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MINI REVIEW

Mycobacterial lipoarabinomannan: an extraordinary lipoheteroglycan with profound physiological effects

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Detailed structural and functional studies over the last decade have led to current recognition of the mycobacterial lipoarabinomannan (LAM) as a phosphatidylinositol anchored lipoglycan with diverse biological activities. Fatty acylation has been demonstrated to be essential for LAM to maintain its functional integrity although the focus has largely been on the arabinan motifs and the terminal capping function. It has recently been shown that the mannose caps may be involved not only in attenuating host immune response, but also in mediating the binding of mycobacteria to and subsequent entry into macrophages. This may further be linked to an intracellular trafficking pathway through which LAM is thought to be presented by CD1 to subsets of T-cells. The implication of LAM as major histocompatibility complex (MHC)-independent T-cell epitope and the ensuing immune response is an area of intensive studies. Another recent focus of research is the biosynthesis of arabinan which has been shown to be inhibitable by the anti-tuberculosis drug, ethambutol. The phenomenon of truncated LAM as synthesized by ethambutol resistant strains provides an invaluable handle for dissecting the array of arabinosyltransferases involved, as well as generating much needed structural variants for further structural and functional studies. It is hoped that with more systematic investigations based on clinical isolates and human cell lines, the true significance of LAM in the immunopathogenesis of tuberculosis and leprosy can eventually be explained.

Key words: CD1/ethambutol/lipoarabinomannan/lipomannan/mycobacteria/phosphatidylinositol mannosides/tuberculosis

Introduction

Mycobacteria have evolved many specific adaptations that enable them to infect and survive within specific host cells. Such host–pathogen interactions are mediated by specialized molecules, in particular, those associated with the unique cell envelope. The essence of the mycobacterial cell wall is the mycolyl arabinogalactan–peptidoglycan complex (mAGP) and the associated lipoarabinomannan (LAM) (Brennan and Nikaido, 1995; Figure 1). mAGP constitutes the underlying core of the wall proper, whereas LAM has been shown to exert profound physiological effects. We review here our current understanding of the structure, biosynthesis, and function of this extraordinary lipoglycan which is likely to be a key virulence factor and drug target in the treatment of mycobacterial diseases including tuberculosis and leprosy.

Early perception of arabinomannan

The existence of an arabinose- and mannose-containing, serologically active polysaccharide in mycobacterial cell wall was first recognized in the 1930s (Chargaff and Schaefer, 1935; Menzel and Heidelberger, 1939; Seibert and Watson, 1941). Some 20 years later, Tsumita *et al.* (1960) and then Ohashi (1970), among others, demonstrated that there are two classes of arabinomannan (AM) in *M.tuberculosis*, namely acylated and nonacylated. Only the "macromolecular lipopolysaccharide" type which contains palmitic and tuberculostearic acids was active in the hemagglutination assay.

Despite this observation, the early perception of AM was mainly that of a neutral polysaccharide, propounded by a series of structural studies by Azuma, Misaki, and coworkers (Azuma *et al.*, 1968; Misaki *et al.*, 1977), who also characterized the related arabinogalactan (AG) of mAGP. Working on a polysaccharide fraction isolated by vigorous alkaline extraction, they concluded that AM consists of an α(1→6)-linked D-Man*p* backbone to which were attached short side chains of α(1→2)linked D-Man*p* residues and α(1→5)-linked D-Ara*f* residues. In addition, a serologically inactive mannan whose structure may resemble that of the mannan core of AM was also isolated.

In independent work, Weber and Gray (1979) isolated and partially characterized an acidic AM from *M.smegmatis* which was shown to comprise approximately 56 arabinosyl residues, 11 mannosyl residues, 2 phosphates, 6 monoesterified succinates, and 4 ether linked lactate groups. After saponification, this polysaccharide could be separated into phosphorylated and nonphosphorylated forms, but there was no mention of fatty acylation. The polysaccharide had the ability to precipitate antisera from rabbits immunized with cell walls of *M.smegmatis*, and it was concluded that both AM and AG shared the common immunodominant epitope, i.e., chains of contiguous α-(1→5)-linked arabinofuranosyl residues.

