1

GENERAL INTRODUCTION
**TUBERCULOSIS**

Tuberculosis (TB) is an airborne and contagious disease caused by *Mycobacterium tuberculosis*. With approximately 1.7 million deaths each year, it is still one of the leading infectious diseases in the world (Table 1). The incidence of TB is highest in South-East Asia, African and Western Pacific regions (79). Further, an estimated 11 to 13% of the TB cases worldwide is among HIV-positive persons (mainly in African regions) (79); TB is a major cause of death in this group. The World Health Organization has started specific programs to stop TB by implementing new projects for improvement of diagnosis, notification of TB cases and treatment. While the global TB mortality rate and prevalence have begun to decline clearly (79), the reduction in incidence rate per capita is slow with about 1% per year since 2004 and absolute numbers of TB cases are still rising due to population growth (79). TB can be cured with a six to nine month course of tuberculostatics, but this long period of drug therapy forms a potent risk factor for ending treatment too early (i.e. lack of compliance) and thereby inducing emergence of multidrug-resistant (MDR-)TB, i.e. TB resistance against the standard first-line drugs (Table 1). MDR-TB is most prevalent in India, China, the Russian Federation, and South Africa (80). Furthermore, up to now 58 countries have reported a least one case of extensively drug-resistant (XDR-)TB against which both first- and second-line drugs are no longer effective and which is extremely difficult to treat (80).

Interestingly, although it has been estimated that about one-third of the world population is infected with *M. tuberculosis*, only 5 to 10% of the infected persons develops the active disease. In the majority of the cases this is pulmonary TB, but other parts of the body can become affected as well. In the lungs, the bacteria infect macrophages (Mφ) and cause formation of granulomas: cellular aggregates including activated Mφ, lymphocytes and fibroblasts which surround the infected cells. On the one hand these granulomas are considered to be important in containment of *M. tuberculosis*, but on the other hand they can also become a reservoir for live bacteria, as mycobacteria have the ability to survive inside the Mφ (66). Hence, in case of non-active disease, the bacterium can still persist in host tissues for many years and cause active disease at a later stage in life.

<table>
<thead>
<tr>
<th>Table 1. Facts on TB. Data for 2009 (79) and for 2008 (80).</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009:</td>
</tr>
<tr>
<td>Incidence* of TB</td>
</tr>
<tr>
<td>Prevalence** of TB</td>
</tr>
<tr>
<td>Mortality among HIV-negative people</td>
</tr>
<tr>
<td>Mortality among HIV-positive people</td>
</tr>
<tr>
<td>2008:</td>
</tr>
<tr>
<td>Incidence of MDR-TB</td>
</tr>
<tr>
<td>Deaths caused by MDR-TB</td>
</tr>
</tbody>
</table>

* **Incidence**: the number of new cases in a population in a given time period, often a year.  
** **Prevalence**: the total number of individuals affected by the disease in a population at a given time.
The only currently available vaccine against TB - Bacillus Calmette-Guérin (BCG) - has been used since 1921 and only provides high protective efficacy against meningitis and disseminated TB in children, but not against pulmonary TB in adults (78). BCG, which is a live attenuated strain of Mycobacterium bovis, may share some of the immunomodulating properties with M. tuberculosis to which it is closely related and therefore not confer full protection (27, 28, 46). Other factors which possibly reduce the efficacy of BCG, are the existence of multiple BCG substrains with different properties (64), e.g. in lipid composition (13), co-infections with either environmental mycobacterial strains which reduce the immunogenicity of BCG (27, 81) or helminths which modulate the immune response (23, 77), and (natural) waning of the immune response. Next to this, unlike M. tuberculosis, M. bovis BCG is not able to enter the cytosolic compartment of Mφ after being phagocytosed which is discussed later in this chapter (72). Noteworthy, BCG does protect against leprosy and Buruli ulcer, which are caused by Mycobacterium leprae and Mycobacterium ulcerans, respectively (78).

