogenesis of lipoarabinomannan and related glycoconjugates has been identified (Table 1), and some of these genes are essential for the survival of

| ORF      | Function                  | Role                                      | References                  |
|----------|---------------------------|-------------------------------------------|-----------------------------|
| PgsA (Rv2612c) | Peptidylglycan synthase | Synthesis of Phosphatidylinositol | Jackson et al. (2000) |
| PimA (Rv2610c) | α(1 → 2)-Mannopyranosyltransferase | Synthesis of PIM1 | Kordulakova et al. (2002) |
| Rv2611c | Acyltransferase | Synthesis of Ac1/AC2-PIM1 | Kordulakova et al. (2003) |
| PimB<sup>a</sup> (Rv2188c) | α(1 → 6)-Mannopyranosyltransferase | Synthesis of Ac1/AC2-PIM2 | Lea-Smith et al. (2008); Mishra et al. (2008b, 2009) |
| MgtA/PimB (Rv0557) | α(1 → 6)-Mannopyranosyltransferase | Synthesis of ManGlcGroAc2 | Tatituri et al. (2007a, b); Mishra et al. (2008b) |
| PimC (Rv02-ORF1) | α(1 → 6)-Mannopyranosyltransferase | Synthesis of Ac1/AC2-PIM3 | Kremer et al. (2002) |
| PimE (Rv1159) | α(1 → 2)-Mannopyranosyltransferase | Synthesis of Ac1/AC2-PIM5 | Morita et al. (2006) |
| MptB (Rv1459c) | α(1 → 6)-Mannopyranosyltransferase | Synthesis of proximal mannan backbone i.e. AC1/AC2-PIM<sub>12-17</sub> | Mishra et al. (2008a) |
| MptA (Rv2174) | α(1 → 6)-Mannopyranosyltransferase | Synthesis of distal mannan backbone i.e. AC1/AC2-PIM<sub>22-25</sub> | Mishra et al. (2007) |
| MptC (Rv2181) | α(1 → 2)-Mannopyranosyltransferase | Adds α(1 → 2)-Manp units on the mannan backbone, and also adds a second mannose cap on ManLAM | Kaur et al. (2008, 2010); Mishra et al. (2011) |
| EmbC (Rv3793) | α(1 → 5)-Arabinofuranosyltransferase | Involved in the synthesis of the α(1 → 5)-arabinan backbone | Zhang et al. (2003); Alderwick et al. (2011) |
| AftC (Rv2673) | α(1 → 3)-Arabinofuranosyltransferase | Adds Araf on α(1 → 5)-arabinan backbone | Birch et al. (2010) |
| AftD (Rv0236c) | α(1 → 3) or α(1 → 5)-Arabinofuranosyltransferase | Either adds α(1 → 3)-Araf units to the non-reducing end of the α(1 → 5)-arabinan branch or synthesizes α(1 → 5) itself | Skovierova et al. (2009) |
| CapA (Rv1635c) | α(1 → 5)-Mannopyranosyltransferase | Adds first mannose cap on ManLAM | Appelmelk et al. (2008) |
M. tuberculosis, therefore representing excellent drug targets. However, the roles of lipoarabinomannan and related glycoconjugates in mycobacterial pathogenicity require the availability of mycobacterial mutants defective in their respective biosynthetic pathways, as most of the studies have been carried out using purified molecules that do not represent the true in vivo condition during infection. The availability of complete genome sequences of several mycobacteria and related actinomycetes and the development of novel tools for genetic manipulation have enhanced these possibilities.

Part II – Interactions with host immune system

Accessibility of lipoglycans to the immune system: localization and trafficking

Lipoarabinomannan and related lipoglycans are not only essential for mycobacterial growth and cell viability (Haites et al., 2005; Kovacevic et al., 2006), but are also thought to be important in the interactions between the mycobacteria and their host. The nature of these host–pathogen interactions is determined by the accessibility of the lipoglycans to the immune system, i.e. can cell wall-bound lipoglycans be recognized by the pattern-recognition receptors (PRRs) of the immune system and how do the lipoglycans traffic, once released from the mycobacterial cell wall?

The localization of PIMs and lipoarabinomannan in the mycobacterial cell envelope has been assessed in multiple ways, including biotin tagging of lipoarabinomannan and extraction of lipids from the cell wall with detergents or by mechanical treatment with glass beads. Because of the strong conditions needed to extract lipoarabinomannan from the cell wall, and the possibility to detect lipoarabinomannan with lipoarabinomannan-recognizing antibodies on whole cells, it was hypothesized that lipoarabinomannan is firmly attached via its MPI anchor to the surface of the cell (Chatterjee & Khoo, 1998). Biotin labeling, assumed to be restricted to the cell surface, showed two fractions of lipoarabinomannan: one anchored to the cytosolic membrane and one in the mycobacterial outer membrane (mycomembrane) (Hoffmann et al., 2008; Pitarque et al., 2008). However, biotin is only a small molecule and may have easier access to lipoarabinomannan more buried in the cell wall as compared with the large PRRs, which may reduce the potential of lipoarabinomannan to be recognized by the immune system. Furthermore, native mycobacterial cells are surrounded by a capsule, which could cover lipoarabinomannan. The mycobacterial capsule mainly consists of polysaccharides and proteins (Daffé & Etienne, 1999). Electron microscopy (EM) with immunogold-labeled cells using ConA and anti-arabinan antibodies, combined with nuclear magnetic resonance studies, showed the presence of mannose-capped arabinomannan (Man-AM; i.e. without the lipid anchor present in Man-LAM) in the capsule (Ortalo-Magné et al., 1995). The capsule has been reported to have only a very low lipid content, among which are small amounts of PIMs and virtually no lipoarabinomannan (Ortalo-Magné et al., 1996a, b). A recent study used immunogold–EM with monoclonal antibodies against PIM₆ (F183-24), and against the mannose cap (55.92.1A1) and the arabinan domain (F30-5) of Man-AM and Man-LAM to detect surface localization of these lipoglycans. Unperturbed mycobacterial cells bearing an intact capsule display good labeling with these antibodies (Sani et al., 2010), which confirms the presence of PIM₆ and Man-AM in the capsule. In contrast, mycobacteria without a surrounding capsule due to growth under perturbing conditions (in the presence of 0.05% Tween-80 and mechanical agitation) hardly become labeled with these antibodies. As lipoarabinomannan is localized in the cell wall and not in the capsule, this suggests limited surface exposure of lipoarabinomannan and related glycans buried in the mycomembrane, even if the capsule is not covering the cell wall. However, the amount of lipoarabinomannan or its accessibility in these cells grown under perturbing conditions has not been assessed further.