LAM, the current structural model

Our present day understanding of the cell wall associated AM and mannan as true lipoglycans, termed LAM and lipomannan (LM), respectively, stems from the seminal work by Hunter, Chatterjee, Brennan, and coworkers (Hunter *et al.*, 1986; Hunter and Brennan, 1990; Chatterjee *et al.*, 1992a). Critically important in these works was the development and successful application of organic solvent extraction procedures followed by phenol-water biphasic wash, to yield LAM/LM in their native acylated states

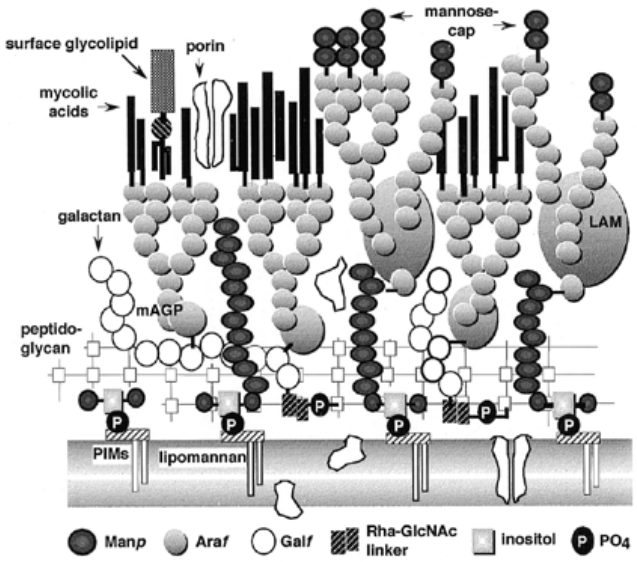

Fig. 1. A chemical model of the mycobacterial cell wall. The three-dimensional and spatial arrangement of the key molecules are largely unknown. It is thought that most mycobacterial cell walls conform to this model with mAGP and LAM as the two principal constituents. The surface glycolipids include a variety of species- and strain-specific glycopeptidolipids, lipo-oligosaccharides, and phenolic glycolipid, the chemical identity and amount of which varies from one species to another.

devoid of most of the proteins and neutral polysaccharides. Currently in the author's laboratory, the extracted LAM and LM are routinely separated as individual entities using size exclusion column chromatography with a matrix of Sephacryl S-200 in the presence of a buffer containing deoxycholic acid, 0.5 M EDTA, 1 M NaCl in 10 mM Tris at pH 8.0 (Chatterjee *et al.*, 1992a). This allows most of the structural analysis, as well as biological studies, to be carried out on relatively pure LAM and LM preparation from various strains of mycobacteria.

Additionally, LAM/LM have been shown to bind to the hydrophobic matrix of octyl Sepharose through their fatty acyl chains at low concentrations of propanol (5–15%, v/v) and salt (≥0.05 M) (Leopold and Fischer, 1993). This property has been exploited to further separate LAM/LM from nonacylated forms or contaminating polysaccharides (Khoo *et al.*, 1996). Alternatively, Leopold and Fischer (1993) demonstrated that LAM and LM can be resolved on such hydrophobic interaction columns. With an increasing propanol gradient, their elution profile was apparently dominated by the large size difference of the glycan moieties and then, secondly, by the number of fatty acids.

Phosphatidylinositol mannoside anchor

To date, our cumulative structural data are consistent with the mannan core in both LAM and LM being based on an α1→6 linked backbone, substituted to varying degrees at position 2 with single α-Man residues, and directly attached to position 6 of the *myo*-inositol of a phosphatidylinositol (PI) anchor (see Figure 2, for schematic model).

In cases where the PI anchor portion of LM and LAM has been examined in detail (Chatterjee *et al.*, 1992a; Venisse *et al.*, 1995), it was found to be indistinguishable from the dimannosylated phosphatidylinositides (PIM_2), the structures of which were established over 30 years ago (Lee and Ballou, 1964). The predominant fatty acyl chains are palmitate (C16:0) and 10-methyloctadecanoate (tuberculostearate, C19), with smaller amounts of C14:0; C17:0; methyl-C17:0; and C18:0. Just as PIM_2 and other higher phosphatidylinositol mannosides (PIMs) were known to carry up to four fatty acyl chains, with additional acylation on the mannoses (Pangborn and McKinney, 1966; Brennan and Ballou, 1967; Khoo *et al.*, 1995a), a portion of the PI mannan core of LAM and LM from *M.tuberculosis* and *M.leprae* was likewise implicated to carry additional fatty acylation on their PIM_2 moiety (Khoo *et al.*, 1995a). This is consistent with the findings of Leopold and Fischer (1993), who resolved LAM and LM from *M.tuberculosis* into species carrying two to four fatty acyl chains on their hydrophobic interaction columns.

