Lipoarabinomannan
MULTIGLYCOSYLATED FORM OF THE MYCOBACTERIAL MANNOSYLPHOSPHATIDYLINOSITOLS*

Delphi Chatterjee, Shirley W. Hunter, Michael McNeil, and Patrick J. Brennan‡
From the Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523

The lipopolysaccharides of mycobacteria, lipoarabinomannan (LAM) and lipomannan (LM), of key importance in host-pathogen interaction, were recently shown to contain a phosphatidylinositol "anchoring domain." We now have established that LAM and LM are based on the phosphatidylinositol mannoses, the characteristic glycosylphospholipids of mycobacteria. Digestion of the arabinoise-free LM with an endo-α1→6-mannosidase yielded evidence for the presence of the 1-(sn-glycerol-3-phospho)-α-myoinositol-2,6-bis-α-D-mannopyranoside unit, indistinguishable from that derived from phosphatidylinositol dimannoside. This same inositol substitution pattern was shown to be present in LAM by methylation analysis before and after dephosphorylation. Positions C-2 and C-6 of the inositol unit of LAM are occupied by mannosyl residues and C-1 by a phosphoryl group. Partial acid hydrolysis of per-O-methylated LAM and comparison by gas chromatography-mass spectrometry of the resulting derivatized oligosaccharides with like products from phosphatidylinositol hexamannoside demonstrated that the C-6 of inositol is the point of attachment of the mannan core of LAM, which consists of an α1→6-linked backbone with considerable α1→2 side chains. Thus, a structural and presumably biosynthetic relationship is established between some of the membranous mannosylphosphatidylinositol oligosaccharides described some 25 years ago and the newly emerging, biologically active lipopolysaccharides of mycobacteria.

Mycobacterial lipoarabinomannan (LAM) has recently assumed unexpected importance in the interaction between mycobacteria and host cell (1). It is highly immunogenic with diagnostic potential and is a key factor in regulating macrophage function (2, 3), which may facilitate survival of mycobacteria within phagocytic cells. It also inhibits the processing of mycobacterial protein antigens by antigen-presenting cells (4) and may abrogate T-cell activation, further ensuring persistence of pathogen within the host. LAM also induces the production of tumor necrosis factor (5, 6) and thus may be responsible for mediating granuloma formation, nerve damage, and tissue necrosis. So profound are some of its biological effects that they have been attributed to endotoxin-like activity or actual endotoxin (7). LAM is considered a mycobacterial virulence factor. The molecule has been studied in the past (8). However, only recently was it realized that LAM, and its arabinoise-free relative, LM, contain phosphatidylinositol (9, 10), and thus are the first prokaryotic versions of the biologically important phosphatidylinositolglycans (11). We now report that LAM and LM are based on the phosphatidylinositol mannoses (PIM), a group of distinct mycobacterial glycosylphospholipids, known since the 1940s and fully described by Ballou and colleagues (12, 13) in the 1960s. Consequently, the profound biological functions of LAM in terms of host-pathogen relationships can now be matched with thoughts on its biogenesis and its physiological implications for mycobacteria.

EXPERIMENTAL PROCEDURES

Preparation of LAM, LM, PIMs, and the Decacylated Derivatives—Preparations containing the majority of cellular LAM, LM, and the more polar of the PIMs were obtained by disruption of armedillo-derived, delipidated Mycobacterium leprae or cultured Mycobacterium tuberculosis H37Ra followed by extraction with 50% ethanol and partitioning between phenol and water (9). The freeze-dried aqueous layer was suspended in a buffer containing 0.2 M NaCl, 0.25% deoxycholate, 1 mM EDTA, 0.02% NaN₃, and 10 mM Tris, pH 8.0, and applied to a column (1.5 × 90 cm) of Sephacryl S-200 which was developed with the same buffer. Polyacrylamide gel electrophoresis, when combined with a periodate-containing silver stain (9), provided an excellent guide of purification. Detergent was removed from fractions as described (14). PIM was purified from the earlier CHCl₃/CH₂OH extracts as described (15). Decacylated versions of all products were obtained by treatment with 0.1 N NaOH and appropriate purification (10, 12).