Although culture filtrate has been reported to contain only trace amounts of lipids (Lemassu & Daffé, 1994), studies with infected macrophages (Mφ) showed intracellular trafficking of PIMs and lipoarabinomannan, suggesting that these glycolipids are substantially released from mycobacteria. Even release into noninfected bystander cells (Xu et al., 1994; Beatty et al., 2000; Rhoades et al., 2003) and subsequent presentation through CD1 glycoproteins (Schaible et al., 2000) has been observed. Furthermore, isolated PIMs and lipoarabinomannan can be incorporated into the endomembranes and plasma membranes of different cell types, a process requiring the MPI anchor and the mannan core (Ilanguamaran et al., 1995; Shabaana et al., 2005; Welin et al., 2008). It can be hypothesized that the lipoglycans released are able to modulate the immune response, for example by interfering with phagosome maturation.

Phagosome maturation arrest

At least two strategies used by M. tuberculosis to survive in Mφ have been described. One is delay of the phagosome maturation, i.e. prevention of fusion of the phagosome with late endosomal and lysosomal organelles, which normally leads to killing and digestion of a pathogen in an acid environment (Armstrong & Hart, 1971; Russell, 2001; Nguyen & Pieters, 2005). The other strategy is based on escape from the phagosome to the cytosol (van der Wel et al., 2007). In the phagosome maturation arrest, a role for
both Man-LAM and PIMs has been implicated (Vergne et al., 2003b; Welin et al., 2008).

The phagosome–lysosome fusion process starts after cytosolic Ca\(^{2+}\) increase. The Ca\(^{2+}\)/calmodulin-dependent PI3-kinase hVPS34 and its modulatory subunit p150 will then generate the membrane-trafficking lipid phosphatidylinositol 3-phosphate (PI3P) on the phagosomal membrane (Vergne et al., 2004a). Both PI3P and early endocytic small GTPase Rab5 mediate in the subsequent recruitment of membrane tethering protein early endosome autoantigen 1 (EEA1) to the phagosome (Vergne et al., 2003a) (Fig. 6). EEA1 plays an essential role in phagosome maturation by interacting directly with syntaxin-6, a soluble NSF attachment protein receptor (SNARE) protein involved in the delivery of cathepsins (lysosomal hydrolases) and V\(_{h}H^+\)-ATPase from the trans-Golgi network to the phagosome (Simonsen et al., 1999). The normal cytosolic Ca\(^{2+}\) increase upon an infection is absent during mycobacterial uptake. This has been hypothesized to lead to a reduced activity of the PI3-kinase hVPS34 and an altered production of PI3P in the case of phagocytosis of mycobacteria (Malik et al., 2000), but the inhibition of phagosome maturation by Man-LAM appears to involve binding to the MMR instead (Kang et al., 2005). Furthermore, a role for macrophage phosphatase SHP-1 has been suggested, which is activated by Man-LAM and impairs Ca\(^{2+}\) signaling (Ono et al., 1997; Knutson et al., 1998; Vergne et al., 2004a).

Another possibility of interference by Man-LAM in phagosome maturation, distinct from blocking the rise in \([\text{Ca}\(^{2+}\)]_{\text{cyt}}\), considers a role for the activation of p38 mitogen-activated protein kinase (p38 MAPK). p38 MAPK activity may indirectly maintain Rab5 in an inactive GDP-bound form (Cavalli et al., 2001; Vergne et al., 2004a). This is consistent with the report that the induction of p38 MAPK reduces the recruitment of Rab5-effector protein EEA1 to the early endosome (Fratti et al., 2003a). Moreover, the level of p38 MAPK activation is significantly increased upon infection with \textit{M. bovis} BCG (Fratti et al., 2003a) and Man-LAM was hypothesized to be a triggering component (Vergne et al., 2004a). However, recently, it has been shown experimentally that p38 MAPK activation is neither induced nor influenced by isolated Man-LAM, and thus must be linked to other mycobacterial components (Welin et al., 2008).

Noteworthy, lipoarabinomannan is incorporated into membrane lipid rafts, a process that is also required for the phagosome maturation arrest (Welin et al., 2008). Lipid rafts are highly dynamic lipid domains, enriched in cholesterol and glycosphingolipids, and that have been associated...
with cell signaling (Simons & Toomre, 2000; Pike, 2009). It has been suggested that the presence of lipoarabinomannan in the endomembrane causes drastic reorganization of the lipid domains and thereby fusion of the lipid vesicles (Hayakawa et al., 2007). However, PI-LAM from avirulent M. smegmatis is also incorporated into endomembranes, although to a lesser extent, but it cannot prevent phagosome–lysosome fusion nor inhibit cytosolic Ca²⁺ increase (Vergne et al., 2003b; Kang et al., 2005; Welin et al., 2008).

Given that the MMR only recognizes the mannose-capped Man-LAM and not Ara-LAM or PI-LAM (Schlesinger et al., 1994), this is further evidence that ligation of the Man-LAM to the MMR is required for the phagosome maturation block, which appears to be restricted to the more virulent Mycobacterium spp. A role for the MMR has been confirmed recently by showing that glycopeptidolipids from M. avium delay phagosome–lysosome fusion by interaction with the MMR and by an MMR siRNA knockdown in human monocyte-derived Mφ, resulting in increased phagosome–lysosome fusion upon M. avium infection (Sweet et al., 2009).