On the other hand, Venisse *et al.* (1995) recently reported the presence of a portion of mannan core without PI anchors existing alongside those which do terminate with PIM_2 moiety, in a deacylated LAM preparation from *M.bovis* BCG, after acid treatment (0.1N HCl, 100°C, 15 min). More intriguing is the identification by the same group of a phosphoinositols-glyceroarabinomannan (abbreviated to PI-GAM by the authors) from *M.smegmatis* which apparently carries a nonacylated phosphoinositol-glycerol at the reducing end. The absence of C16 and C19 fatty acids in their PI-GAM preparation was in support of the conclusion drawn from detailed NMR analysis but was contrary to most other studies on LAM from *M.smegmatis* where fatty acylation can be readily demonstrated (D. Chatterjee, unpublished observation).

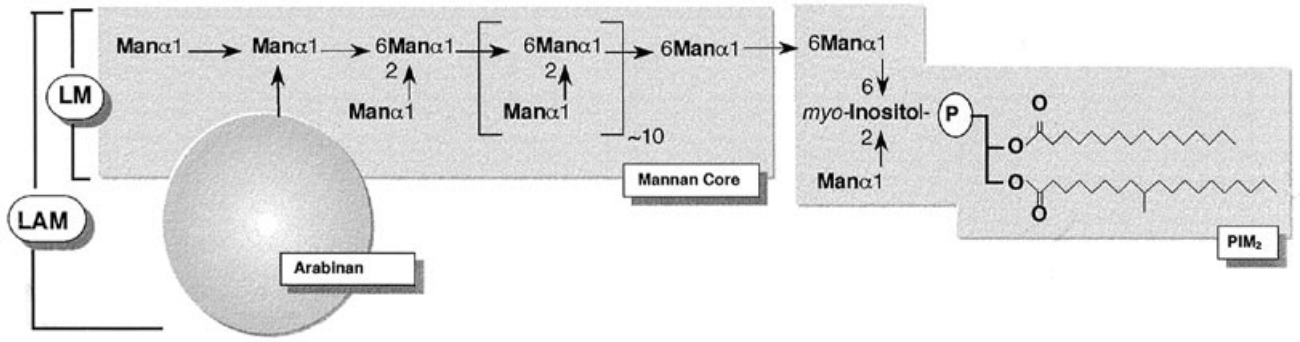

Fig. 2. Schematic visualization of the structural relationship of LAM, LM, and PIM_2.

From these studies, it is almost certain that there are heterogeneity and species/strain variations in the position and degree of fatty acylation on LAM. However, by virtue of their extraction and purification procedures, as well as electrophoretic mobility on SDS–PAGE, LAM and LM are without doubt true lipoglycans, and a majority, if not all, do carry a PIM_2 anchor. Loss of fatty acylation or the PI anchor probably represent experimental artefacts, or degradation in the normal or induced turnover and secretion of AM into the culture medium (Lemassu and Daffé, 1994).

From PIM_2 to mannan core

The biosynthesis of PIMs of *M.tuberculosis* was pursued as early as 1967 (Brennan and Ballou, 1967). The fundamental pathway that emerged from this early work was: **PI** → **PIM_1**→ **PIM_2**→ **PIM_3** → **PIM_4**→ **PIM_5** → **PIM_6**, with GDP-mannose as the mannosyl group donor (the subscript on M denotes the number of mannose residues). It was believed that PIM_6 is not on the direct pathway to the formation of LAM but is an end product. On the other hand, PIM_2 (or possibly PIM_3 and PIM_4) could be extended to a linear α1–6 mannan with a chain length of approximately 16 residues, by sequential transfer of α-mannose residues from GDP-mannose to PI via particulate microsomal enzymes (Besra *et al.*, 1997). However, heptaprenyl-P-Man (C_{35}-P-Man) and the decaprenyl-P-Man (C_{50}-P-Man) have since been discovered (Yokoyama and Ballou, 1989), and these are now believed to be also the mannosyl donors in the initial polymerization of the mannan core of LAM (Besra and Brennan, unpublished observations).