Preparation of [³H]Ins-LM—A mixture of [³H]Ins-LAM and [³H]Ins-LM was generated as described (10), and complete separation of the two was achieved by chromatography on a column of DEAE-Sephacel in 0.01 M Tris-HCl, pH 7.4, containing 1% Triton X-100 (10). [³H]Ins-dLM was generated by treating the parent product with 0.1 N NaOH and purification on a column of Bio-Gel P-2 in 0.1 N CH₃COOH (10).

Enzymatic Digestion of LAM—[³H]Ins-dLM (25 mg) was digested with exo-α-mannosidase as described (10). The reaction mixture was boiled, centrifuged, and applied to a column (1 × 100 cm) of Bio-Gel P-10 in 0.1 N CH₃COOH (10). The [³H]Ins-containing fractions were collected, combined, dried (22 mg), and further digested for another 72 h with endo-α-(1→6)-mannosidase as described by Nakajima et al. (16). The product was applied three times to a 2-ml column of mixed bed resin each time recovering the [³H]Ins-containing material with 2 N HCOOH.

Dephosphorylation of LAM, LM, and PIMs—Purified dLAM, dLM, and dPIM, (2–3 mg) dried and in 12 × 25-mm propylene tubes, were treated with 250 µl of precooled 48% aqueous HF at 1 °C for 36 h.

*This work was supported by Grant AI 18357 and Contract NO1 AI-08074 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health and a grant (to D. C.) from the World Health Organization (WHO) Special Programmes for Research and Training in Tropical Diseases. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be addressed.

1 The abbreviations used are: LAM, lipomannan; dLAM, decacylated LAM; LM, lipomannan; dLM, decacylated lipomannan; PI, phosphatidylinositol; PIMs, phosphatidylinositol mannoses; PIM6, phosphatidylinositol hexamannoside; dPIM6, decacylated phosphatidylinositol hexamannoside, dPIM, decacylated phosphatidylinositol dimannoside, i.e. Gro-P-Ins-Man6; PIM6, phosphatidylinositol hexamannoside; dPIM6, decacylated phosphatidylinositol hexamannoside, dPIM, decacylated phosphatidylinositol dimannoside, i.e. Gro-P-Ins-Man6; IM, inositolmannoside; Manol, mannnitol; Me, O-CH₃; Et, O-C₂H₅; t, terminal; EI, electron impact; GC, gas chromatography; MS, mass spectrometry.
The HF was removed by continuous evaporation over KOH and the products applied to columns of Bio-Gel (P-2 for PIM and P-100 for LAM and LM). Fractions were assayed for carbohydrate, organic-, and inorganic-P, and the carbohydrate-containing P-free fractions were dried prior to further analysis.

Sugar Compositional and Linkage Analysis—Products of the deacetyl- and dephosphorylation steps were freeze-dried, methylated with CH3I (17), and applied to cartridges of SEP-PAK (Waters Associates, Inc., Milford, MA) for removal of reaction products (14). The CH3CN and CH3OH eluates were combined and samples converted to the respective alditol acetates for GC/MS analysis (14). In addition, the intact native LAM, PIM, and PIM were methylated under neutral conditions with tert-butylpyridine and methyltrifluoromethane sulfonate under Argon for 2 h at 50 °C (18). Per-Me-LAM was dialyzed exhaustively against H2O and lyophilized. The per-Me-PIM and -PIM were purified on columns of Sephadex LH-20 (17).