As mammalian phosphoinositide P13P plays an important role in phagosome maturation, next to lipoarabinomannan, other mycobacterial P1-analogs, PIMs and lipomannan, were investigated as well for their possible interference in phagosome maturation. While for lipomannan no role in phagosome maturation arrest could be detected (Kang et al., 2005), PIMs do have an effect, although in a way distinct from Man-LAM (Vergne et al., 2004b). Similar to lipoarabinomannan, PIMs can be incorporated into lipid rafts and, moreover, the addition of PIMs competitively inhibits lipoarabinomannan insertion (Ilanguamaran et al., 1995; Welin et al., 2008). Although PIMs seem to reverse the effect of lipoarabinomannan of preventing endosomal fusions (Welin et al., 2008), however, indications exist of a different role of PIMs in the phagosome maturation arrest. PIMs do prevent phagosome acidification, but not by reducing the recruitment of syntaxin-6 (Fratti et al., 2003b; Vergne et al., 2004b). Instead, PIMs induce the acquisition of endosomal SNARE protein syntaxin-4 and the transferrin receptor (Fratti et al., 2003b; Vergne et al., 2004a, b) (Fig. 6). Transferrin and its receptor are recycling endosomal markers involved in iron delivery (Clemens & Horwitz, 1996; Sturgill-Koszycki et al., 1996). While Man-LAM arrests phagosome maturation by blocking the recruitment of late endosomal and lysosomal markers, PIMs appear to stimulate fusion with early endosomes and thereby retrieve nutrients necessary for mycobacteria residing in the phagosomal compartments (Kelley & Schorey, 2003; Vergne et al., 2004b). This process is also Rab5-dependent (Gorvel et al., 1991), in particular when Rab5 activity is rate limiting, but whether PIMs affects Rab5 directly or indirectly is not yet known (Vergne et al., 2004b).

Interestingly, also in the effect that PIMs exert on the phagosome maturation, the MMR seems to play a role. While the lower-order PIMs (PIM₁) are not recognized by the MMR, the MMR has a high affinity for higher-order PIMs (PIM₃ and PIM₄) (Torrelles et al., 2006). This is consistent with the report that only in Mφ stimulated with higher-order PIMs was a significant increase in phagosome–lysosome fusion seen upon MMR blockade (Torrelles et al., 2006). Thus, although PIMs and Man-LAM influence the phagosome maturation by distinct mechanisms, both may involve recognition by the MMR. This indicates a balance between Man-LAM preventing maturation into the phagolysosome on the one hand and PIMs stimulating early endosomal fusion to retrieve nutrients on the other (Vergne et al., 2004b).

Inhibition of phagosome maturation by pathogenic Mycobacterium spp. may be a critical first step for their intracellular survival. Mycobacteria probably display several mechanisms to prevent lysosomal transfer, of which one is interference of Man-LAM in the phagosome maturation process. A M. marinum mutant that only produces lipoarabinomannan devoid of mannose caps showed a significant increase in colocalization with phagolysosomes in murine Mφ as compared with its parent strain. However, the absolute numbers remained low in this assay (7.4%, 12.0% and 5.0% for the wild-type, mutant and complemented strain, respectively), and importantly, no significant differences in bacterial survival were observed (Appelmelk et al., 2008). Other mechanisms of phagosome maturation blockade, independent of Man-LAM, have been reported, for example the secretion of SapM by M. tuberculosis, a lipid phosphatase that hydrolyzes the P13P on the endomembranes (Vergne et al., 2005), and the secretion of a eukaryotic-like serine/threonine protein kinase G (PknG) (Walburger et al., 2004).

Clusters of differentiation (CD)1

CD-1 glycoproteins have been identified as important antigen-presenting molecules of the immune system, next to major histocompatibility complex (MHC) class I and II molecules. While MHC class I and II present peptide antigens, CD1 molecules present glycolipids, thereby covering the presentation of a large variety of both self as well as microbial antigens (Young & Moody, 2006; de Libero & Mori, 2009). In mycobacterial infection, CD1 ensures the presentation of the glycolipids unique to the mycobacterial cell wall to activate CD1-restricted T cells and is thereby involved in shaping the immune response (Porcelli et al., 1998; Sieling et al., 1999; Barral & Brenner, 2007).

Human CD1 molecules are expressed by a variety of antigen-presenting cells (APC) and can be divided into three groups: CD1a, CD1b and CD1c together form group 1, and
CD1d and CD1e form group 2 and group 3, respectively. Murine homologs for group 1 CD1 molecules have not been identified, but mice do express CD1d. Group 1 CD1 molecules present lipids to a clonally diverse T-cell population in which the precursors have unique specificity for a single antigen (Barral & Brenner, 2007). The expression of group 1 CD1 on isolated human myeloid APC is hardly detectable, but it is upregulated to high levels within a couple of days after infection with *M. tuberculosis* or activation by mycobacterial lipids (Roura-Mir et al., 2005) (Felio et al., 2009). This demonstrates an apparent role of antigen presentation by the group 1 CD1 in the clonal expansion of T cells and, hence, the adaptive immune response against mycobacterial infection (Roura-Mir et al., 2005; Barral & Brenner, 2007). CD1d presents lipids to CD1d-restricted natural killers T (NKT) cells including the subset of clonally less diverse invariant NKT cells that display a rapid innate-like response (Barral & Brenner, 2007). In contrast to group 1 CD1, CD1d molecules are constitutively expressed and are reported to be downregulated during mycobacterial infection, confirming their association with the innate immune response (Roura-Mir et al., 2005; Moody, 2006). CD1e is only restricted to myeloid dendritic cells (DCs) and is not expressed at the cell surface and thus does not present antigens to TCRs. In this review, we focus on CD1b and CD1d, because these CD1 molecules bind and present PIMs and related lipoglycans. Group 2 CD1d has been reported to only bind lower-order PIMs, PIM₂ and PIM₆, but not lipomannan or Man-LAM (Fischer et al., 2004; Zajonc et al., 2006). In contrast, group 1 CD1b binds several mycobacterial lipid including PIM₂ and Man-LAM (Sieling et al., 1995; Prigozy et al., 1997; Ernst et al., 1998).