Within this context, little is known about the status of LM, whether it represents another end product from a common biosynthetic pathway, or whether it is an intermediate and a substrate for arabinosylation. Nor is it known whether addition of a single mannose stub along the linear α1–6 mannan backbone bears any significance to the regulation of biosynthesis from PIMs to LM and LAM. The mannan cores from LAM of both *M.tuberculosis* Erdman (Chatterjee *et al.*, 1993) and *M.bovis* BCG (Venisse *et al.*, 1995) were inferred to be highly branched and estimated to be around 20 mannose residues in total, with considerable heterogeneity with respect to the exact length and degree of branching. The LM of Erdman appeared to be much longer than that of the corresponding LAM mannan core, as estimated from one MALDI-MS analysis (Khoo and Chatterjee, unpublished observations). In contrast, both LAM mannan core and LM from *M.smegmatis* were shown to be averaging about 26 residues (Khoo *et al.*, 1996) and only about half of the α6-mannan backbone residues are further substituted at position 2. Despite detailed NMR and chemical analysis, the exact position and the number of the attachment site(s) for the arabinan chains have never been defined. This is further compounded by the putative presence of additional phosphorylation on the mannan core (Venisse *et al.*, 1995; Khoo and Chatterjee, unpublished observations).

Arabinan motifs and capping functions

While the PI mannan core may be entirely embedded within the cell wall, the arabinan of LAM has been demonstrated to be exposed on the surface and directly implicated in the immunopathogenesis of leprosy and tuberculosis (Brennan *et al.*, 1990). The seminal work, on what has been called arabinofuranosyl-terminated LAM (AraLAM) from a rapidly growing strain of

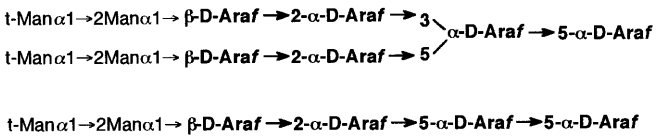

Fig. 3. The nonreducing termini of the arabinan in LAM. The branched Ara_6 and Ara_4 motifs are shown here with the most abundant cap, namely Man_2, which therefore yields Man_4Ara_6 and Man_2Ara_4 as the most abundant components in the endoarabinase digestion products of ManLAM.

Mycobacterium (Chatterjee *et al.*, 1991), revealed two distinct types of nonreducing termini, which would give rise to the linear Ara_4 and branched Ara_6 motifs (Khoo *et al.*, 1995b) when digested with a novel endoarabinanase obtained from a soil *Cellulomonas* species by selective culturing on mycobacterial arabinogalactan (McNeil *et al.*, 1994) (Figure 3).

Interestingly, the same two terminal arabinan motifs were subsequently found to be extensively (≈ 70%) capped with residues of α-Man*p* in LAM from the virulent Erdman strain of *M.tuberculosis*, a product which was then termed ManLAM (Chatterjee *et al.*, 1992b). The mannose-caps consist exclusively of mono-, di-, and trimers of α-D-mannoses (Manα1→ [2Manα1]$_{0,1,2}$ →) directly linked to C-5 of the terminal β-D-Ara*f*. Upon digestion with endoarabinanase, ManLAM yielded Man_4Ara_6 and Man_2Ara_4 as the most abundant products, along with Ara_2 deriving from, presumably, the inner chains which link the termini together and attach them to the PI-mannan core (Chatterjee *et al.*, 1993).

The initial speculation that mannose-capping is restricted to virulent strains of *M.tuberculosis* was not supported by more recent structural studies. It has since been shown that LAMs from all *M.tuberculosis* strains examined (Erdman, H37Rv and H37Ra), as well as the attenuated *M.bovis* BCG vaccine strain, are mannose-capped with the extent of capping varying between 40–70% (Prinzis *et al.*, 1993; Venisse *et al.*, 1993; Khoo *et al.*, 1995b). A considerably smaller proportion of the nonreducing termini of LAM from *M.leprae* were also found to terminate with mannoses, whereas those from the fast growing *M.smegmatis* are not (Khoo *et al.*, 1995b; Gilleron *et al.*, 1997).

In addition, a novel inositol phosphate capping motif was identified on a minor portion of the otherwise uncapped arabinan termini of AraLAM, which may partly account for its characteristic biological properties associated with macrophage activation (Khoo *et al.*, 1995b). Based on one-dimensional ^{31}P and two-dimensional (^{1}H-^{31}P) heteronuclear studies (Gilleron *et al.*, 1997), these phosphoinositide motifs were further shown to occupy the terminal β-d Ara*f* motifs of the AraLAMs. About four such phosphoinositide motifs were found to be present per molecule of AraLAM of which three were found to be mild alkaline labile. These recent studies resolved the earlier reports on the presence of alkaline stable and labile inositol phosphate on AraLAM from fast growing species (Hunter and Brennan, 1990) but still left the succinyl and lactyl substituents (Weber and Gray, 1979; Hunter *et al.*, 1986) unaccounted for.

