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MYCOBACTERIAL LIPIDS : A HISTORICAL PERSPECTIVE

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1. ABSTRACT

Mycobacterial lipids have been studied for more than 70 years, due to the fascinating diversity of their structures and biological activities. A historical perspective, and the present status on the structure and activity of major lipids of the outer envelope of mycobacterial cells are presented : mycolic acids, which are main constituents of the cell wall, and glycolipids known for toxic or immunological properties (cord factor, SL, DAT, PGL, GPL, LOS, LAM). As far as possible, it was tried to distinguish between experimentally established knowledge and currently accepted speculations.

2. INTRODUCTION

Till the use of the powerful antituberculous drug isoniazid in 1952, tuberculosis was one of the great killer diseases of humankind. It recently re-emerged as a health problem in impoverished populations in rich countries. Thus, this is not surprising that the study of mycobacterial lipids, which was initiated more than 7O years ago under the direction of R.J. Anderson, is still an active field.

The goal of the initial cooperative investigations was to "secure compounds of reasonable purity for biological studies and to determine the chemical composition of the various substances". This goal is still to be reached since, while tremendous progress in chromatography, mass spectrometry and NMR spectroscopy allowed to nearly fulfill the chemical part of the project, the biological side is far from completion. For instance, the knowledge on lipid mycobacterial biosynthesis lags far behind that on their fine structure, and a part of what we think we know about the biological activities of these lipids is mere speculation .

What follows is a brief historical survey of the development of knowledge on mycobacterial lipids, the yin and the yang in the field, and to briefly underline the respective contributions of some of the scientists that were engaged in what often was a long term transatlantic contest .

There are several excellent reviews on mycobacterial lipids. For more details, the reader can look for instance in references (1) and (2).

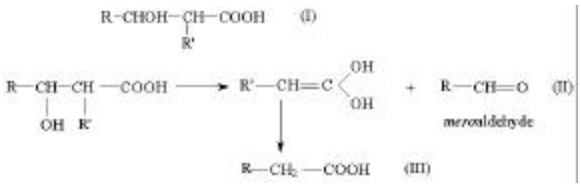

Figure 1. Mycolic acid (I) and its pyrolytic cleavage

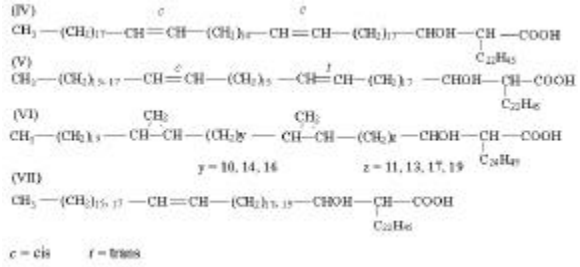

Figure 2. Structures of alpha-mycolic acids (IV) *M. Phlei*; (V) *M. smegmatis*; (VI) *M. tuberculosis*; (VII) alpha'-mycolic

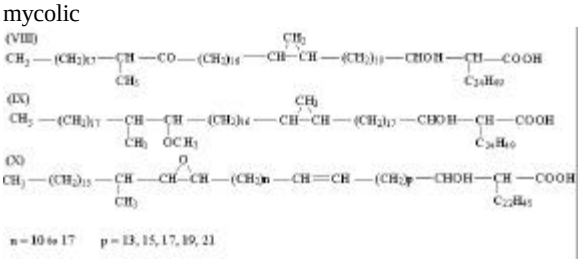

Figure 3. Mycolic acids bearing oxygenated function in their long (*mero*) chain (VIII) oxo-or keto; (IX) methoxylated; (X) epoxy

3 . MYCOLIC ACIDS

3 .1. Historical introduction

By prolonged saponification of the waxes of tubercle bacillus, an acid called *mycolic acid* was isolated by Anderson in 1939 (3). The formula $C_{88}H_{172}O_4$, which shows a deficit of 4 hydrogen atoms, although no unsaturation could be detected, was first attributed to this acid. By heating under reduced pressure to a temperature of 280-350°C, mycolic acid gave a distillate in a yield of 23.6%, identified to hexacosanoic acid. The presence of mycolic type acids was demonstrated in bovine and avian strains, a saprophytic strain (*M. phlei*) and another one falsely considered to be *M. leprae*.