The structure of CD1 molecules has similarities to the MHC class I molecules, but shows some important differences in its binding groove, which is deeper and facilitates the binding of two acyl chains as present in the MPI anchor of PIMs and Man-LAM (Zeng et al., 1997; Porcelli et al., 1998; Fischer et al., 2004). While the lipid anchoring in the hydrophobic CD1 groove is relatively nonspecific, the TCR recognizes the hydrophilic carbohydrate head group of the antigens with high specificity (Moody et al., 1997). As compared with CD1b, additional interactions between the center of the binding groove of CD1d and the polar head group of the PIM are of additive importance for the formation of a stable glycolipid complex and subsequent T cell recognition (Zajonc et al., 2006). In the presentation of PIM₄ by CD1d, the two additional α(1→6)-linked Man₅ residues are probably oriented away from the binding groove (Zajonc et al., 2006). Considering the low abundance of PIM₄ in the mycobacterial cell wall in contrast to (diacylated) PIM₂ (Gilleron et al., 2001), presentation of PIM₄ by CD1d may not be of high biological significance. For group 1 CD1b, mycobacterial antigens with head groups much larger than present in PIM₂ have been described, which raises questions regarding how these large carbohydrates fit in the narrow space between the TCR and CD1 (Young & Moody, 2006). Higher-order PIM₆ needs processing into the smaller PIM₂ before being able to stimulate CD1b-restricted T cells. A role in this antigen processing has been implicated for CD1e, because the presence of CD1e is required for the activation of CD1b-restricted T cells by PIM₆, and not by PIM₂ (de la Salle et al., 2005). As mentioned above, CD1e does not present lipid antigens at the cell surface, but probably aids in endosomal/lysosomal α-mannosidase activity to produce PIM₂ by binding PIM₆ similar to the other antigen-presenting CD1 molecules (de la Salle et al., 2005). Secondly, CD1e may facilitate the loading of other CD1 molecules (de Libero & Mori, 2009). How Man-LAM is presented in the interaction between the TCR and the CD1b-Man-LAM complex has not yet been resolved. Man-LAM may be partly digested similar to PIM₆ (Ernst et al., 1998), which is most likely, as already PIM₆ with its short carbohydrate head group requires processing. Of note, CD1b and Man-LAM do colocalize in the cell (Prigozy et al., 1997) and CD1b is able to bind Man-LAM (Ernst et al., 1998). Two other options have been suggested by Young & Moody (2006). One possibility is flattening of the glycan part of Man-LAM between the TCR and CD1, so that only one or two carbohydrate units are positioned directly between the TCR and CD1. Multiple TCR-docking orientations may play a role in this as well. In the second option, Man-LAM is not presented by CD1b, but stimulates the process of CD1-dependent T cell activation indirectly via the mechanisms discussed below (Young & Moody, 2006).

CD1b is the predominant group 1 CD1 molecule present in the late endosomes/lysosomes and MHC class II compartments (Prigozy et al., 1997; Ernst et al., 1998; Schaible et al., 2000) and shares with MHC class II molecules the requirement for acidification in order to function (Benaroch et al., 1995; Sugita et al., 1999). PIMs and Man-LAM have been observed to be released in the phagosomes of infected cells and transported into the same intracellular compartments (Xu et al., 1994; Prigozy et al., 1997; Schaible et al., 2000). The low pH in these compartments causes conformational changes in the structure of CD1b including relaxation of certain parts of its binding groove to facilitate subsequent antigen loading (Ernst et al., 1998; Sugita et al., 1999; Kronenberg & Sullivan, 2008; Relloso et al., 2008; de Libero & Mori, 2009). As described above, PIMs and Man-LAM interfere in phagosome maturation and in particular Man-LAM has been shown to prevent endosomal acidification (Fratti et al., 2001). Hence, PIMs and Man-LAM likely impede their own presentation by CD1b. On the other hand, mycobacterial lipids have been shown to induce the transcription and expression of group 1 CD1 glycoproteins at the surface of the APC by signaling through TLR-2.
(Roura-Mir et al., 2005; Moody, 2006). Possible lipids involved were reported to be PIM2 and Ara-LAM extracted from the mycobacterial cell wall (Roura-Mir et al., 2005). However, PIM2 and Ara-LAM are poor TLR2 ligands as discussed in the next section (Nigou et al., 2008). Copurified lipopeptides, which are more potent inducers of TLR2 signaling, may also have induced CD1 expression in this assay (Nigou et al., 2008; Zahringer et al., 2008).

Mycobacteria are able to interfere with the immune response against mycobacterial infection including the modulation of peptide antigen presentation by MHC class I and II molecules (Kauffman & Schaible, 2005). Therefore, the lipid antigen presentation via four different CD1 glycoproteins forms an important alternative mechanism to induce an effective immune response (Sugita et al., 1999). Although the function and expression of CD1 molecules can be impaired by mycobacteria or mycobacterial components such as capsular z-glucan (Gagliardi et al., 2007, 2009; Balboa et al., 2010), the many distinct pathways for antigen sampling from various intracellular localizations and their subsequent presentation circumvent the immune evasion strategies exploited by mycobacteria (Sugita et al., 1999; Kauffman & Schaible, 2005; de Libero & Mori, 2009). Furthermore, both group 1 and group 2 CD1 presentation of lipid antigens seem to play a potential role in the protection against tuberculosis by vaccination with BCG (Watanabe et al., 2006; Venkataswamy et al., 2009).

**TLRs**

Three TLRs have been implicated to play a role in the mycobacterial infection: TLR2, TLR4 and TLR9 (Ozinsky et al., 2000; Quesniaux et al., 2004a; Jo, 2008). PIMs, lipomannan and lipoarabinomannan have all been examined for signaling via TLR2 and via TLR4, of which an overview is given here.

Lipopeptides are the major ligands for TLR2 (Brightbill et al., 1999), but MPI-anchored mannosylated lipoglycans can signal via TLR2 as well, depending on their degree of acylation and mannosylation (Gilleron et al., 2006; Doz et al., 2007; Nigou et al., 2008). TLR2 dimerizes with either TLR1 or TLR6 in order to function (Ozinsky et al., 2000). TLR1/TLR2 heterodimers mainly recognize triacylated lipopeptides, while the diacylated forms bind TLR2/TLR6 (Takeuchi et al., 2002; Akira & Takeda, 2004). Lipoglycan-induced signaling occurs via the TLR1/TLR2 complex (Elass et al., 2005; Gilleron et al., 2006; Nigou et al., 2008). A positive relation exists between the length of the mannann chain and the ability of the lipoglycan to activate TLR2 (Nigou et al., 2008). The lipoglycan bearing the largest accessible mannan chain (i.e. not substituted with an arabian domain) – lipomannan – showed to be a potent inducer of TLR2-signaling (Quesniaux et al., 2004b), although this activity is restricted to the tri- and tetraacylated forms (Ac1/Ac2LM) (Gilleron et al., 2006; Doz et al., 2007). Next to the induction of cytokines, Mø stimulated with lipomannan displayed increased production of matrix metalloproteinase (MMP)-9 (Elass et al., 2005). This was due to the downregulation of the transcription of the MMP-9 inhibitor, tissue inhibitor of metalloproteinases-1, and dependent on TLR2. This implies a role for lipomannan in tissue destruction by MMP-9 during mycobacterial infection via interaction with TLR2 (Elass et al., 2005). Furthermore, lipomannan induces granuloma macrophage fusion in an *in vitro* granuloma model in a TLR2-dependent way (Puisségur et al., 2007).