LAM as a heterogeneous lipoglycan

It is now well appreciated that LAM from any single source is heterogeneous in size, branching pattern, acylation, and phosphorylation, on both the arabinan and mannan portions. Thus, any structural feature that can be physicochemically defined is a weighted average of the composite molecular species. The

extreme heterogeneity is evident from the broad diffuse band observed on SDS–PAGE analysis of LAM and LM (Hunter *et al.*, 1986), as well as from several recent MALDI-MS studies which afforded an indication of the mean distribution of true molecular weight. It was shown that native LAM from *M.bovis* BCG and *M.tuberculosis* gave a broad peak centered at 17.3 kDa, and 16.7 kDa after deacylation, with a reported size distribution of 4 kDa heterogeneity (Venisse *et al.*, 1993). Likewise, the molecular weight of LAM from *M.smegmatis* was estimated to be around 15 kDa from the MALDI mass spectrum of the permethylated samples (Khoo *et al.*, 1996). Depending on the species/strains, the total arabinose averages around 70 - 80 residues, with different degrees of branching and relative amounts of the Ara_4 and Ara_6 terminal motifs. In fact, the latter feature, as estimated from high pH anion exchange chromatography (HPAEC) analysis of the endoarabinanase digestion products, becomes an important criterion in distinguishing the arabinan of LAM and AG (Khoo *et al.*, 1996).

Ethambutol resistance and truncated LAMs

Despite apparent structural similarity, the biosyntheses of the arabinans of AG and LAM are differentially inhibited by the anti-tuberculosis drug ethambutol (Emb). It has been demonstrated that the incorporation of [^{14}C]glucose into the cell wall arabinan of *M.smegmatis* was immediately inhibited upon addition of Emb to young cultures but that into LAM was not apparent until after 1 h of exposure (Takayama and Kilburn, 1989; Deng *et al.*, 1995; Mikusová *et al.*, 1995). Furthermore, when grown in the presence of Emb, an Emb-resistant mutant derived from *M.smegmatis* by consecutive passage in media containing increasing concentrations of Emb, apparently made "normal" cell wall AG but while the LAM was truncated (Mikusová *et al.*, 1995). Truncation in the structure was subsequently demonstrated as primarily a consequence of selective and partial inhibition of the synthesis of the linear Ara_4 terminal motif, which constitutes a substantial portion of the arabinan termini in LAM but not in AG (Besra *et al.*, 1995; Khoo *et al.*, 1996).

Similar truncation of LAM was observed when *M.smegmatis* was transfected with plasmids containing the *emb* region from *M.avium* which encodes for Emb resistance. Sequence analysis indicated that there are three genes in this region, *embR*, *embA*, and *embB*, and that the translationally coupled *embA* and *embB* genes are necessary and sufficient to confer an Emb-resistant phenotype when expressed in *M.smegmatis* on a multicopy vector (Belanger *et al.*, 1996). Thus, Emb resistant strains were derived by electroporation with the plasmids pAEB 148 (containing *embR*, *embA*, and *embB*) and pAEB 109 (containing only *embA* and *embB*). A cell-free system developed by Lee *et al.* (1995) was previously shown to be effective in using the lipid carrier, decaprenylphosphoarabinose (DPA), as a donor of arabinose in the polymerization of arabinan. The incorporation of radiolabeled Ara into a polymer of arabinan was inhibited to a maximum level of 70% when increasing amounts of Emb were added to the reaction mixture containing membrane fractions of *M.smegmatis*. A comparative study showed that similar extracts from the Emb resistant recombinant strains carrying pAEB148 and pAEB109 were only inhibited to about 30–35% and 55–60%, respectively (Belanger *et al.*, 1996). These results indicated that *embA* and *embB* gene are associated with high level of Emb-resistant arabinosyltransferase activity and that *embR* is required for maximum resistance.

Based on the known structure of arabinan, it may be speculated that the polymerization of arabinan is essentially an $\alpha 1 \rightarrow 5$ elongation of the arabinan chains punctuated by $\alpha 3$-branching. The linear terminal Ara_4 motif is a consequence of nonbranched termination with $\beta 2$-Ara, whereas the terminal Ara_6 motif is the branched counterpart. Thus, Emb may be inhibiting all or most of the arabinosyltransferases involved in the biosynthesis of arabinan, a phenomenon not unexpected, given that all individual arabinosyltransferases are likely to recognize and utilize the same donor such as DPA, and hence containing structurally homologous active sites. The differential effect of Emb in eliciting synthesis of truncated LAMs but normal AG in the resistant strains is a consequence of the differential requirement of these two arabinan-containing components in growth. Selection for growth in culture in the presence of Emb entails that the mutant or recombinant must now be able to make functional AG, whereas a defective LAM is tolerable, at least for *in vitro* growth. These would translate into a more stringent requirement for the $\alpha 3$-branching arabinosyltransferase (or the composite biosynthesis machinery specifically required for making Ara_6); a target needs neutralization by overexpression or mutation in order to grow. In the presence of Emb, the competition between branching and elongation would then be distorted in favor of the branching Ara_6 terminal motif, resulting in the phenomenon of truncated LAM.