![Figure 1 Scanning electron micrograph of Mycobacterium tuberculosis. Magnification 15549x. Picture by J.H. Carr.](image)

**The mycobacterial cell envelope**

*M. tuberculosis* is a rod-shaped bacterium ranging from 2-4 μm in length and 0.2-0.5 μm in width (Figure 1). The cell envelope of mycobacteria consists of a plasma membrane, a periplasmic space, and an arabinogalactan-peptidoglycan layer to which mycolic acids are covalently attached (Figure 2) (14). Except for some slight differences, the mycobacterial peptidoglycan is similar to the most common type found in Eubacteria (14). The following layers are more unusual and found only in the phylogenetic group

---

of Actinomycetales to which the genus of Mycobacterium belongs. The arabinogalactan layer is made up of a polymer of arabinofuranosyl (Araf) and galactofuranosyl (Galp) residues and connects the peptidoglycan layer with the mycolic acids (16). Mycolic acids are high-molecular-weight fatty acids composed of two unbranched hydrocarbon chains, a shorter α-branch and a longer meromycolate moiety, with a total size of 60 to 90 carbon atoms (4). They form a very heterogeneous group due to variations in chain length, presence of functional groups on the meromycolate branch, and the number and type of double bonds (4). Furthermore, mycolic acids are part of trehalose 6,6′-dimycolate (TDM/cord factor), one of the most abundant glycolipids in the mycobacterial cell wall (57). TDM is an important virulence factor, and responsible for the cord-like appearance of M. tuberculosis cells (37). Together with the arabinogalactan-peptidoglycan layer, the mycolic acids form a stable skeleton in the mycobacterial cell wall (14). In addition to the covalently linked mycolic acids and the TDMs, the cell wall further contains several other (glyco)lipids, most of which are specific for mycolates (i.e. mycobacteria and closely related species with mycolic acids), such as the sulfolipids, lipoarabinomannan (LAM) and glycopeptidolipids (7).
The outermost layer surrounding the mycobacterial cell wall is the capsule (Figure 2), which exists of glycans (α-glucan, arabinomannan, mannan) and proteins (45), but has a very low lipid content (59).

Recently, the layer of mycolic acids and extractable lipids was proven to form a lipid bilayer, the mycobacterial outer membrane (Figure 2) (36, 82), though this cell wall organization was postulated in 1982 already (48). Models have been proposed in which mycolics acid are fully inserted into the inner leaflet of the outer membrane, or only partly, stabilizing the membrane with their long meromycolate chain (36, 82). In all models, the extractable lipids form the outer leaflet, but are present in the inner leaflet as well with their polar carbohydrate headgroup facing the opposite direction. Mycobacterium was previously regarded as a Gram-positive bacterium (7, 14), but consequently, this view had to be adjusted. The presence of a second outer membrane makes the mycobacterial cell wall more reminiscent of a Gram-negative bacterium, although several differences exist (e.g. in thickness of the different layers, composition of the cell wall) (36). Hence, classification of Mycobacterium as either Gram-positive or –negative would not be correct.

Together, these unique properties of the mycobacterial cell envelope make it into an impermeable, protective barrier for the bacterium, which uses protein porins and various secretion systems for efficient nutrient acquisition and protein secretion (7, 19). Furthermore, the glycolipids and glycans in the cell wall and capsule may have key functions in the Mycobacterium-host interaction (15, 22).

Mannosylated structures in the mycobacterial cell wall: lipoarabinomannan and related glycolipids

One of the intriguing and complex lipids found in the mycobacterial cell envelope is the glycolipid lipoarabinomannan (LAM). Similar to the unusual cell wall structures, glycolipids related to LAM are also only found in certain species within the group of Actinomycetales (54). First, the immunologically active polysaccharide arabinomannan (AM), was discovered, which was reported to consist of D-mannopyranose (Manp) and D-arabinofuranose (Araf) residues (1, 49). Part of the isolated AM appeared to be acylated and later on LAM was identified as a true glycolipid containing phosphate, inositol, and acylated glycerol in addition to the glycan component AM (11, 38, 39). Mycobacterial LAM essentially consist of three segments: a mannosylated phosphatidylinositol (MPI-)anchor, a mannan core (30-35 Manp residues), and a branched arabinan domain (≈ 60 Araf residues) (8, 11, 54), which will be discussed here in this order (Figure 3).