In the group of PIMs, both Ac1/Ac2PIM2 and Ac1/Ac2PIM6 have been reported to signal via TLR2, irrespective of their acylation pattern (Jones et al., 2001; Gilleron et al., 2003). Further, in two studies, cellular activation via TLR2 by non-mannose-capped lipoarabinomannan (PI-LAM/ Ara-LAM) from rapidly growing species has been observed (Means et al., 1999; Underhill et al., 1999), but not for *M. tuberculosis* or BCG-derived Man-LAM. In addition, an inflammatory response induced by PI-LAM from *M. smegmatis* in mice appeared to be TLR2 dependent (Wieland et al., 2004). However, in a comparative study of all lipoglycans, Ac1/Ac2PIM2, PI-LAM and Ara-LAM were shown to be poor inducers of TLR2 signaling as compared with lipomannan and Ac1/Ac2PIM6 (Nigou et al., 2008). This is consistent with an earlier study showing that in contrast to lipomannan, neither Ara-LAM from *M. chelonei* nor Man-LAM and Ac1/Ac2PIM2 from *Mycobacterium kansasii* mediate TLR2-dependent activation (Vignal et al., 2003). Moreover, chemical degradation of the arabinan domain of Man-LAM from *M. kansasii* restored its ability to induce cytokine secretion via TLR2, which suggests that the arabinan domain prevents the proper interaction of Man-LAM with TLR2 (Vignal et al., 2003). This was confirmed by a recent study by Birch et al. (2010) in which lipoarabinomannan containing a truncated arabinan domain from an *M. smegmatis* AftC knockout mutant showed enhanced TLR2 signaling as compared with wild-type lipoarabinomannan. The positive effects of lipoarabinomannan in the earlier reports could be due to contamination of the lipoarabinomannan extract with lipopeptides (Nigou et al., 2008; Zahringer et al., 2008; Geurtsen et al., 2009; Birch et al., 2010). Overall, the data indicate that lipomannan, and in a minor respect PIM6, are the only significant TLR2 ligands from this group of mycobacterial lipoglycans.

For TLR4, only a few mycobacterial lipoglycans have been reported as ligands. Using nonactivated Mø, only tri- and tetra-acylated lipomannan from *M. tuberculosis* and *M. bovis* BCG, respectively, show signaling via TLR4 (Gilleron et al., 2006; Doz et al., 2007). In contrast to TLR2, which can only signal by the recruitment of adaptor proteins, Myeloid...
A few studies report on an immunosuppressive effect of lipomannan, Ac1LM and PIMs on lipopolysaccharide-activated Mφ (Quesniaux et al., 2004b; Doz et al., 2007, 2009). These inhibitory effects were independent of their signaling via TLR2, which partially compensated the decrease of proinflammatory cytokine secretion (Quesniaux et al., 2004b; Doz et al., 2007). Which receptor-ligand ligation is then responsible for the immunosupresion is not yet known, but it is unlikely to be the MMR (Doz et al., 2007): in contrast to the higher-order PIMs, the MMR recognizes lipomannan and Ac1/ Ac2PIM2 only with low affinity (Torrelles et al., 2006), while they show similar inhibitory effects on lipopolysaccharide-activated Mφ (Doz et al., 2009). Importantly, neither lyso-PIM (monacylated PIM) nor PI were inhibitory in this assay, indicating the requirement of mannosyl moiety and a diacylated MPI anchor (Doz et al., 2009). Of note, in all these experiments, lipopolysaccharide was used, which is a major ligand for TLR4. PIMs might interfere in the interaction between lipopolysaccharide and TLR4, thereby causing its inhibitory effect, but this seems unlikely (Doz et al., 2009). However, lipopolysaccharide is a nonmycobacterial ligand, which makes this assay system rather artificial for the comprehension of TLR-dependent immunomodulation by PIMs and related lipoglycans during mycobacterial infection. Finally, the immunomodulatory properties of Man-LAM on both lipopolysaccharide-activated Mφ and DCs have been described as well (Geijtenbeek et al., 2003; Pathak et al., 2005). These effects of Man-LAM can be attributed to its binding by the MMR and DC-SIGN, respectively, which will be discussed in the next sections.

**In vivo** studies using TLR knockout mice are not fully in agreement on whether TLRs are crucial for mycobacterial clearance and host defense (Drennan et al., 2004; Ferwerda et al., 2005) or not (Sugawara et al., 2003a, b; Nicolle et al., 2004; Holscher et al., 2008). Importantly, TLR signaling via the MyD88 pathway has been shown in multiple studies to be dispensable for the induction of an efficient adaptive immune response (Fremond et al., 2004; Nicolle et al., 2004; Ryffel et al., 2005; Holscher et al., 2008). A critical role for TLRs then has been hypothesized in the early recognition and killing of mycobacteria (Quesniaux et al., 2004a; Ryffel et al., 2005; Jo, 2008). However, this innate immune response mainly depends on MyD88 and may also involve a MyD88-mediated pathway different from via TLR (Feng et al., 2003; Holscher et al., 2008), which is the type I interleukin–1 receptor (IL–1R1)-mediated signaling (Fremond et al., 2007; Reiling et al., 2008). Alternatively, TLR2 activation may have a second function as a regulator by preventing an exaggerated inflammatory immune response in a later stage of mycobacterial infection (Drennan et al., 2004; Suitmuller et al., 2006; Jo, 2008; Harding & Boom.

### Table 2. Overview of reports on the activation of TLR2 by LM

| LM type | Cells | Dependence shown for | Effect | References |
|---------|-------|----------------------|--------|-----------|
| Mc-LM, Mk-LM | THP-1 Mφ | TLR2+CD14 | Release TNF and IL-8 | Vignal et al. (2003) |
| Mc-LM, Mk-LM, BCG-LM | BMDM Mφ | TLR2 | Release TNF and NO | Quesniaux et al. (2004b) |
| Mc-LM, Mk-LM, BCG-LM | THP-1 Mφ | TLR2 | expression MMP-9 | Elas et al. (2005) |
| BCG-LM | THP-1, BMDM Mφ | TLR1/2 | Release TNF | Gilleron et al. (2006) |
| BCG-Ac1LM | THP-1, BMDM Mφ | TLR1/2, TLR4 | Release TNF | |
| Mc-LM, Mk-LM, Ms-LM, Mtb-LM | human PBMC | TLR2 | Mφ fusion into MGC in *in vitro* granuloma | Puissengur et al. (2007) |
| BCG-Ac1LM | BMDM Mφ | TLR2, TLR4 | Release TNF and NO | Doz et al. (2007) |
| BCG-Ac2LM | BMDM Mφ | TLR4 | Release TNF and NO | |
| Mtb-Ac1(2)LM | BMDM Mφ | TLR4 | Release TNF | |
| Mtb-LM, BCG-LM | HEK/CD14/TLR2, THP-1 Mφ | TLR1/2 + CD14 | Activation NF-κB | Nigou et al. (2008) |