Orientation of LAM in cell envelope

The observation that a full size mature LAM is not a requisite for mycobacterial growth in culture does not discount the biological significance in its partial inhibition. It may be argued that in order for LAM to effectively induce and/or suppress the proper immune response in the host (see following sections), a fully functional LAM is required and that most of its function will be critical depending on the integrity of its terminal arabinan motifs, its exposure on the surface and perhaps active secretion. To date, it is still unclear how LAM is associated with the cell wall. By virtue of its extraction methods, LAM appears to be firmly, but not covalently, attached to the walls. Furthermore, monoclonal antibodies to LAM recognize whole mycobacterial cells in ELISA experiments, suggesting that at least part of the molecule is situated on the exterior of the cell, accessible to the environment (Gaylord *et al.*, 1987).

Two possible situations have been hypothesized: (1) LAM is anchored in the plasma membrane by its "lipid anchor," and protrudes through the thickness of the wall so that its terminal arabinose or mannose-capped arabinose units are accessible to the outside (McNeil and Brennan, 1991); (2) LAM is incorporated by its PI-anchor into the outer leaflet of a proposed outer-membrane analogue in mycobacteria, and along with other polar wall-associated lipids makes up this membrane (Rastogi, 1991). Both these hypotheses are consistent with external presentation of terminal arabinose or mannose caps. The relatively strong conditions needed to release LAM from mycobacteria seem to favor the first hypothesis, but there is no strong evidence to support either hypothesis. A third possibility is that LAM has no permanent situation in the envelope, but is essentially a secreted molecule which passes through the envelope, so that the envelope-associated population merely represents those molecules in transit between a probable site of synthesis in the plasma membrane and the exterior of the mycobacterial cell. Lemassu and Daffé (1994) further demonstrated the existence of non-PI containing arabinomannan in the so-called capsular polysaccharide associated with

M.tuberculosis which consists of mannose-capped arabinan motifs essentially identical to ManLAM. This raised the possibility that recognition by antibody of the whole cell was directed against these extracellular arabinomannans which may also be partly responsible for the many biological activities attributed to LAM.

Biological functions of LAM

LAM exhibits a wide spectrum of immunomodulatory functions, but the biological implication of the *in vitro* immunological data is not always clear. Using AraLAM, ManLAM, and *M.leprae* LAM, the early data obtained include LAM-induced abrogation of T-cell activation (Kaplan *et al.*, 1987); inhibition of various IFN-γ-induced functions including macrophage microbicidal and tumoricidal activity (Sibley *et al.*, 1988), scavenging of potentially cytotoxic oxygen free radicals (Chan *et al.*, 1991); inhibition of protein kinase C activity (Chan *et al.*, 1991); and evocation of a large array of cytokines associated with macrophages such as α-TNF (Moreno *et al.*, 1988, 1989; Barnes *et al.*, 1992a; Chatterjee *et al.*, 1992c; Adams *et al.*, 1993), granulocyte-macrophage-CSF, IL-1a, IL-1b, IL-6, and IL-10 (Barnes *et al.*, 1992b).

In these studies, AraLAM was consistently shown to be much more potent in evoking α-TNF and other responses, relative to ManLAM. Similarly, although both LAMs elicited immediate early response genes (including c-fos, JE, KC) in murine bone marrow–derived macrophages (Roach *et al.*, 1993), only AraLAM induced both α-TNF and a potentially lethal TNF-dependent NO response (Roach *et al.*, 1995). Recently, AraLAM and not ManLAM was found to induce interleukin 12 (IL-12) expression in murine macrophages which may thus drive naive T-cells toward T-helper type 1 (Th1) cell development (Yoshida and Koide, 1997). The earlier hypothesis linking the mannose-capping function of LAM to attenuation in immunopotency and thus serving as virulence factor, was somewhat weakened by the subsequent realization that LAMs from *M.tuberculosis*, irrespective of virulence status, are all mannose-capped (Khoo *et al.*, 1995b). Furthermore, the activity of AraLAM may be entirely due to its inositol phosphate capping, a feature not found on LAMs from pathogenic *M.tuberculosis* or *M.leprae* (Khoo *et al.*, 1995b; Gilleron *et al.*, 1997).