The MPI-anchor consists of phospho-myoinositol group substituted with acylated glycerol, and one Manp residue at the C2-position of the inositol. The mannan core is attached at the C6-position of the inositol (42). Besides the C1 and the C2 of the glycerol, the MPI-anchor can be further acylated at two more positions: 1) the C6 of the Manp attached to the C2 of the inositol, and 2) the C3 of the inositol (53) (Figure 3). The major fatty acids observed in LAM are tuberculostearic acid and palmitic acid. However, differently acylated forms, varying both in degree and composition of
acyl chains and also depending on *Mycobacterium* species, co-exist in the cell wall (54). The mannan core consists of a linear chain of α(1→6)-linked Man₆ which are partly substituted at their C2-position with a single Man₆ residue (11) (Figure 3). One exception is LAM from *Mycobacterium chelonae*, in which the mannan core of LAM is substituted with α(1→3)-linked Man₆ instead of α(1→2)-linked Man₆ (35). In LAM from various *M. tuberculosis* and *M. bovis* BCG strains, the branching degree of the mannan core (i.e. ratio substituted Man₆ : total Man₆ of mannan core) was determined to be 60 to 70%, while a little lower branching degree was reported for LAM from

![Figure 3](image-url-3)

**Figure 3.** Structure of LAM. Figure is based on published structures (8, 54). LAM consists of a MPI-anchor (A), a mannan core (B), and a branched arabinan domain (C). R₁, R₂, R₃, and R₄ indicate potential sites of acylation. LAM can be capped with one to three mannose residues (ManLAM), phospho-inositol caps (PILAM) or be completely uncapped (AraLAM) depending on *Mycobacterium* species. All three forms are shown here at random positions (D). The structure of LM is very similar to LAM devoid of the arabinan domain (i.e. A + B). CapA (Rv1635c) adds the first mannose residue of the mannose cap on ManLAM to arabinan termini (18). Rv2181 both substitutes the mannan core with single α(1→2)-Man₆ as well as elongates the monomannoside cap with one or two Man₆ (40, 41). PIM₄, consisting of a MPI-anchor with two Man₆ residues attached, is the precursor for LAM and LM, as well as for PIM₅. The branching point between PIM₄ and LAM/LM biosynthesis is very likely PIM₅, which is formed by the MPI-anchor mannosylated with in total four Man₆ residues (A plus the first three α(1→6)-linked Man₆ at the C6-position of Ins). Mannosyltransferase PimE (Rv1159) is required to add the fifth α(1→2)-Man₆ (52) and possibly the sixth α(1→2)-Man₆ to produce PIM₆ and PIM₇, respectively (Chapter 5, Figure 1). The current view on the biosynthesis of LAM, LM and PIMs is reviewed in more detail in Chapter 7.
The arabinan domain consists of a linear chain of α(1→5)-linked Araf which is branched at some points at the C3-positions with α-Araf residues. These branches can be either linear [(Ara)_n] or bifurcating [(Ara)_m], but always terminate with β-Araf-(1→2)-α-Araf→ (Figure 3) (9). Interestingly, the arabinan domain structure of LAM shows similarities to the arabinan in the arabinangalactan layer of the cell wall (9, 49). Some of the branching points in the arabinan domain have been reported to be substituted at their C2-positions with succinyl groups (one to four per LAM molecule) in LAM isolated from several M. bovis BCG strains (17).

Depending on the Mycobacterium species, the non-reducing arabinan termini can be decorated further. In LAM of several pathogenic species such as M. tuberculosis, as well as LAM of the non-pathogenic but the closely related BCG strain, the arabinan domain is capped with one to three α(1→2)-linked Manp-residues (the mannose cap) forming ManLAM (Figure 3) (12, 76). Both the degree of capping varies between species from 1 to 10 caps per LAM molecule (54), as well as the capping motifs of which the dimannoside one is the most abundant (61). This mannose cap has been hypothesized to play an important role in immunomodulation (31, 56) which is discussed in the next section. In non-pathogenic species also non-capped LAM (AraLAM) and LAM capped with phospho-inositol (PILAM) have been identified: in M. chelonae (35) and M. smegmatis (43), respectively.