Generation of Per-O-alkyl Oligosaccharide Fragments—The per-Me-IM and the dephosphorylated per-Me-LAM were subjected to partial depolymerization (19) as follows. Samples (2 mg) were dissolved in 2 m CF3COOH (500 µl), hydrolyzed for 2 h at 90 °C, reduced with NaBH4, dried thoroughly, ethylated with CH3I, and the per-O-alkylated fragments purified by SEP-PAK prior to GC/MS analysis.

GC/MS, FAB/MS, and NMR—GC/MS was performed on HP-1 (Hewlett-Packard, Avondale, PA) or DB-23 (J&W Scientific, Rancho Cordova, CA) 12-m capillary columns. The temperature gradient programs for resolution of alditol acetates and per-O-alkylated oligosaccharides have been described (19). The Hewlett-Packard 5970 mass selection detector in the electron impact or selective ion mode was used to record masses. FAB/MS was performed on a VG 707 extra high frequency mass spectrometer (19). A 300 MHz Bruker spectrometer was employed for H and 13C NMR studies; chemical shifts are quoted with reference to aceton. A 500 MHz Bruker spectrometer was used for 31P NMR, and chemical shifts were related to external 80% H3PO4.

RESULTS

Purification of LAM, LM, and PIMs—A key development in the present demonstration of a progressive structural link between PIMs, LM, and LAM was the satisfactory resolution of the three. Initial extraction of M. leprae or M. tuberculosis H37Ra with organic solvents or detergent favored removal of the simpler PIMs, notably PIM, the dominant member (12, 20). PIM and the deacylated product (dPIM; Gro-P-Ins-Man,) were readily purified (Fig. 1) and characterized (20); the structure described by Lee and Ballou (20) for Gro-P-Ins-Man showed the expected molecular mass of 1488 Da. Methylation analysis (Fig. 2D) revealed the presence of a Manp (1, 5-Ac2-3, 4, 6-Me-Manol; m/z 102, 118, 129, 161, 162, 205), 6-linked Manp (1, 5, 6-Ac2-3, 4, 6-Me-Manol; m/z 102, 118, 129, 162, 189, 233), 2-linked Manp (1, 2, 5-Ac3-3, 4, 6-Me-Manol; m/z 129, 130, 161, 190), and a Manp (see below). Accurate quantitation of the relative amounts of these derivatives was not possible under the conditions. Nevertheless, the combined evidence supported the structure proposed for PIM as the seminal work of Lee and Ballou (13) (Fig. 2A). Also, this rich source of PIMs a scarce product in previous work (13) and its key physical properties, were key factors in the subsequent analysis of LAM.

The Presence of the Gro-P-Ins-Man Unit in LAM—The availability of a bacterial endo-a-D-mannosidase, capable of cleaving a-1-6-linked b-Manp residues (16), which are known to dominate the mannan backbone of LAM and LM (10), the opportunity to look specifically for a PIM unit in LAM/LM. Initial digestion of [3H]Ins-dLM with an exo-a-D-mannosidase and purification of the product on Bio-Gel in 0.1 N CH3COOH (10) resulted in an oxomannosidase-resistant [3H]Ins-containing product (Fig. 3A), analysis of which had previously suggested a linear a-1-6-linked nona- decamannoside with some single t-a-Manp side chains attached to C-2 (10). This product proved susceptible to endo- 