Nevertheless, in a recent study using human fetal microglial cells, both ManLAM and AraLAM were shown to have TNF-α stimulating properties, suggesting that the source of macrophages may be an important determinant of the response to different LAMs (Peterson *et al.*, 1995). Thus, only studies involving ManLAM on human cell lines may be considered to bear real semblance to infection *in vivo* and pathogenesis. The granulomatous immune response in tuberculosis is characterized by delayed hypersensitivity and is mediated by various cytokines released by the stimulated mononuclear phagocytes, including TNF-α and IL-1b. ManLAM, free of LPS contamination, has been shown to stimulate mononuclear phagocytes to release TNF-α, IL-1b, and IL-6 (Zhang *et al.*, 1993, 1994). It was thought that IL-6 may play a role in clinical manifestations and pathological events of tuberculosis infection as it was identified in the granulomas in animal models of BCG infection. In a subsequent study, it was demonstrated that there is a striking upregulation by ManLAM of IL-8 mRNA expression in alveolar macrophages from patients with pulmonary TB (Zhang *et al.*, 1995). IL-8 synthesis and release is an early response of macrophages after phagocytosis of *M.tuberculosis* which serves to attract both acute and chronic inflammatory infiltrates associated with necrotizing granulomas in lung tissue and thus participates in the process of containment of the pathogen. In addition, ManLAM from the virulent strains of *M. tuberculosis* (Erdman and H37Rv), but not AraLAM, could stimulate phagocytosis by interacting with the human macrophage mannose receptor (Schlesinger, 1993; Schlesinger *et al.*, 1994, 1996). Thus, mannose-caps on ManLAM of *M.tuberculosis* strains may mediate efficient binding and entry, and regulate the initial events of phagocytosis as well as survival within the host macrophages.

Migration and t-cell recognition of LAM

Despite many of the well documented activities of LAMs on cells of lymphomonocytic origin, mechanisms by which LAMs mediate these effects are poorly understood. Although most of the immunobiological activities are thought to be directly elicited by the terminal carbohydrate head group, such as the mannose capping on ManLAM or phosphoinositol capping on the AraLAM, it has been shown that the lipid moiety is nonetheless essential to maintain its functional integrity (Barnes *et al.*, 1992a).

In a recent elegant report (Llangumaran *et al.*, 1995), it was demonstrated that LAM could integrate directly into the host cell membrane through its PI anchor, an event that could be competitively inhibited by PIM_6. In the same study, it was also shown that LAMs were preferentially incorporated into plasma membrane domains enriched in endogenous GPI-linked proteins and interfere with the lateral mobility of the GPI-linked Thy-1 surface glycoproteins in the plane of the membrane. The acyl chains on LAM were found to be critical for LAMs to interact with the target cell membrane. Thus, LAM was claimed to be inserted directly into the plasma membrane bilayer of target cells through the acyl chains of its PI anchors without apparent involvement of the surface receptors. This interaction is quite distinct from the interaction of the ManLAM with macrophage mannose receptor involved in the phagocytosis of LAM-coated microspheres (Schlesinger *et al.*, 1994). Not only can the mannose receptor mediate the initial uptake and internalization, but it may also deliver LAM to late endosomes for eventual presentation to T-cells by CD1b molecules (Prigozy *et al.*, 1997).

T-cell recognition of LAM and other mycobacterial hydrophobic nonpeptide antigens have been the major findings leading to the currently accepted phenomenon of major histocompatability complex (MHC)-independent pathways for antigen presentation (Melián *et al.*, 1996; Jullien *et al.*, 1997). In studies directly related to LAM (Sieling *et al.*, 1995), two αβ+, CD4-, CD8- (double negative, DN) T-cell lines derived, respectively, from a leprosy skin lesion and the peripheral blood of normal donors were found to be responsive to LAM/LM/PIMs in the presence of CD1b-expressing antigen presenting cells. Presentation of these lipoglycan antigens required internalization and endosomal acidification, but was independent of both class I and class II MHC molecules. Significantly, the presence of an acylated PI unit and the mannan core with α1→2 Man*p* residues was required and that only LAM, but not enterobacterial lipopolysaccharides from *E.coli*, lipophosphoglycans from *Leishmania donovani*, or lipoteichoic acids from *Streptococcus pyogenes*, was reactive against the derived DN T-cells. Furthermore, the T-cells derived from leprosy skin lesion only responded to LAM from *M.leprae*, whereas those from normal donors recognized LAMs from both *M.tuberculosis* Erdman and *M.leprae*. This suggests that although the lipid units are required, probably for binding to the hydrophobic α_1 and α_2 domains of CD1 which correspond spatially to the peptide-binding groove in MHC molecules