Ms, *Mycobacterium smegmatis*; Mk, *Mycobacterium kansasi*; Mc, *Mycobacterium chelonae*; Mtb, *Mycobacterium tuberculosis*; BCG, *Mycobacterium bovis* BCG; Ac1/Ac2LM, tri-/tetra-acylated LM (note: in some articles written as Ac3/Ac4LM); BMDM, bone marrow-derived macrophages; PBMC, peripheral blood mononuclear cells; MGC, multinucleated giant cells.
2010). Pattern recognition by multiple TLRs does contribute to the control of mycobacterial infection (Bafica et al., 2005; Ryffel et al., 2005; Jo et al., 2007), but may play a less significant role than originally assigned (Reiling et al., 2008).

**DC-SIGN**

In contrast to the interaction between mycobacteria and Mφ, which can be mediated by several different receptors (e.g. complement receptors, CD14, MMR, scavenger receptor) (Ernst, 1998), binding of mycobacteria to DCs is for the largest part mediated by DC-SIGN (CD209) (Tailleux et al., 2003). DC-SIGN is a type II transmembrane tetrameric C-type lectin containing one carbohydrate recognition domain per monomer. It was originally hypothesized that via interaction with DC-SIGN, mycobacteria modulate the immune response and thereby escape immune surveillance (Geijtenbeek et al., 2003; van Kooyk & Geijtenbeek, 2003). However, the exact role of DC-SIGN in tuberculosis infection has not yet been clarified and is still under debate (Neyrolles et al., 2006; Ehlers, 2009; Tanne & Neyrolles, 2010). Studies on the relation between the level of expression of DC-SIGN in human populations and susceptibility to tuberculosis are contradicting each other, varying from a protective effect to increased susceptibility to tuberculosis by high DC-SIGN expression or no correlation at all (Barreiro et al., 2006; Gomez et al., 2006; Ben-Ali et al., 2007; Vannberg et al., 2008).

In a binding study using a DC-SIGN-expressing cell line, the lectin showed a high preference for the *Mycobacterium* spp. from the tuberculosis complex, while it bound little or not to strains from outside the complex (Pitarque et al., 2005). On the basis of differences in the mannose-capping degree of lipoarabinomannan between these species and the fact that DC-SIGN only recognizes lipoarabinomannan if it is mannose-capped, it was expected that Man-LAM would establish the interaction between mycobacteria and DC-SIGN as present on DCs (Geijtenbeek et al., 2003; Maeda et al., 2003). Furthermore, DC-SIGN has a high-affinity ω(1 → 2)-linked Manp residue, which increases with chain length (Koppel et al., 2004). The predominant mannose cap on Man-LAM of *M. tuberculosis* and *M. bovis* BCG consists of ω(1 → 2)-linked dimannosides, and ω(1 → 2)-linked trimannosides are also present (Pitarque et al., 2005). Surprisingly, an *M. bovis* BCG mutant, which only produces lipoarabinomannan without mannose cap, did not bind less to DC-SIGN and DCs as compared with wild-type BCG (Appelmelk et al., 2008); thus, ligands for DC-SIGN other than Man-LAM must be present in the cell wall.

Next to Man-LAM, PIMs have been examined for their binding to DC-SIGN as well (Torrelles et al., 2006; Driessen et al., 2009). In particular, the higher-order PIMs (PIM3 and PIM4), with terminal ω(1 → 2)-linked Manp residues reminiscent of the mannose cap of Man-LAM, were of interest.

| Mycobacterial ligands for DC-SIGN | Discussed in |
|----------------------------------|-------------|
| ManLAM                           | Geijtenbeek et al. (2003); Pitarque et al. (2005); Appelmelk et al. (2008) |
| ManAM                           | Pitarque et al. (2005) |
| LM (higher-order) PIMs         | Pitarque et al. (2005) |
| 19- and 45-kDa antigen*       | Pitarque et al. (2005) |
| Mannosylated proteins*         | Driessen et al. (2009) |
| Capsular α-glucan             | Geurtsen et al. (2009) |

*Mannosylated lipoprotein (19 kDa) and mannosylated glycoprotein (45 kDa).

1 Unidentified proteins from *Mycobacterium bovis* BCG whole-cell lysates probed SDS-PAGE/immunoblot with a DC-SIGN-Fc construct. Note: a ConA affinity capture assay identified > 30 putative mannosylated proteins in *Mycobacterium tuberculosis* culture filtrate (Gonzalez-Zamorano et al., 2009), which constitute potential ligands for DC-SIGN.

