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SUMMARIZING DISCUSSION

Interaction between mycobacterial ManLAM and the host immune response

Mycobacterium tuberculosis appears a 'successful' pathogen as it is estimated that around one-third of the world population is infected. Only 5 to 10% of the infected population develops active tuberculosis (TB), but the bacteria are not always eradicated from the body in the other cases. *M. tuberculosis* is able to survive inside human cells leading to a latent, asymptomatic form of TB-infection, but can still become 'reactivated' TB at a later stage in life. Hence, the *Mycobacterium*-host interactions are both interesting, and, considering the need for new drugs and more effective vaccines against TB, important to study. The complex and thick mycobacterial cell wall contains large amounts of glycans, proteins, and lipids with known or hypothesized roles in the targeting or modulating of the host immune response, of which one is mannose-capped lipoarabinomannan.

Simultaneously with the discovery of C-type lectin DC-SIGN as the major receptor on dendritic cells (DCs) for *M. tuberculosis*, the mannose cap of lipoarabinomannan (ManLAM) appeared essential for the recognition of LAM by DC-SIGN (10, 42). Next to alveolar macrophages (Mφ), alveolar DCs form a reservoir for mycobacteria (5, 22) and as antigen-presenting cells, DCs are important in the regulation of the immune response (19). Furthermore, infection with *M. tuberculosis* induces expression of DC-SIGN on alveolar Mφ, while Mφ stay DC-SIGN-negative in case of non-mycobacterial lung diseases or in healthy lungs (41). Hence, the characteristics of the interaction between on the one hand *Mycobacterium* and ManLAM, and on the other hand DCs and DC-SIGN are of interest in the understanding of the immune response against *M.* tuberculosis: is ManLAM the key ligand in the interaction between mycobacteria and DC-SIGN? Does inhibition of this interaction prevent or reduce TB-infection? And is the mannose cap on LAM a virulence factor restricted to the slow-growing pathogenic *Mycobacterium* species?

ManLAM and other mycobacterial ligands for DC-SIGN

Although the mannose cap on ManLAM is essential for the binding of purified LAM to DC-SIGN, it appeared not essential for the binding of whole *Mycobacterium* cells to DC-SIGN and DCs (**Chapter 2**). Mycobacteria evidently bear multiple ligands for DC-SIGN at their cell surface. Next to ManLAM, we discovered PIM_6 (**Chapter 5**) and α-glucan as ligands for DC-SIGN (12). Other ligands are lipomannan (LM), mannosylated proteins, and arabinomannan (ManAM; similar glycan structure as ManLAM, but without the MPI anchor) in the capsule. These three ligands (with 19 kDa and 45 kDa antigens as examples of mannosylated proteins) have all been shown to be able to inhibit binding of *M. tuberculosis* to DC-SIGN (29). On SDS-PAGE/immunoblot, ManLAM and PIM_6 seemed the major ligands in *Mycobacterium bovis* BCG cell lysates when probed with a DC-SIGN-Fc construct (**Chapter 5**). As the *M. bovis* BCG double mutant deficient in the biosynthesis of both PIM_6 and the mannose cap on LAM (Δ*capA*Δ*pimE*) did still not show any reduction in binding to DC-SIGN

and DCs as compared to the wild-type strain, neither ManLAM nor PIM_6 appeared determining ligands on the mycobacterial cell surface in the *Mycobacterium*-DC-SIGN interaction (**Chapter 2** and **Chapter 5**). Noteworthy, recently tested cell lysates probed with DC-SIGN-Fc on SDS-PAGE/immunoblot using alternative experimental conditions (i.e. a different type of Tricine SDS-PAGE gel (35)) did also show similarly strong staining on immunoblot at the position of LM (≈ 15 kDa; unpublished results, R. Ummels), which was not observed previously (**Chapter 5**). Importantly, weak staining with DC-SIGN-Fc on all immunoblots could be observed of (mannosylated) proteins (**Chapter 5** and unpublished results, R. Ummels). To obtain insight in the role of mannosylated proteins in the interaction of mycobacteria with DC-SIGN, a knockout in the protein mannosyltransferase (PMT; gene *Rv1002c*) would be useful, as it catalyzes the first steps of protein O-glycosylation (49). At least for the 45 kDa-antigen, protein glycosylation reminiscent of the mannose cap has been identified, i.e. one to three α1,2-linked mannosyl residues at the different glycosylation sites (6). Unfortunately, the PMT-gene *Rv1002c* seems essential in *M. tuberculosis* based on absence of *Rv1002c*-mutant in a large random transposon mutant library (33).