(Porcelli, 1995), fine specificity in binding may reside in the glycan epitopes. More recently, three other DN T-cell lines from normal donors were found to recognize protease resistant mycobacterial lipoglycan antigens in the context of CD1c (Beckman *et al.*, 1996). Interestingly, one T-cell line was also found to respond specifically to *M.leprae* LAM and not the structurally similar *M.tuberculosis* LAM.

The implication of CD1-restricted T-cell recognition of LAM/LM is unclear at present. T-cells activated by LAM secrete proinflammatory cytokines and are cytolytic (Sieling *et al.*, 1995). The presence of *M.leprae* LAM-specific DN T-cells in localized leprosy skin lesions indicates a role for this novel antigen presentation pathway in host defense. It has been suggested that broad recognition of nonpolymorphic antigen presenting molecules may be important in the acquisition of cell mediated immunity to persistent intracellular pathogens that synthesize a diverse range of unusual lipoglycans, including LAM. A model of LAM and CD1b trafficking through the endosomal compartments of antigen presenting cells was recently proposed. The colocalization of the mannose receptor, LAM, and CD1b in the MHC class II antigen loading compartment (MIIC) prompted the suggestion that the mannose receptor delivers LAM for loading onto CD1b in MIIC, followed by the trafficking of LAM-CD1b complex to cell surface (Prigozy *et al.*, 1997). This pathway therefore links recognition of microbial antigens by a receptor of the innate immune system to the induction of adaptive T-cell responses, and is relevant to LAM shed by infecting mycobacteria in lung cavities. Alternatively, LAM may be released into mycobacteria laden phagosomes and reaches the endocytic pathway by intracellular trafficking through other lysosomal-like vesicles (Xu *et al.*, 1994).

In addition to direct recognition, LAM may also specifically induce human peripheral blood T-cell chemotaxis. It was shown that the culture supernatants from human alveolar macrophages infected *in vitro* with virulent *M.tuberculosis* could induce T-cell migration (Berman *et al.*, 1996). Much of the migratory activity present in the supernatants could be blocked using a monoclonal antibody against LAM, suggesting that LAM is one of the chemotactic factors released. Furthermore, both AraLAM and *M.tuberculosis* ManLAM, but not LAM from *Mycobacterium bovis*, BCG, could induce T-cell chemotaxis *in vitro*. Thus, as in the case of CD1-restricted T-cell recognition, fine structural differences among LAMs from different sources can be discriminating and important when considering their biological functions and potency in eliciting host immune responses. The seemingly heterogeneous nature may perhaps conceal a well evolved adaptation enabling the intracellular pathogen to fine tune a perfect balance against the host defense system for its survival and propagation.

Concluding remarks

We end this review with some thoughts concerning the structure-to-function frontier. We have introduced a historical perspective on how our laboratory, starting with fascinating problems in the field of structural definition, has gradually become involved in the issues of biosynthesis, immunology, and pathogenesis. One of the vital contributions which we bring to such progress is a sensitivity for precisely defined structures. Much has been learned about the chemistry and biology of LAM in the past few years, and yet much more remains to be defined, as is obvious from this review. It is felt that for a true appreciation, future work on delineating the roles of LAM in immunopathogenesis needs to be focused on *in vivo* studies involving human cell lines whenever possible. On the other hand, we now consciously direct our next phase of structural studies on LAMs obtained from clinical isolates with a variety of virulence and drug resistant profiles. It is hoped that we can eventually delineate the structural motifs which contribute as virulence factors and/or protective epitopes and thereby derive effective drugs and vaccines against tuberculosis.

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Abbreviations

AG, arabinogalactan; AM, arabinomannan; AraLAM, non-mannose capped LAM; DN T-cell, αβ+, CD4-, CD8- double negative T-cells; Emb, ethambutol; ManLAM, mannose-capped LAM; LAM, liporabinomannan; LM, lipomannan; PI, phosphatidylinositol; PIM_x, phosphatidylinositol mannosides with subscript "x" denotes the number of mannose residues.

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