As expected, DC-SIGN recognizes the higher-order PIMs with high affinity as compared with the lower-order PIMs (Boonyarattanakalin et al., 2008; Driessen et al., 2009). Besides PIMs, other potential ligands in the mycobacterial cell wall for DC-SIGN have been identified: lipomannan, Man-AM and mannosylated lipoproteins 19 and 45 kDa (Pitarque et al., 2005), and more recently, capsular α-glucan (Geurtsen et al., 2009) (a list is provided in Table 3). All these compounds have been shown to bind DC-SIGN and/or inhibit binding of *M. tuberculosis* to DC-SIGN. This suggests that binding of mycobacteria to DC-SIGN and DCs is not mediated by one dominant ligand, but that there is a redundancy in ligands for DC-SIGN present on the cell surface (Pitarque et al., 2005). This was supported by the observation that even a double knockout *M. bovis* BCG strain that neither produces the mannose cap on lipoarabinomannan nor higher-order PIMs did not show any reduction in binding to DC-SIGN and DCs as compared with its parent strain (Driessen et al., 2009). Moreover, this mutant strain did not induce an altered cytokine profile in DCs (Driessen et al., 2009). All of the reported ligands for DC-SIGN can be found in DC-SIGN nonbinding *Mycobacterium* spp. as well. Hence, other factors probably determine the binding to DC-SIGN instead of only the presence or the absence of one or a few ligands. The affinity of DC-SIGN for specific *Mycobacterium* spp. may be related to differences in the mannosylation pattern of potential ligands, the relative abundance of various compounds at the cell surface (in particular, ω(1 → 2)-linked Manp-containing compounds), cell wall structure and/or capsule composition. Finally, not all potential ligands, when cell wall bound, may be accessible to DC-SIGN and thus of equal importance for the binding of mycobacteria to DC-SIGN.
To investigate the DC-SIGN-dependent modulation of the immune response by mycobacteria or their mannosylated lipoglycans, several in vitro and in vivo studies have been performed. Purified mycobacterial Man-LAM, not Ara-LAM, inhibits both lipopolysaccharide- as well as BCG-induced maturation of DCs by reducing the expression of MHC class II and costimulatory molecules (CD80, CD83 and CD86) as compared with untreated lipopolysaccharide- or BCG-activated cells (Geijtenbeek et al., 2003). Furthermore, Man-LAM has been shown to induce the secretion of anti-inflammatory IL-10 in lipopolysaccharide-activated DCs (Geijtenbeek et al., 2003). CD83 and CD86). As compared with untreated lipopolysaccharide- or BCG-activated cells (Geijtenbeek et al., 2003). Moreover, Man-LAM has been shown to induce the secretion of anti-inflammatory IL-10 in lipopolysaccharide-activated DCs (Geijtenbeek et al., 2003). Ligation of Man-LAM to DC-SIGN appears to interfere with lipopolysaccharide signaling via TLR4 by the activation of serine-threonine kinase Raf-1, which subsequently leads to acetylation of NF-κB subunit p65. Acylation of p65 prolongs and enhances the transcription of IL10, thereby increasing IL-10 production (Gringhuis et al., 2007). Together with a study on mice lacking SIGN receptor (SIGNR)-1, a murine homolog for DC-SIGN, which displayed a stronger T helper 1 response upon M. tuberculosis infection and a reduced level of IL-10 production (Wieland et al., 2007), this led to the initial thought that the interaction with DC-SIGN is beneficial for mycobacteria.

Recently, it has been shown that Man-LAM alters the cytokine profile in lipopolysaccharide-activated DCs by increasing the secretion of not only IL-10, but also of proinflammatory IL-12 and IL-6 (Gringhuis et al., 2009). Binding of Man-LAM or mannose-containing pathogens like M. tuberculosis induces the recruitment of effector proteins to a DC-SIGN signalosome, which is required for the activation of Raf-1, followed by enhancement of the transcription of the genes encoding IL-12 and IL-6 in a similar manner as for IL-10 (Gringhuis et al., 2009). Noteworthy, lipopolysaccharide is a nonmammalian bacterial ligand and both Man-LAM (Geijtenbeek et al., 2003) as well as intact mycobacteria (Driessen et al., 2009) do not induce IL-10 secretion in nonactivated DCs. In the context of mycobacterial infection, further research on interference of DC-SIGN signaling induced by Man-LAM with signaling via TLR2 by for example BCG-Ac1LM or the 19 kDa antigen would be of interest.

A study using mouse DCs showed a higher induction of suppressor of cytokine signaling-1 expression by SIGNR1 stimulation as compared with signaling via TLR2 (Srivastava et al., 2009). It is important to mention that seven murine homologs for human DC-SIGN are known. Each homolog differs in certain properties from the human DC-SIGN, among which are the specific mannos- and fucose-structures it recognizes, and this may alter their functions significantly (Powlesland et al., 2006; Tanne et al., 2009). Strikingly, human DC-SIGN transgenic mice exhibited decreased pathology and prolonged survival during mycobacterial infection (Schaefer et al., 2008). A clear protective role has recently also been shown for the murine ortholog SIGNR3 (Tanne et al., 2009). Resistance to M. tuberculosis infection was impaired in SIGNR3-deficient mice, but not in mice lacking SIGNR1 and SIGNR5 (Tanne et al., 2009).

DC-SIGN specifically recognizes the pathogenic Mycobacterium spp. from the tuberculosis complex (Pitarque et al., 2005). By expressing many ligands for DC-SIGN, DC-SIGN is the most important uptake receptor for mycobacteria to infect DCs. However, concluding from these last studies, DC-SIGN-binding appeared not to be mainly beneficial for the pathogens, and DC-SIGN may function primarily in the protection of the host. How DC-SIGN signaling exactly optimizes the immune response against mycobacteria, i.e. by strengthening the proinflammatory response or by preventing excessive inflammation, remains to be established (Ehlers, 2009) (Fig. 7).

Mannose receptor

The MMR (CD206) is a type I transmembrane monomeric C-type lectin with eight carbohydrate recognition domains. In addition to the complement receptors, the MMR mediates for a large part in the phagocytic uptake of mycobacteria by Mφ (Schlesinger, 1993). The MMR recognizes virulent M. tuberculosis strains Erdman and H37Rv, but not avirulent H37Ra, which has been suggested to be caused by subtle structural differences in Man-LAM (Schlesinger, 1993; Schlesinger et al., 1996). Furthermore, the MMR also shows a low affinity for nontuberculosis mycobacteria M. smegmatis, M. phlei and M. kansassi, of which the first two species only bear lipoarabinomannan without a mannose cap (Astarie-Dequeker et al., 1999). Although the presence of a mannose cap on Man-LAM is essential for the recognition of lipoarabinomannan by the MMR (Schlesinger et al., 1994), it does not completely explain the differences in MMR-mediated uptake by Mφ between the Mycobacterium spp. (Schlesinger et al., 1996; Astarie-Dequeker et al., 1999; Appelmelk et al., 2008). Next to the recognition of the mannose cap on Man-LAM, the MMR furthers binds higher-order PIMs, and PIMs, with a preference of the triacylated species above the tetra-acylated ones, and glycopeptidolipids, but not to lower-order PIMs, lipomannan and Ara-LAM or PI-LAM (Schlesinger et al., 1994; Villeneuve et al., 2005; Torrelles et al., 2006). Because of their structural similarities, mannosylated proteins and (phosphorylated) arabinomannans and mannan may be involved as well (Ortalo-Magné et al., 1995; Dobos et al., 1996; Maes et al., 2007; Torrelles et al., 2008a). Man-LAM and related lipoglycans all contribute to the MMR-mediated phagocytosis of mycobacteria (Villeneuve et al., 2005; Torrelles et al., 2008b). Overexpression of Mt-ManB in M. smegmatis results in the overproduction of PIMs, lipomannan and...
lipoarabinomannan. Subsequent increased association with Mφ is probably due to the higher amount of higher-order PIMs, as lipoarabinomannan from M. smegmatis does not have a mannose cap (McCarthy et al., 2005).