Recently, four novel mycobacterial DC-SIGN-ligands have been reported, which are the proteins 60 kDa chaperonin-1 (Cpn60.1), DnaK, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and lipoprotein LprG (2). Interestingly, only the latter one, LprG is bound by the glycan binding site of DC-SIGN. Hence, the other three proteins probably represent a carbohydrate- and Ca^{2+} -independent DC-SIGN ligation. Noteworthy, treatment of *M. bovis* BCG with a low concentration of periodate (10 mM) to destroy carbohydrate rings only at the very surface-exposed structures without affecting cell viability (29), did significantly reduce binding of the bacteria to DC-SIGN (unpublished results, S. Kroeze). As also calcium-chelator EDTA is able to reduce the binding for approximately 80% (2), the Ca^{2+}-dependent binding of DC-SIGN to the mycobacterial cell surface glycans constitutes the major part in the ligation of DC-SIGN by mycobacteria.

Another possible candidate in mediating *Mycobacterium*-DC-SIGN interaction is mycobacterial cord factor: trehalose 6,6'-dimycolate (TDM). Recently, (non-mycobacterial) high trehalose concentrations (5 to 25 mM) were demonstrated to inhibit binding of DC-SIGN to various ligands (20). Trehalose (α-D-glucopyranosyl-(1□1)-α-D-glucopyranoside; a non-reducing disaccharide) shares stereochemistry at 3- and 4-OH groups with mannose (**Chapter 1**: Figure 4C). Trehalose is part of mycobacterial cord factor and thereby a major element of the mycobacterial cell envelope. However, the main receptor for mycobacterial TDM has recently been reported to be the C-type lectin Mincle (21, 39), and formal proof that TDM is a DC-SIGN-ligand is currently lacking.

Concluding, it can be hypothesized, that although the presence and/or binding to DC-SIGN of individual mycobacterial compounds may vary, all different types of DC-SIGN-ligands together -glycans, glycolipids, and mannosylated proteins- on the mycobacterial surface may contribute to the binding of *Mycobacterium* to DC-SIGN.

Evidently, mycobacteria express a redundant number of ligands for DC-SIGN in the cell wall. Interestingly, although the biosynthesis of DC-SIGN-ligands is not restricted to *M. tuberculosis* and even non-pathogenic environmental *Mycobacterium smegmatis* expresses DC-SIGN-ligands among which PIM_6, regarding whole bacterial cells, cellular DC-SIGN displays the highest affinity for *Mycobacterium* species of TB-complex (29). What the exact properties of *M. tuberculosis* are that enhance its recognition by DC-SIGN as compared to weakly or non-DC-SIGN-binding species, has still not been fully elucidated and remains an open and puzzling question. Beside the presence or absence of specific ligands, differences in structure and composition of the cell envelope and subsequent differences in accessibility for DC-SIGN to potential ligands, might also play a role.

A mutant in *M. tuberculosis* which still should be examined for its interaction with DC-SIGN, is a *Rv2181*-knockout. *Rv2181* encodes the mannosyltransferase responsible for the α1,2-mannosyl substitutions of the mannan core of LAM as well as for the elongation of the mannose cap on ManLAM (24, 25) (**Chapter 1**: Figure 3). Although the mannan core of LAM is not recognized by DC-SIGN (**Chapter 2** and (10)), this might be different for the mannan core of ManAM. It has been demonstrated that LAM of *M. smegmatis* Δ*aftC* of which the arabinan domain is truncated, displays strongly enhanced binding by DC-SIGN-Fc (1). Hence, it seems that the arabinan domain shields the mannan domain for interacting with DC-SIGN. In ManAM, the MPI-anchor is absent and the mannan core may be more accessible to DC-SIGN as compared to the mannan core of ManLAM. Thus it can be hypothesized that ManAM as well as 'capless' AM in the capsule of *M. bovis* BCG Δ*capA* contribute significantly to the binding of mycobacteria by DC-SIGN. A homolog for *Rv2181* does exist in DC-SIGN non-binder *M. smegmatis* (*MSMEG_4247*) and is involved in the addition of mannosyl substitutions on the mannan core of LAM as well (24). However, in contrast to Rv2181 of *M. tuberculosis* and its homologs in *M. bovis* BCG and *M. marinum*, MSMEG_4247 did not elongate the monomannoside caps present on LAM of *M. smegmatis* complemented with the *M. marinum capA*-gene (**Chapter 2**). This indicates that *M.tuberculosis*-Rv2181 has a broader function as compared to its homolog in *M. smegmatis*. At the cell surface of a *Rv2181*-mutant strain, and even more likely in a *pimE-Rv2181* double-knockout, the α1,2-linked mannose structures which mediate in *Mycobacterium*-DC-SIGN, might be significantly reduced, i.e. causing a reduction in binding of the bacteria by DC-SIGN, but this is to be tested yet.