A second period in the history of mycolic acid could be defined, from 1949 to about 1960, characterized by the intensive use of chromatography which revealed the multiplicity of mycolic acids present in each strain of mycobacteria (4, 5). Their general structure was determined (figure 1, formula I), explaining the pyrolytic reaction. The production of C_{26} or C_{24} fatty acids (formula III) by pyrolysis depended on the origin of the bacteria : hexacosanoic acid was produced by pyrolysis of acids from human and bovine strains; tetracosanoic acid production, in the case of avian and saprophytic strains. Mycolic acids were defined as high molecular weight fatty acids with a hydroxy group in position 3 and a long chain in position 2 (4).

The third and last period of mycolic acid history took profit of the use of NMR spectroscopy and mass spectrometry. By working on a mycolic acid fraction from *M. smegmatis*, in 1964 the group of Etemadi (6) was able to give the first complete structure of a mycolic acid molecule (formula V). Later on it was shown that cyclopropane rings could exist instead of the double bonds (leading to an acid devoid of unsaturation) : for example acids from *M. kansasii* (7) and M. tuberculosis (8). The fine structures of such acids were determined.

3.2. Structure : present status

Mycolic acids isolated from mycobacteria are called mycolic acids or eumycolic acids, to distinguish them from the lower terms, coryno-mycolic acids (from *Corynebacterium*) or nocardo-mycolic acids (from *Nocardia*).

The prefix alpha has been attributed to the acids first eluted from a column of adsorption chromatography. In some strains of mycobacteria, shorter molecules of mycolates are present (C60 instead of C80); they are called alpha'-mycolic acids.

Other mycolates, eluted after alpha-mycolates, are called according to the oxygen functions which exist in the mero moiety (formula II) : keto- (or oxo-) mycolates, methoxy-mycolates, epoxy-mycolates, carboxy-mycolates (often called dicarboxy-mycolates). They are easily analysed (as methyl esters) by thin-layer chromatography, and their pattern is a great help to discriminate between some closely related species of mycobacteria.

Diunsaturated mycolic acids can be split by oxidative ozonization: the first structure of a mycolic acid, the alpha-smegma-mycolic acid (V), was established (6).

The cyclopropane rings of alpha-mycolic acids have the *cis* geometry, but when the next carbon atom is substituted by a methyl group, the fixation of the methyl (from methionine) occurs on a *cis* double bond, which provokes its displacement by one carbon atom and its passage to the *trans* geometry.

3.2.1. Mycolic acids without oxygen function in the mero part (alpha-mycolic acids, figure 2)

They are major mycolic acids in most mycobacterial species, for instance in *M. phlei* (9), *M. smegmatis* (6) and *M. tuberculosis* (10-12).

Lower homologs (alpha'-mycolic acids) are found in some species (figure 2, formula VII). These acids, about C_{60}, represent up to about 25% of the total mycolic acids of the cell. The first alpha'-mycolic acid was isolated from *M. smegmatis* (C_{62}, C_{64}) (13). They are also present in *M. chelonei* (C_{64}), *M. fortuitum* (C_{68}), *M. parafortuitum* (C_{58} - C_{60}), *M. vaccae* (C_{58} - C_{60}). In *M. fallax*, *M. triviale* and *M. brumae*, only alpha-mycolic acids are present (14).

3.2.2. Mycolic acids with an oxygenated function in the mero part (figure 3)

Keto- (or oxo-) mycolic acids (15, 16; formula VIII) and methoxylated mycolic acids (17, 18; formula IX) were known for long. More recently, (omega-1)-methoxy acids were identified in species from the environment (19).