**Fig. 1.** Demonstration of the purity of the key mycobacterial products. A, two µg each of purified LAM, LM, and PIMs from M. leprae were applied to polyacrylamide gels as described (10). Gels were stained with an AgNO3-periodic acid reagent (9). B, thin layer chromatography of 1) the Gro-P-Ins-Man, from M. tuberculosis and 2) the Gro-P-Ins-Man, from M. leprae on silica gel in 1-butanol/pyridine/acetic acid/water (5:5:1:3). The plate was charred with 10% H2SO4. M. W., molecular weight.
dLAM, and Gro-P-Ins-Man₆ were methylated under neutral conditions (22) in which the phosphodiester bond is stable. Unlike partially methylated acylic alditol acetates, cyclic derivatives do not yield readily interpretable EI mass spectra. The one derivative from Gro-P-Ins-Man₆ that could not be identified as a mannitol acetate was shown to have a molecular weight by GC/MS in the chemical ionization mode \((\text{M}+\text{NH}_4)^+\), \(m/z\) 366, indicative of 1,2,6-Ac₃-3,4,5-Me₃-Ins, the expected product (13). The Ins derivative from LAM was shown to be identical to that from Gro-P-Ins-Man₆ by coelution from two different capillary GC columns (HP-1 and DB-23) and an identical EI/MS fingerprint \((m/z\) 256, 212, 87, 75). Thus, it was concluded that the Ins unit of LAM, like those from PIM₂ and PIM₆, is substituted at C-1, C-2, and C-6. In order to differentiate between the phosphorylated and mannosylated positions, dLAM was dephosphorylated with HF, and the products applied to a column of Bio-Gel P-100, in \(H_2O\). The retained P-free carbohydrate fractions (also free of the majority of Ara) were pooled, methylated, hydrolyzed, reduced, acetylated, and compared with like products obtained from per-Me-IM₆ by GC/MS. The one non-acyclic acetate derived from LAM was identical to that obtained from PIM₆ and was identified as 2,6-Ac₂-1,3,4,5-Me₄-Ins. Accordingly, the substitutions on the Ins unit of LAM were considered to be identical to these in the PIMs.

In the more highly mannosylated PIMs, multiple glycosylation occurs at the C-6 position of Ins while a single, constant \(\alpha\)-Manp residue occupies C-2 (12, 13, 20, 23). Seeking the same arrangement in LAM, the per-O-alkylated fragments of PIM₆ were first generated by subjecting per-Me-IM₆ to partial acid hydrolysis, with \(\text{NaBH}_4\) ethylation with \(C_2[H]_2\) and GC/MS analysis. The strategy was to apply the known GC/MS features of these fragments to the search for like fragments in LAM. The total ion chromatogram of the products from per-Me-IM₆ and the mass spectra of two of the key fragments/compounds, both per-O-alkyl oligomannosyl inositols, are shown in Fig. 5, and the properties of the full range of per-O-alkyl oligomannosyl alditols and per-O-alkyl oligomannosyl inositols are presented in Table I. The position

\[ \text{FIG. 2. Structural characteristics of PIM}_6\text{ from }M.\text{ tuberculosis. A, proposed structure of Gro-P-Ins-Man}_6\text{. B, }^1\text{H NMR (500 MHz) of Gro-P-Ins-Man}_6\text{. C, }^{13}\text{C DEPT-NMR of Gro-P-Ins-Man}_6\text{. D, GC/MS of the products of methylation analysis of per-Me-IM}_6\text{ and FAB/MS of per-Me-IM}_6\text{ (inset).} \]
of the substitutents on Ins (either OCH₃, OC₂H₅, or Man) were deduced from the known substitution pattern on Ins of the PIMs rather than from primary mass spectral data, as indicated above. Nevertheless, by relying on GC/MS analysis and available information on the structure of the PIMs, it was possible to identify all of the ensuing Ins-containing dimers, trimers, and tetramers and also the per-O-alkyl oligomannosyl mannitols, and attribute MS features to each of them. The most important fragments/compounds arising from these experiments were 1, 4, 8, 10, 13, and 14 (Table I).

These, if present in LAM, would help demonstrate the presence of a single terminal Man unit at C-2 on Ins and growth of the mannosyl chain at C-6.