ManLAM in mycobacterial infection

As conceived dominant ligand for DC-SIGN, ManLAM was hypothesized to mediate the escape from immune surveillance by mycobacteria via DC-SIGN (10, 11). As mentioned above, unexpectedly, ManLAM appeared not to be the dominant ligand on the mycobacterial cell surface for DC-SIGN and the *M. bovis* BCG Δ*capA* mutant strain was neither reduced in cellular DC-SIGN-binding nor did the mutant strain show a phenotype in *in vivo* mice infection studies (**Chapter 2**). Furthermore, in an

independent study, neither a clear enhancement nor a clear reduction in protection against TB-infection was observed by vaccinating mice with the *M. bovis* BCG Δ*capA* strain as compared to the wild-type strain (9), which indicates minor influence of the mannose cap on the immune response induced. However, *M. bovis* BCG may not be a good model to study TB-infection as in contrast with *M. tuberculosis*, *M. bovis* BCG is not able to escape to the cytosol after phagocytosis Mφ and DCs (48). Hence, although *M. bovis* BCG probably is bound similarly well by DC-SIGN as compared to *M. tuberculosis*, the subsequent course of infection is not completely similar. Therefore, *M. tuberculosis* Δ*capA* might show a different phenotype *in vivo*. In particular, as a possible role for the mannose cap of ManLAM in phagosome-lysosome fusion has been reported ((40, 50, 51), discussed in **Chapter 7**). In murine Mφ infected *in vitro* with *M. marinum* Δ*capA*, a small, but significant increase in phagosome-lysosome fusion was observed as compared to infection with *M. marinum* wild-type (**Chapter 2**). In a similar assay by another group however, no differences in phagosome-lysosome fusion could be detected when murine Mφ were infected with *M. bovis* BCG Δ*capA* (9).

Although ManLAM may not be the determining ligand in *Mycobacterium*-DC-SIGN ligation, together with the other DC-SIGN-ligands present on the mycobacterial cell surface, ManLAM signals via DC-SIGN and can induce specific changes in the immune response (7). Various results have been obtained in co-incubation studies of ManLAM with DCs. In LPS-activated DCs, ManLAM has been reported to enhance or reduce production of the pro-inflammatory cytokine IL-12p70 in a mannose-cap dependent way ((15) and (28), respectively). Furthermore, ManLAM enhances production of anti-inflammatory cytokine IL-10 in LPS-activated DCs in *in vitro* assays (10, 15), but not in DC-SIGN-expressing alveolar Mφ from TB-patients *ex vivo* (41). Importantly, all these studies were performed in the presence of the non-mycobacterial TLR4-ligand LPS as ManLAM by itself does not induce a response, while cross-talk between DC-SIGN and TLR2 (in addition to TLR4) may also be very relevant to study in relation to TB-infection. Moreover, IL-10 cannot be detected in BAL fluids from patients with TB (41). Hence, these *in vitro* assays, including the DCs-infection experiments in **Chapter 2** and **Chapter 5** can indicate potential effects of ManLAM on the immune response upon infection with *M. tuberculosis*, but not completely clarify the role of DC-SIGN in an *in vivo* TB-infection, in particular in the lung.

One big hurdle for studying DC-SIGN *in vivo* are the many differences between human DC-SIGN and the murine SIGN-homologs of which there are seven (30). Both human DC-SIGN expressed in transgenic mice, and murine SIGNR3, which is regarded as the homolog most similar to human DC-SIGN, were shown to contribute to host defense during TB-infection in mice (34, 43), opposite to the pathogen-favorable role initially hypothesized for DC-SIGN. However, SIGNR3 is redundant in controlling long-term infection and differs from human DC-SIGN in signaling as it is dependent on tyrosine kinase Syk (43), while human DC-SIGN does not (14). In contrast to SIGNR3, an immunosuppressive function for SIGNR1, which also recognizes mycobacterial mannosylated structures, has been reported in suppression of T cell activity in mice

(52). But then, similar to SIGNR3, SIGNR1 is also redundant in controlling the *in vivo* infection with *M. tuberculosis* (52). Hence, as multiple murine SIGNR-lectins exist with seemingly different roles in TB-infection, results obtained from studies on (one of the) murine SIGNR cannot easily be extrapolated to humans and human DC-SIGN.