LAM shares its MPI-anchor with related glycolipids lipomannan (LM) and phosphatidylinositol mannosides (PIMs) (10, 42). LM is similar to LAM without the arabinan domain (Figure 3, A+B) (7). PIMs consist of a MPI-anchor substituted with one to six Manp residues (PIM₁ to PIM₆) (Figure 3 and Chapter 5, Figure 1). The major PIM species found in mycobacteria are PIM₁ and PIM₆, (42, 67) with PIM₆ being an end product, and PIM₂ both end product as well as precursor for PIM₆, LM, and LAM (42, 51). The most striking feature of PIM₆ is its terminal α(1→2)-linked tri-mannoside, which is reminiscent of the mannose cap on ManLAM. Hence, PIM₆ may have some of the immunomodulatory properties of ManLAM in common.

In contrast to AM, LAM is absent from the capsule, but anchored via its lipid portion in the cell wall (11). Only for PIMs, small amounts can be found in the capsule (58, 60). Two cell wall fractions of LAM have been described, parietal and cellular LAM (17, 33), which were initially thought to have separate localizations in the cell wall because of a difference in difficulty to extract the LAM from the cell wall (17). However, due to higher degree of acylation (17), cellular LAM is probably just more firmly attached in the cell wall as compared to parietal LAM rather than localized differently (62). Based on the current model of the mycobacterial cell wall, LAM can be either inserted in the plasma membrane or in the mycobacterial outer membrane, in which the glycan part of the latter fraction is thought to be surface-exposed (62) (Figure 2). Importantly, interactions of LAM, LM, and PIMs with the host immune system, depend on their accessibility to host immune receptors.
Immunomodulation by *M. tuberculosis* and ManLAM: interaction with host-receptor DC-SIGN

The bacterium's ability to persist in host tissues for many years, which is also referred to as latency, is dependent on at least two different processes. The first is the ability of the bacterium to inhibit phago–lysosome fusion after being ingested by Mφ. Normally, ingested bacteria are contained within phagosomes which later on fuse with lysosomes leading to bacterial destruction and subsequent presentation on MHC complexes. However, pathogenic mycobacteria interfere with this process by actively blocking phago–lysosome fusion thereby creating a unique niche in which they are able to survive for many years (65). For the past decades, the central dogma has been that pathogenic mycobacteria exclusively reside within this phagosomal compartment until they are released from the cell and cause active disease. However, recently, this view was challenged by the observation that *M. tuberculosis*-containing phagosomes rapidly fused with lysosomes in both monocyte-derived dendritic cells (DCs) and Mφ (72). Yet, at day 2 post infection, *M. tuberculosis* was able to escape from the phago–lysosomes into the cytosol where they were able to replicate (72). This same phenomenon was observed for *Mycobacterium leprae* but not for the vaccine strain *Mycobacterium bovis* BCG or for heat-killed mycobacteria, suggesting that the process is specific for pathogenic mycobacteria. Although these findings contradict earlier observations and await independent confirmation, they may have important implications for the current view on host–mycobacterial interactions.

A second feature that supports the ability of pathogenic mycobacteria to cause chronic infections is their capacity to suppress host immune responses. To this end, DCs form an interesting target due to their central role in the induction of adaptive immunity. Although early data already indicated that mycobacteria influence DC functioning, the underlying mechanisms remained poorly understood. Major progress into this field was made when it was found that the interaction between *M. tuberculosis* and DCs is almost exclusively dependent on the binding to Dendritic Cell-Specific ICAM3-Grabbing Non-Integrin (DC-SIGN) (31, 70). Although DCs express a wide variety of other pattern-recognition receptors (PRRs) that can recognize pathogen-associated molecular patterns (PAMPs), such as the MR and dectin-1, only antibodies directed against DC-SIGN could block the *Mycobacterium*–DC interaction (70). Comparable results were obtained with the *M. bovis* BCG vaccine strain (32, 70).