Selected ion GC/MS analysis and coinjection GC were applied to the population of per-O-alkyl oligoglycosyl alditols and inositols arising from LAM. Thus, recognition of the diagnostic ions (m/z 252 and 312) for compound 1 (t-Manp-(1→2)-Ins-2Et; see Table I for nomenclature), combined with the correct Rt value (10.7 min), served to demonstrate the presence of this monomannosylinositol in LAM. The characteristic ions of compound 8 (m/z 437, 456, 516), a dimannosyl inositol (t-Manp-(1→2)-Ins-(6→1)-Manp-6Et), combined with the correct Rt value (18.4 min), served to demonstrate the presence of this particular fragment in LAM. Compounds 10 and 13 were also shown to be present in LAM based on the selective ions m/z 252, 312, 516, and m/z 437 and 701, respectively. These particular fragments clearly demonstrated that the Man unit at C-2 of the Ins unit of LAM was not further extended, and, also, that the dimannosyl unit, Et-6α-D-Manp-(1→6)α-D-Manp, must have been attached to the 6-position of Ins. Interestingly, fragment 14, Et-2α-Manp-(1→6)α-D-Manp-(1→6)α-D-Manp-(1→6)-Ins-2Et, arising from PIM8, was absent from LAM. Instead, two later eluting, new tetrasaccharides emerged as monitored by m/z 252 and 456, with the following probable structures (Table I).

Et-6α-Manp-(1→6)α-D-Manp-(1→6)α-D-Manp-(1→6)-Ins-2Et

In summary, recognition of these key fragments and others (Table I, footnote d) demonstrate that only a single α-Manp is on C-2 of the Ins of LAM and the extended mannan emanates from C-6. The evidence was not definite as to the structure of the mannan core beyond the initial Ins-linked mannobiose unit. However, several of the distinctive fragments arising from LAM but absent from PIMs combined with previous evidence (10) strongly suggest both 1→6-type chain extension in addition to branching at the 2-position within the mannan core.

**DISCUSSION**

In 1939, Anderson (24) isolated from the human tubercle bacillus a phospholipid fraction, which on hydrolysis yielded glycerophosphoric acid, mannose, and the hexahydric alcohol "inositide." Alkaline saponification of the phospholipid yielded a "phosphorus-containing glycoside," which on dephosphorylation produced inositol dimannoside. Some 25 years later, Lee and Ballou (13) arrived at the complete structure of the family of phosphatidylinositol mannosides from *M. tuberculosis* and *M. phlei.* They showed unequivocally that the mannosides were attached glycosidically at positions 2 and 6 of the myo-inositol ring (20) and that chain elongation occurred at the latter position (13). The present paper, again 25 years later, demonstrates that the biologically important lipopolysaccharides of mycobacteria, lipoarabinomannan, and lipomannan are based on these same phosphatidylinositol mannosides and are probably the products of yet further glycosylation. The postulated structure of LAM from *M. tuberculosis* H37Ra is shown in Fig. 6.
used in defining the non-reducing ends of LAM (14, 26) also was based less on independent direct evidence but more provided evidence of a linear α-1-6-linked mannan backbone through comparison with the substituted inositol obtained of arabinan to the mannan backbone are not known. These experiments arose convincing evidence of the presence of enzymes such as the PI-specific phospholipase from PIM, and thus on earlier evidence (20), a not unreasonable approach given the unambiguity of the earlier work. The susceptibility of LM to endomannosidase was crucial and fortunate in view of the resistance of LAM/LM to other key susceptibilities. A variation of the partial depolymerization strategy of the Gro-P-Ins-Mann unit in LM and, by implication, in LAM. A variation of the partial depolymerization strategy used in defining the non-reducing ends of LAM (14, 26) also provided evidence of a linear α-1-6-linked mannan backbone with a considerable degree of mannosyl α1-2 branching, altogether reminiscent of the short homomannosyl oligosaccharide described by Maitra and Ballou (25). Nevertheless, the frequency of such α1-2 branch points on the mannan backbone of LAM and the issue of whether they are single t-Man or mannobiose units has not yet been resolved.