Overall, DC-SIGN is evidently not solely an escape route for *M. tuberculosis*. DC-SIGN seems to have a function in protection against *M. tuberculosis*, possibly in controlling bacterial load and/or in preventing an exaggerated immune response (8). However, DC-SIGN still is the main cell entry route for mycobacteria into DCs, which subsequently can become a reservoir of live bacteria next to Mφ. Studying the role of DC-SIGN by creating a mutant *M. tuberculosis* strain which is not bound by DC-SIGN turned out to be a mission impossible. At least a multiple knockout strain in which the biosynthesis of several DC-SIGN-ligands is impeded, would be required, and this strain might then be affected in cell growth and/or interaction with other receptors as well.

One highly expressed receptor on DCs and Mφ with a similar recognition pattern as DC-SIGN, is the MMR. The MMR binds higher-order PIMs and ManLAM, but not AraLAM or PILAM (37, 45). Furthermore, the MMR displays binding preferences for specific *M. tuberculosis* strains (virulent strains Erdman and H37Rv, but not for avirulent H37Ra), which, similar as observed for DC-SIGN-binding, cannot be completely ascribed to the presence or not of a mannose cap on LAM: LAM of both H37Rv and H37Ra carry mannose caps (38). Also for the MMR, functions in regulation of the immune response upon mycobacterial infection have been reported (32, 45). Considering this preferential binding by MMR for virulent *M. tuberculosis* strains plus the above mentioned observation that DC-SIGN binds the *Mycobacterium* species of the TB-complex with the highest affinity (29), it can be hypothesized that the heavily mannosylated cell surface of mycobacteria is evolved as mechanism for host adaptation (47). Mannose-dependent *Mycobacterium*-host interactions might lead to a more balanced immune response (8), but may also favors a chronic (latent) TB-infection rather than resulting in acute infection (47). Two clinical isolates of *M. tuberculosis* (HN885 and HN1554) with truncated, less-exposed ManLAM and a reduced amount of higher-order PIMs in the cell wall, exhibited lower association to the MMR and Mφ as compared to *M. tuberculosis* strain Erdman (46). Interestingly, after phagocytosis by Mφ, the HN885 and HN1554 strains replicate at faster rate as compared to the more heavily mannosylated Erdman strain, which indicates that these strains might be hypervirulent and in an *in vivo* infection would progress into active TB (46, 47). However, additional differences were observed in the cell wall composition of these strains and furthermore, MMR-independent phagocytosis is reduced as well (46). Hence, differences in virulence or course of infection *in vitro* and *in vivo* between the *M. tuberculosis* strains cannot unambiguously be attributed to differences in degree of cell surface mannosylation. Next to this, it was already noticed that in the presence of serum, the complement receptors are the preferred route of cell entry by Mφ *in vitro* instead of the MMR (36). *In vivo* the conditions are even more complex and many other receptors not depending on mannose-recognition, e.g. Mincle and

the complement pathway, are present that potentially can mediate in lung cells the entry of mycobacteria. To determine the relevance of mannose-dependent interactions in the establishment of the infection, an isogenic pair of wild-type and hypo- or hypermannosylated strain should be studied, e.g. *M. tuberculosis* wild-type versus a *pimE-Rv2181* double-knockout strain which has reduced α(1,2)-linked mannosyl structures on its cell surface. Another strategy is to specifically inhibit mannose-dependent host-pathogen interactions by masking the mannosylated mycobacterial cell surface with an exogenous compound.