Human DC-SIGN is a carbohydrate-binding and Ca\(^{2+}\)-dependent C-type lectin receptor primarily expressed by dendritic cells (Figure 4A). Its carbohydrate recognition domain (CDR) contains the characteristic amino acids triplet EPN (in one-letter amino acid-code) typical for mannose and fucose recognizing C-type lectins (20, 21) (Figure 4B). Indeed, DC-SIGN recognizes N-linked high-mannose oligosaccharides and branched fucosylated structures, however fucosylated structures have not been

---

identified in the mycobacterial cell wall. In an effort to identify the ligand responsible for binding to DC-SIGN, it was found that binding of both monocyte-derived DCs and HeLa-derived P4 cells expressing DC-SIGN to \textit{M. tuberculosis} could almost completely be inhibited by pre-incubating the cells with ManLAM, suggesting that this glycolipid forms an important ligand for DC-SIGN (70). In a different study, the importance of the mannose cap in LAM-DC-SIGN interaction was determined using two different assay systems: 1) binding of a DC-SIGN-Fc construct to ManLAM and ‘capless’ AraLAM coated onto ELISA-plates (hence, with ‘monomeric’ DC-SIGN), and 2) binding of fluorescent beads coated with either ManLAM or AraLAM to DCs and a transfected K562 cell line both expressing DC-SIGN (31) (i.e. cellular DC-SIGN which can form tetramers). It was shown that the interaction of ManLAM with DC-SIGN was dependent on the terminal mannose caps as the ‘capless’ AraLAM did not bind to DC-SIGN (31). Later on, these findings were confirmed by the demonstration that DC-SIGN specifically interacts with polyacrylamide (PAA)-coupled neo-glycoconjugates that resemble the mannose cap of ManLAM as shown in Figure 4C (44). DC-SIGN-binding of these glycoconjugates with varying cap length — (man)\textsubscript{1} ara-PAA, (man)\textsubscript{2} ara-PAA, and (man)\textsubscript{3} ara-PAA — was examined again in the two mentioned assays systems: ELISA-plate format and fluorescent bead adhesion assay (44). Next to the presence of a mannose cap, cap length appeared an essential factor in DC-SIGN-recognition in DC-SIGN displaying a strongly decreased affinity for (man)\textsubscript{1} ara-PAA as compared to the (man)\textsubscript{2} ara-PAA and (man)\textsubscript{3} ara-PAA glycoconjugates (44). Interestingly, ManLAM is mainly found in pathogenic, slow-growing mycobacteria, whereas AraLAM is only found in avirulent, rapid-growing species, suggesting that ManLAM-DC-SIGN interactions may be important for mycobacterial pathogenesis. These data, together with the finding that also PILAM poorly inhibits \textit{M. tuberculosis}-DC-SIGN interactions, led to the hypothesis that DC-SIGN can discriminate between mycobacterial species through the recognition of the mannose caps on ManLAM (47). However, later on, this view was found to be too simplistic as it was demonstrated that not all mycobacterial species that contain ManLAM are bound by cellular DC-SIGN (61). This was not due to intrinsic differences between the different ManLAMs, as it was shown that the purified ManLAMs, also the ones from the species that did not bind DC-SIGN, could all efficiently block the binding of \textit{M. tuberculosis} to DC-SIGN (61). Furthermore, experiments addressing the cell-surface exposure of ManLAM showed that between species only minor differences exist (61). These data strongly suggested that ManLAM was not the only DC-SIGN ligand present on mycobacteria and further investigations led to the discovery of at least four additional ligands: LM, mannose-capped AM and two mannosylated glycoproteins (19 and 45 kDa antigens) (61). In a later study, also the PIMs were shown to bind to DC-SIGN (71).