Thus, the critical question of a structural relationship between the PIMs and LAM has been addressed. The presence of the two fatty acyl functions, stearyl and tuberculostearyl, previously found exclusively associated with the PIMs (15), has already been shown in LAM (9). Of considerable significance, a lipomannan from Propionibacterium freudenreichii was also recently shown to contain inositol, glycerol, phosphate, and fatty acids (27), pointing to a covalently linked phosphatidylinositol in an organism phenotypically related to Mycobacterium. Thus, LAM and LM join the PIMs of mycobacteria as members of the biologically important glycosylphosphatidylinositol in an organism phenotypically related to Mycobacterium. Thus, LAM and LM join the PIMs of mycobacteria as members of the biologically important glycosylphosphatidylinositol (11). What bearing this fact may have on the powerful biological activity of LAM is not known but is presumed to be significant considering that many of the host-parasite interactions (29). In particular, the key identification of the substituted inositol derivative arising from LAM/LM was based less on independent direct evidence but more through comparison with the substituted inositol obtained from PIM, and thus on earlier evidence (20), a not unreasonable approach given the unambiguity of the earlier work. The susceptibility of LM to endomannosidase was crucial and fortunate in view of the resistance of LAM/LM to other key enzymes such as the PI-specific phospholipase C (10). From these experiments arose convincing evidence of the presence of the Gro-P-Ins-Man$_n$ unit in LM and, by implication, in LAM. A variation of the partial depolymerization strategy used in defining the non-reducing ends of LAM (14, 26) also provided evidence of a linear α-1-6-linked mannan backbone with a considerable degree of mannosyl α1-2 branching, altogether reminiscent of the short homomannosyl oligosaccharide described by Maitra and Ballou (25). Nevertheless, the frequency of such α1-2 branch points on the mannan backbone of LAM and the issue of whether they are single t-Man or mannobiose units has not yet been resolved. Thus, the critical question of a structural relationship between the PIMs and LAM has been addressed. The presence of the two fatty acyl functions, stearyl and tuberculostearyl, previously found exclusively associated with the PIMs (15), has already been shown in LAM (9). Of considerable significance, a lipomannan from Propionibacterium freudenreichii was also recently shown to contain inositol, glycerol, phosphate, and fatty acids (27), pointing to a covalently linked phosphatidylinositol in an organism phenotypically related to Mycobacterium. Thus, LAM and LM join the PIMs of mycobacteria as members of the biologically important glycosylphosphatidylinositol (11). What bearing this fact may have on the powerful biological activity of LAM is not known but is presumed to be significant considering that many of the host-parasite interactions (29).

**TABLE I**

| Fragment from IM$_n$ (No.),* | GC $R_{t}$ | Diagnostic ions (m/z) | Deduced structure* | Presence in LAM* |
|-----------------------------|-----------|----------------------|-------------------|-----------------|
| 1  | 10.70 | 220, 252, 312 | t-Man-(1→2)-Ins-6→Et | Yes |
| 2  | 11.37 | 187, 219, 274 | t-Man-(1→2)-Manol | No |
| 3  | 12.24 | 107, 165, 197, 206, 238, 274, 334, 375 | t-Man-(1→2)-Man-(1→6)-Manol | Yes |
| 5  | 12.51 | 88, 238, 274, 331, 375 | t-Man-(1→6)-Manol | No |
| 6  | 17.20 | 187, 219, 274, 274, 391, 538 | t-Man-(1→2)-Man-(1→2)-Manol | No |
| 7  | 18.20 | 70, 165, 238, 274, 442, 478, 538, 579 | t-Man-(1→2)-Man-(1→2)-Man-(1→6)-Manol | No |
| 8  | 18.40 | 187, 219, 238, 437, 456, 516 | t-Man-(1→2)-Ins-(6→1)-Man-6→Et | Yes |
| 9  | 19.38 | 206, 238, 274, 334, 442, 579 | t-Man-(1→2)-Man-(1→6)-Manol | No |
| 10 | 19.60 | See Fig. 5B | t-Man-(1→2)-Ins-(6→1)-Man-6→Et | Yes |
| 11 | 22.90 | 187, 219, 274, 334, 391, 423, 478, 538 | t-Man-(1→2)-Man-(1→2)-Man-(1→6)-Manol | No |
| 12 | 23.90 | 206, 238, 274, 410, 442, 478, 538, 682 | t-Man-(1→2)-Man-(1→2)-Man-(1→6)-Manol | No |
| 13 | 24.90 | See Fig. 5C | t-Man-(1→2)-Ins-(6→1)-Man-6→Et | Yes |
| 14 | 26.05 | 238, 252, 312, 456, 516, 650 | t-Man-(1→2)-Man-(1→6)-Man-6→Et | No |