In view of that, we investigated the effect of mannose-binding lectin Cyanovirin-N (CV-N), which has been shown already to block mannose-dependent cell entry of HIV-1, on *Mycobacterium* in *in vitro* and *in vivo* infection (**Chapter 6**). ManLAM appeared to be the major ligand for CV-N on the mycobacterial surface, and interestingly, although ManLAM is not the dominant ligand for cellular DC-SIGN, CV-N was able to reduce *in vitro* binding of the bacteria to DC-SIGN and monocyte-derived DCs. In contrast with the DCs, CV-N did not have any effect on the binding of mycobacteria to Mφ *in vitro*. As CV-N did inhibit binding of mycobacteria and mycobacterial ligands to a Fc-construct of the MMR in an ELISA-format assay, this indicates that cell entry via the MMR is not the primary uptake route for the monocyte-derived Mφ *in vitro*. More importantly, coating the mycobacterial cell surface with CV-N, even when a ratio CV-N:mycobacterial cells was used higher than in the *in vitro* assays, did not prevent or delay pulmonary TB-infection in mice up to four weeks post infection. This suggests that, at least in short-term, acute TB-infection, mannose-dependent *Mycobacterium*-C-type lectin interaction is neither critical for cell entry by the pathogen nor does it affect the bacterial load in the host (**Chapter 6**). Hence, although several other reasons can be thought of for the lack of effect by CV-N in *in vivo* TB-infection, inhaled drug therapy that targets the mannosylated surface of *M. tuberculosis* only to inhibit C-type lectin-mediated uptake will likely be ineffective in prevention of or reducing pulmonary infection and dissemination to other organs.

The results obtained with CV-N-coating of mycobacteria seems partly in line as well as partly contradictory with two previous studies, which have examined the protective effect of monoclonal antibodies directed against the glycan part of LAM (18, 44). In the first study, a murine IgG3-mAb 9d8 against arabinomannan was coated on intracheally administered bacteria (44). Although the mAb does protect against TB (enhanced survival of TB-infected mice), it has no effect on bacterial load in the lung (44). Hence, the protective effect of mAb 9d8 appears not to be due to preventing arabinomannan-dependent host-pathogen interactions and subsequent lung cell entry. However, in a later study, both an IgG1 antibody (mAb SMITB14) directed against the glycan part of LAM as well as its $F(ab')_2$-fragment alone were shown to be protective and to reduce bacterial load in the lung when injected either intravenously or intranasally (18). The protective effect by the $F(ab')_2$-fragment alone is obviously Fc-receptor-independent and mediated solely by ligation to glycan part of LAM (18). This indicates that binding of the $F(ab')_2$-fragment of this mAb might reduce bacterial load in the lung by inhibition

of cell entry, although it was also suggested that binding of the $F(ab')_2$-fragment might interfere with the LAM-mediated inhibition of phagosome-lysosome fusion (reviewed in **Chapter 7**) and in this way possibly exert its protective effect (18). Of note, the exact epitopes recognized by the mAbs in these studies is not known (13, 18, 44), which can explain the differences in the outcome of these passive protection studies.

Concluding, ManLAM or related mannosylated structures can be potential vaccine candidates, e.g. as mucosal vaccine eliciting an IgA memory response, but the protective properties cannot solely rely on inhibition of mannose-dependent cell entry. Using the whole glycolipid ManLAM as a vaccine is not the first choice as it is poorly immunogenic and may have undesired side effects as it can modulate the immune response, but the glycan part, AM, conjugated to a carrier protein (e.g. tetanus toxoid) have been reported to be protective when coadministered with additional adjuvant or as booster vaccine after primary BCG vaccination (reviewed in (23)).

Mannose caps: virulence factors or decorative elements?

The mannose cap of LAM does not appear to be a dominant virulence factor of *M. tuberculosis*. This cap is also expressed by non-virulent strains, such as strain H37Ra and *M. bovis* BCG. On the other hand, the expression of the longer, di- and tri-mannoside caps is restricted to specifically the slow-growing group of *Mycobacterium* species that harbours the most pathogenic ones with might only one exception (*Mycobacterium holsaticum*) (**Chapter 4**). Taking further in consideration the fact that cap-like α1,2-linked mannose structures can also be found in high amounts in all *Mycobacterium* species as part of PIM_6 and the mannan core of LM, LAM and AM, and that small amounts of monomannoside caps seem present on LAM of some rapid-growing *Mycobacterium* species as well (**Chapter 4**), this raises several questions: what is the function of these mannosylated glycolipids? Does the mannose cap on ManLAM of the slow-growing mycobacteria have a distinctive function in immunomodulation? And how did the mannose cap evolve?