One of the functions of DC-SIGN is to mediate in the capture and destruction of microbial pathogens and in their presentation to lymphocytes to induce successful immune responses. However, an increasing amount of evidence suggests that pathogens like \textit{M. tuberculosis}, also exploit DC-SIGN to subvert host immune responses (73, 74).
Figure 4 DC-SIGN and the mannose cap of ManLAM. (A) Schematic representation of DC-SIGN. DC-SIGN is a prototype type II transmembrane protein consisting of one carboxy-(COOH-) terminal carbohydrate recognition domain (CRD), followed by a neck domain consisting of one incomplete and seven complete tandem repeats (numbered 1 – 7.5) fused to a transmembrane domain (TMD) and ending with an amino- (NH₃⁺-) terminal cytoplasmic tail. The latter harbours internalization and recycling motifs, such as a di-leucine (LL) and a tri-acidic (EEE) motif, and an incomplete immunoreceptor tyrosine- (Y-) based activation motif (ITAM). The complete extracellular domains form tetramers on the cell surface (25, 50). DC-SIGN has multiple functions. DC-SIGN is involved in the priming of T-cell responses and facilitates dendritic cell homeostasis by controlling extravasation into peripheral tissues (29). Next to this, it mediates the capture, destruction and presentation of microbial pathogens to induce immune responses. (B) Ribbon diagram of the CRD of DC-SIGN. The CRD of DC-SIGN contains the characteristic amino acids triplet EPN (Glu-Pro-Asn) typical for mannose and fucose recognizing C-type lectins (20, 21). Furthermore, the CRD harbours three Ca²⁺ ions, of which one -the principal Ca²⁺- is common to all Ca²⁺-dependent C-type lectins and dictates the recognition of specific carbohydrate structures. The principal Ca²⁺ interacts with four amino acids; Glu347, Asn349, Glu354 and Asn365. Disulfide bonds are indicated by an asterisk. Figure was adapted from Feinberg et al. (26) with permission from AAAS. (C) Structure of the mannose cap as present on ManLAM. It consists of up to three α(1→2)-linked Manp residues, which are attached to arabinan. The affinity of DC-SIGN for the mannose cap increases with the number of Manp residues (44). Important for the interaction of α(1→2)-linked Manp structures with the CRD of DC-SIGN has been reported to be the vicinal, equatorial 3- and 4-OH groups of the mannosyl residues (21, 24). These form coordination bonds with the principal Ca²⁺ and hydrogen bonds with amino acids of which Val351 appeared essential (24, 30).

ManLAM was not only shown to bind to DC-SIGN but was also known to modulate DC cytokine secretion by suppressing the production of pro-inflammatory interleukin (IL)-12 and/or upregulating the production of anti-inflammatory IL-10, thereby inhibiting Th1-type immune responses (31, 56). Interestingly, Geijtenbeek et al.
observed that ManLAM, but not AraLAM, induces IL-10 secretion in LPS-primed DCs (31). By using monoclonal antibodies, they demonstrated that the phenomenon was dependent on DC-SIGN (31). Recently, the molecular signaling pathway underlying this modulatory activity was identified. DC-SIGN appeared to interfere with signaling induced via other PRRs, i.e. toll-like receptors (TLRs), and thereby enhances the transcription of the gene encoding IL-10 (34). Furthermore, a study with mice lacking SIGNR1 —one of the murine homologs of DC-SIGN— showed that these mice had stronger T-helper 1 responses to *M. tuberculosis* and a reduced level of IL-10 (72). However, no differences in bacterial load and mouse survival rates could be detected (72). Interestingly, *M. tuberculosis* seems to actively induce the expression of DC-SIGN by alveolar Mφ. It has been shown that in patients with TB, up to 70% of the alveolar Mφ express DC-SIGN. By contrast, the lectin was hardly detected in alveolar Mφ from healthy individuals or in patients with unrelated lung pathologies (69). Moreover, promoter polymorphisms that influence DC-SIGN expression have been associated with an altered susceptibility to mycobacterial infections (3, 75).

OUTLINE OF THESIS

The aim of the work in this thesis was to study the interaction between mannosylated glycolipids in the mycobacterial cell wall and the host immune system with the main focus on the mannose cap of ManLAM. As a ligand for DC-SIGN, ManLAM is hypothesized to have a key function in modulating the host immune response via this lectin.