A role for the MMR has been implicated in many of the immunomodulatory effects by Man-LAM and the related glycans described above. Next to uptake of mycobacteria by Mφ via the MMR, the MMR is also involved in the transport of glycolipids to uninfected bystander cells and intracellular trafficking to the late endosomal compartments (Prigozy et al., 1997). However, in DCs, uptake of both mycobacteria as well as of the glycolipids is performed mainly by DC-SIGN, leaving a minor role for the MMR (Schaible et al., 2000; Geijtenbeek et al., 2003; Driessen et al., 2009). Binding of Man-LAM to the MMR has been further linked to the delay of phagosome maturation (described in more detail in the corresponding section above) (Kang et al., 2005), the induction of MMP-9 by M. tuberculosis and Man-LAM similar to that observed for TLR2 activation by lipomannan (Rivera-Marrero et al., 2002) and the expansion of regulatory T cells (Tregs) (Garg et al., 2008). Furthermore, DCs from MMR knockout mice secrete more IL-12p40 upon infection with M. tuberculosis as compared with DCs from wild-type mice (Ehlers, 2009). Thus, the MMR seems to have an immunosuppressive function on DCs, which was earlier shown by cross-linking the MMR on DCs with anti-MMR antibodies and several natural ligands (Chieppa et al., 2003). Finally, in Mφ, Man-LAM suppresses lipopolysaccharide-induced IL-12p40 secretion via the induction of IL-1 receptor-associated kinase (IRAK)-M expression (Pathak et al., 2005). IRAK-M negatively regulates TLR signaling (Kobayashi et al., 2002; Io et al., 2007) and its induction is probably triggered by binding of Man-LAM to the mannose receptor (Pathak et al., 2005).

Concluding, the MMR signaling may induce an anti-inflammatory response. A study by Torrelles et al. (2008b) reported, for clinical isolates of M. tuberculosis (HN885 and HN1554) with both truncated Man-LAM and a reduced amount of higher-order PIMs, a significant decrease in association with the MMR as compared with the Erdman strain, while uptake via the CR was not altered. Phagocytosis via the MMR may shape the nature of the immune response, and it was suggested that due to its immunosuppressive activity, uptake via the MMR may lead to a latent infection instead of active disease (Torrelles et al., 2008b). Related to this, an M. tuberculosis pimB-mutant strain (Rv0557/mgtA), which expresses less lipomannan and Man-LAM in its cell wall, showed an increased rate of macrophage death upon infection as compared with its parent strain (Torrelles et al., 2009). This does not necessarily mean increased virulence, but may even be advantageous for the host as the more virulent strains have been reported to inhibit apoptosis (Torrelles et al., 2009). Although the pimB-mutant phenotype could not be linked to reduced association with the MMR, the presence of high amounts of PIMs, lipomannan and Man-LAM results in a less inflammatory immune response and indicates adaptation of the mycobacteria to their host (Torrelles et al., 2009; Torrelles & Schlesinger, 2010) (Fig. 7).

Concluding remarks

The entire repertoire of enzymes and genes involved in the biogenesis of lipoarabinomannan and related
glycoconjugates has almost been identified. However, unlike *C. glutamicum*, many of the genes involved in biosynthesis of these molecules in *M. tuberculosis* are essential for its survival (Sassetti et al., 2003; Kaur et al., 2009; Guerin et al., 2010; G.S. Besra, unpublished data) like its cousin *M. smegmatis* (Jackson et al., 2000; Kordulakova et al., 2002; Guerin et al., 2009; Skowierova et al., 2009), and therefore represent excellent drug targets. Furthermore, the identification of enzymatic machinery is now leading to the biophysical analysis of the three-dimensional structures of these enzymes and the identification of inhibitors, which may develop into novel drugs against tuberculosis. In addition, very little is known about the regulation of the biosynthesis of these glycoconjugates and their respective presentation on the mycobacterial cell wall; such studies may lead to the identification of few more interesting and potential targets. To date, serine–threonine kinases have been suggested in the regulation process of mycobacterial glycosyltransferases (Alderwick et al., 2006b; Molle & Kremer, 2010). However, further studies are required to unravel the detailed mechanism of regulation in terms of the species, composition, heterogeneity, size and length of these polymers.

PIMs, lipomannan and Man-LAM all display several immunomodulatory properties by interaction with different receptors of the immune system. Their activity depends on their degree of acylation and mannosylation. While lipomannan is mainly associated with TLR signalling, the higher-order PIMs and Man-LAM are recognized by the C-type lectins DC-SIGN and the MMR. Interestingly, alveolar Mφ constitutively express the MMR (Wileman et al., 1986), but DC-SIGN expression is only induced in alveolar Mφ in the lungs of tuberculosis-infected patients (Taillieux et al., 2005), which may influence the disease pattern in an important, but yet to be determined way. Both C-type lectins show comparable recognition specificities for the mannosylated glycolipids, and as receptors CD14 (Pugin et al., 1994) and pulmonary surfactant protein (SP)-A (Sidobre et al., 2000) and SP-D (Ferguson et al., 1999, 2006) also bind Man-LAM, the interactions of mycobacteria with all these PRRs may enhance or dampen inflammatory signals and thereby determine the nature of the immune response (Ernst, 1998; Torrelles et al., 2008a). Anti-inflammatory signaling via the interaction of the glycolipids with the host immune system can be regarded as strategies of the mycobacteria to escape immune surveillance (Torrelles & Schlesinger, 2010), but may also be vital in the prevention of an exaggerated inflammatory response (Jo, 2008). As only 5–10% of the tuberculosis-infected persons develop the active disease, the glycolipids may play a crucial role in host adaptation of the mycobacteria and hence, a balanced immune response resulting in a latent tuberculosis infection (Fig. 7).

**Acknowledgements**

G.S.B. acknowledges support in the form of a Personal Research Chair from Mr James Bardrick, Royal Society Wolfson Research Merit Award, as a former Lister Institute-Jenner Research Fellow, the Medical Research Council and The Wellcome Trust (081569/Z/06/Z). The authors thank Drs Apoorva Bhatt and Jeroen Geurtsen for critically reading the manuscript.

**Authors’ contribution**

A.K.M. and N.N.D. contributed equally to this work.

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