* See Fig. 5.
* Retention time on 12 m HP-1 column programed as described (19).
* The nomenclature used is illustrated by the following examples: fragment 1, t-Man-(1→2)-Ins-6→Et = 2,3,4,6-tetra-O-CH$_3$-Man-(1→6)-1,3,4,5-tetra-O-CH$_3$-6-O-[C$_5$[H$_8$]-Ins; fragment 8, t-Man-(1→2)-Ins-(6→1)-Man-6→Et = 2,3,4,6-tetra-O-CH$_3$-Man-(1→2)-1,3,4,5-tetra-O-CH$_3$-6-O-[C$_5$[H$_8$]-Man. Thus, in this scheme, the Et(O-C$_5$[H$_8$]) group points to a position of prior glycosidic linkage. All Man residues are α-D-Manp.
* Evidence for the presence of the Ins-containing fragments 1, 4, 8, 10, and 13 in LAM is discussed in the text.

None of the fragments from IM$_n$ that contained linear 2-linked Man residues with a considerable degree of mannosyl α1-2 branching, altogether reminiscent of the short homomannosyl oligosaccharide described by Maitra and Ballou (25). Nevertheless, the frequency of such α1-2 branch points on the mannan backbone of LAM and the issue of whether they are single t-Man or mannobiose units has not yet been resolved. Thus, the critical question of a structural relationship between the PIMs and LAM has been addressed. The presence of the two fatty acyl functions, stearyl and tuberculostearyl, previously found exclusively associated with the PIMs (15), has already been shown in LAM (9). Of considerable significance, a lipomannan from Propionibacterium freudenreichii was also recently shown to contain inositol, glycerol, phosphate, and fatty acids (27), pointing to a covalently linked phosphatidylinositol in an organism phenotypically related to Mycobacterium. Thus, LAM and LM join the PIMs of mycobacteria as members of the biologically important glycosylphosphatidylinositol (11). What bearing this fact may have on the powerful biological activity of LAM is not known but is presumed to be significant considering that many of the biological activities of LAM are dependent on full acylation of the molecule (2, 28). On the other hand, the nature of the non-reducing ends of LAM, which consist of branched arabinans in an avirulent strain of M. tuberculosis (14) and linear α1-2-linked oligomannosyl units in a virulent strain, have profound effects on such biological phenomena as the release of tumor necrosis factor. Thus, rather than the glycosylphosphatidylinositol, a functional paradigm for LAM may be the lipoteichoic acids and lipomannans of Gram-positive bacteria in which the lower regions of the molecule are also key to host-parasite interactions (29).

Fig. 6. Postulated structure of LAM. Details of the attachment of arabinan to the mannan backbone are not known.
Acknowledgments—We thank C. E. Ballou for a gift of the endo-
α1→6-mannosidase, Marilyn Hein for preparing the manuscript, and
Carol Marander for graphics.