First the role of the mannose cap in the interaction between DC-SIGN and whole *Mycobacterium* cells was investigated. Therefore, mutant *Mycobacterium* strains were created in which the gene responsible for the addition of the first mannosyl residue of the cap to the arabinan domain was knocked out (18) (*capA*, Figure 1). Next, these mutant strains were assessed in a range of *in vitro* and *in vivo* assays to determine their interactions with DC-SIGN and to test for a possible altered course of infection or changes in the induction of immune responses as compared to the parent strains (Chapter 2).

In Chapter 2, a random transposon mutant library in *Mycobacterium marinum* -a *Mycobacterium* species closely related to *M. tuberculosis*- was screened on colony blot with a mannose cap-specific monoclonal antibody. This type of screening can be used to identify enzymes that have a direct function in the capping of LAM with mannose, such as CapA, but in addition can also reveal additional proteins which act at an earlier stage in the biosynthesis of LAM, or have a more regulatory role. Therefore, the mutant library described in Chapter 2 was screened further for ‘capless’ mutants (Chapter 3).

*Mycobacterium* species can be divided on basis of their growth rate in two groups: the slow-growing and rapid-growing species. Distinction on basis of 16s rRNA gene sequencing places the species in two phylogenetic groups that are almost identical to
the ones based on growth rate, and of which the slow-growing one appears to have evolved most recently (63, 68). Interestingly, the presence or absence of a mannose cap on LAM of the different species seems also to be related to these same groups as well. For several slow-growing species, ManLAM has been shown to be present in their cell wall, while only ‘capless’ LAM (AraLAM or PILAM) has been detected in the few rapid-growing species which have been tested so far (11, 54). The most virulent pathogens can be found in the group of the slow-growers, and the presence of a mannose-cap may be related to growth speed and/or pathogenicity. To gain more insight into the role of the mannose cap, we tested a large range of Mycobacterium strains including multiple rapid-growing species, for the presence of mannose-capped LAM in their cell wall (Chapter 4).

As in particular the higher-order PIM₆ are reminiscent of the mannose cap, the role of the PIMs in the Mycobacterium-DC-SIGN interaction was studied as well. An earlier study showed already that PIMs are bound by DC-SIGN (71), but in this study the specific affinity of DC-SIGN for differently mannosylated PIMs had not become clear. Therefore, various PIMs - both synthetic and purified from the mycobacterial cell wall - were analyzed for their recognition by DC-SIGN and DCs. To further address the contribution of PIM₆ in the binding of Mycobacterium to DC-SIGN, a M. bovis BCG mutant strain was created deficient in the production of PIM₆ (ΔpimE; (52)) as well as a double knockout (ΔcapAΔpimE) devoid of both PIM₆ and the mannose cap its LAM (Chapter 5).

As DC-SIGN and the PRR macrophage mannose receptor (MMR) are important pulmonary phagocytic receptors for M. tuberculosis and both recognize manno-containing structures, targeting the mycobacterial mannosylated cell surface may prevent phagocytosis via these receptors and alter the fate of mycobacteria entering the lung. Cyanovirin-N (CV-N), a lectin isolated from cyanobacterium Nostoc ellipsosporum (6), has been shown to recognize mannosylated compounds with a specificity comparable to DC-SIGN (5). Furthermore, CV-N has been reported to inhibit cell entry of the Human Immunodeficiency Virus (HIV-)1 by binding to its mannosylated proteins (2, 6). The ability of CV-N to block mannose-dependent interaction of M. tuberculosis with Mφ and DCs was investigated in vitro and in vivo (Chapter 6).

Since its discovery, an enormous amount of data have been reported on the structure, biosynthesis, and immunomodulating properties of ManLAM and related glycolipids. An overview is given in Chapter 7.

Finally, the role of ManLAM and related glycolipids in mycobacterial infection is summarized and re-evaluated on basis of the studies in this thesis and other reports in Chapter 8.
REFERENCES


32. Geijtenbeek TB et al. (2003) Pathogens target DC-SIGN to influence their fate DC-SIGN functions as a pathogen receptor with broad specificity. APMIS 111: 698-714.


46. Madura LJ et al. (2007) BCG stimulated dendritic cells induce an interleukin-10 producing T-cell population with no T helper 1 or T helper 2 bias in vitro. Immunology 121: 276-282.