LAM, LM and PIMs are first of all components of the mycobacterial cell envelope with a role in cell wall integrity (**Chapter 7** and reviewed in (16)). Besides this, the mycobacterial cell has a preference of producing higher-order glycolipids above the smaller structures: in a *M. smegmatis* inositol auxotroph mutant strain, inositol-deprivation in culture medium leads first to depletion of PI and PIM_2 in the cell wall, followed by PIM_6 (17). While depletion of PIM_2 does not have major effects on the cell wall, loss of PIM_6 affects cell wall integrity and composition significantly and results in loss of cell viability (17). Furthermore, the lipoprotein LpqW has been identified as regulator of PIM/LAM-biosynthesis in favor of LAM (4, 26). A *M. smegmatis lpqW*-mutant strain which is unable to produce LAM, but has a wild-type PIM-composition, is not stable and displays altered colony morphology when grown on culture medium (26). This strain is consistently outgrown by the *lpqW* double mutants in which an additional, evidently compensatory, spontaneous mutation in *pimE* has occurred (4). In the *lpqW-pimE* double knockout strain, PIM_4 -precursor of both PIM_6 and LM/LAM-

is both used for LAM-production and end product as biosynthesis of PIM_6 is blocked (27). In this latter strain, colony morphology and cell growth are restored to wild-type phenotype, revealing the important roles of LpqW in channeling PIM_4 into LAM-biosynthesis and of LAM in cell wall integrity (4). Hence, LAM and related glycolipids are essential cell wall components. Still not all genes involved in the biosynthesis of ManLAM have been identified and probably more unknown genes involved in the tight regulation of the biosynthesis exist which might be difficult to discover (**Chapter 3** and **Chapter 7**).

Although the basic structure of LAM is conserved in the genus *Mycobacterium*, small, but possible crucial, differences exist, of which one is the presence, type, and number of caps on the LAM-molecule. The di- and tri-mannoside caps on LAM have likely evolved with the emergence of slow-growing mycobacteria in which mannosyltransferase Rv2181 gained an extra function in elongation of the monomannoside cap next to substitution of the mannan core LAM with single mannosyl residues (**Chapter 4**). Many immunomodulatory properties for ManLAM have been described in which the mannose cap appeared essential (**Chapter 7**), and thus a distinctive role for these specific α1,2-linked mannosyl residues constituting the mannose cap can be postulated, but then in addition to other functions of LAM and related lipoglycans in the mycobacterial cell wall. Of these immunomodulatory effects of ManLAM attributed to the mannose cap, some might also be due to other subtle differences in LAM (e.g. size of the different domains, acylation) as often LAM from different species has been used as comparison (e.g. PILAM from *M. smegmatis*). Noteworthy, in the debate on cell surface exposition of LAM and its accessibility to host immune receptors (**Chapter 7**), a recent paper showed the active release of membrane vesicles by mycobacteria, including release within host cells (31). Interestingly, analysis of the contents of these vesicles revealed the presence of LAM among other lipids and proteins (31), confirming that LAM is released from the mycobacterial cell and in this way can potentially exert its effect on the immune system of the host.

Although ManLAM and its mannose cap, may not be the primary determinants of the virulence of a *Mycobacterium* strain, ManLAM can 'assist' virulent species in establishment of the infection and/or enhance intracellular survival by modifying the host response. In particular the longer di- and tri-mannoside caps can be seen in this light, as these caps appear restricted to the group of slow-growing mycobacteria which includes the most in number and the most severe pathogenic species (**Chapter 4**). However, the role of the mannose cap as virulence factor seems redundant as *M. bovis* BCG wild-type and the *capA*-mutant behave similarly in the host (**Chapter 2**), but this could well be different for *M. tuberculosis* Δ*capA*. Redundancy in strategies exploited by mycobacteria to infect their host and subsequently to sustain the infection, appears reflected in the host immune system. A recent study shows functional redundancy at the level of pattern recognition receptors (PRRs), including C-type lectins, in the long-term control of mycobacterial infection (3). As each of the PRRs has its own ligand specificity, a broad range of mycobacterial molecules can be recognized by the host and

induce an immune response against the pathogen. This also appeared the case in the CV-N-study in **Chapter 6**: although *M. tuberculosis* expresses a redundant number of multiple mannosylated surface components suggesting a role in host adaptation for these structures, (partial) inhibition of mannose-dependent host-pathogen interactions by CV-N did not have any effect on the course of an *in vivo* TB-infection.

Concluding, as it is estimated that one-third of the world population is infected with *M. tuberculosis* but only 5-10% develops active disease, the many ways in which humans and mycobacteria can interact, of which one is via ManLAM, are most often beneficial for both host and pathogen.

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