REFERENCES
1. Brennan, P. J., Hunter, S. W., McNeil, M., Chatterjee, D., and
Daffe, M. (1990) in Microbial Determinants of Virulence and
Host Response (Ayoub, E. M., Cassell, G. H., Branche, W. C.,
Jr., and Henry, T. J., eds) pp. 55–75, American Society for
Microbiology, Washington, D. C.
2. Sibley, L. D., Adams, L. B., and Krahenbuhl, J. L. (1990) Clin.
Exp. Immunol. 80, 141–148
3. Chan, J., Fan, X., Hunter, S. W., Brennan, P. J., and Bloom, B.
R. (1991) Infect. Immun. 59, 1755–1761
4. Moreno, C., Mehliert, A., and Lamb, J. (1988) Clin. Exp. Immunol.
74, 206–210
5. Moreno, C., Taverne, J., Mehliert, A., Bate, C. A. W., Brealey, R.
J., Meager, A., Rook, G. A. W., and Playfair, J. H. L. (1989)
Clin. Exp. Immunol. 76, 240–245
6. Barnea, P. F., Fong, S.-J., Brennan, P. J., Twomey, P. E., Ma-
zumber, A., and Modlin, R. L. (1990) J. Immunol. 145, 140–
154
7. Molloy, A., Cavenderack, G., Levis, W. R., Cohn, Z. A., and
Kaplan, G. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 973–977
8. Weber, P. L., and Gray, G. L. (1979) Carbohydr. Res. 74, 259–
278
9. Hunter, S. W., Gaylord, H., and Brennan, P. J. (1986) J. Biol.
Chem. 261, 12345–12351
10. Hunter, S. W., and Brennan, P. J. (1990) J. Biol. Chem. 265,
9272–9279
11. Ferguson, M. A. J., and Williams, W. F. (1988) Annu. Rev.
Biochem. 57, 285–320
12. Ballou, C. E., Vilkas, E., and Lederer, E. (1963) J. Biol. Chem.
238, 69–76
13. Lee, Y.-C., and Ballou, C. (1965) Biochemistry 4, 1395–1404
14. Chatterjee, D., Bozic, C., McNeil, M., and Brennan, P. J. (1991)
J. Biol. Chem. 266, 9652–9660
15. Brennan, P., and Ballou, C. E. (1967) J. Biol. Chem. 242, 3046–
3056
16. Nakajima, T., Maitra, S. H., and Ballou, C. E. (1976) J. Biol.
Chem. 251, 174–181
17. Stellner, K., Saito, H., and Hakomori, S.-I. (1973) Arch. Biochem.
Biophys. 155, 464–472
18. Camphausen, R. T., McNeil, M., Jardine, I., and Brennan, P. J.
(1987) J. Bacteriol. 169, 5473–5480
19. Daffe, M., Brennan, P. J., and McNeil, M. (1990) J. Biol. Chem.
265, 6734–6743
20. Lee, Y. C., and Ballou, C. (1964) J. Biol. Chem. 239, 1316–1327
21. Johansson, C., Kordel, J., and Drakenberg, T. (1990) Carbohydr.
Res. 207, 177–183
22. Prehm, P. (1980) Carbohydr. Res. 78, 372–374
23. Ballou, C. E., and Lee, Y. C. (1964) Biochemistry 3, 682–685
24. Anderson, R. J. (1939) Prog. Chem. Org. Nut. Prod. 3, 149–202
25. Maitra, S. K., and Ballou, C. E. (1976) Biochim. Biophys. Res.
Commun. 73, 1101–1108
26. Chatterjee, D., Lowell, K., Rivoire, B., McNeil, M. R., and Bren-
nan, P. J. (1992) J. Biol. Chem. 267, 6234–6239
27. Sutcliffe, I. C., and Shaw, N. (1989) FEMS Microbiol. Lett. 59,
249–252
28. Gaylord, H., Brennan, P. J., Young, D. B., and Buchanan, T. M.
(1987) Infect. Immun. 55, 2660–2663
29. Wicken, A. J., and Knox, K. W. (1990) Biochim. Biophys. Acta
604, 1–26