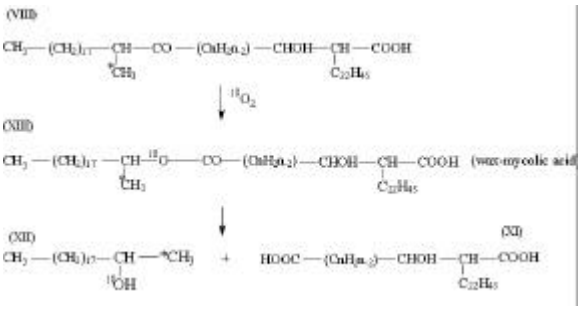

Figure 4. Carboxymycolic acids and their biogenesis. (VIII) keto mycolic acid (first precursor). (XI) carboxy mycolic acid. (XII) eicosanol. (XIII) "wax" mycolic acid (oxygenated precursor)

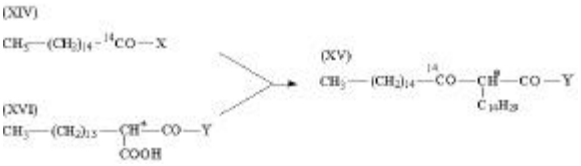

Figure 5. Corynmycolic acid biosynthesis. The "mycolic acid condensation" step postulated as resulting from a reaction between a plamitoyl derivative and a tetradecyl-malonyl derivative

Epoxy-mycolic acids (formula X) contain a *trans*-epoxy ring in the *mero* part of their molecule (20). They are also present in *M. farcinogenes*, *M. senegalense*, *M. chitae* and *M. smegmatis* .

Carboxy - mycolic acids and wax-mycolic acids (figure 4), are the most adsorbed of all the mycolic acids. They have a second carboxyl group and a lower molecular weight (C_{60}-C_{68}). They are often called "dicarboxylic mycolic acids". The structure (XI) has been attributed to these acids (21, 22). They occur in strains that contain a mixture of eicosanol-2 and octadecanol-2 (formula XII).

It was suggested that a keto-mycolic acid could be transformed into esters of carboxy-mycolic acids (wax-esters) and eicosanol-2 (or octadecanol-2) by a reaction of Baeyer-Villiger type (23). This scheme was strengthened by the isolation of wax-esters of mycolic acids from *M. paratuberculosis* (XIII) (24) and by the incorporation of ^{18}O (from $^{18}O_2$) into the wax-esters (25 ; figure 4).

The stereochemistry of the group characteristic of mycolic acids (formula I) studied in coryno-mycolic acid was established as tetradecyl-2*R* hydroxy-3*R* octadecanoic acid (26). Later the configuration 2*R*, 3*R* was also found for the mycolic acids of *M. tuberculosis*, *M. fortuitum*, *M. marinum* and *M. ulcerans* (27-30).

In the methoxy-mycolic acid of the strain Test of *M. tuberculosis*, it has been shown that the group methyl-methoxy (in the *mero* part) has the configuration *erythro* and is *S,S* (30).

3.3. Biosynthesis

The knowledge on mycolic acid biosynthesis lags far behind that on their structure. A few « acellular » systems of biosynthesis were described (31-34), but none of the main enzymes involved has been clearly identified.

Several models have been postulated for the chemical mechanisms of the different steps of mycolic acid biosynthesis. Two distinct steps are commonly postulated, namely chain elongation and mycolic condensation; however it has not yet been proven which of these two steps precedes the other.

3.3.1. Condensation

Working on corynomycolic acid, it was shown that 2 molecules of palmitic acid were condensed one with the other (figure 5) (35). By using a cell-free system (from *C. diphtheriae*) and 1-^{14}C-palmitate (formula XIV) as substrate, a C_{32}-keto ester (formula XV), the mono-(tetradecyl-2' keto-3'-octadecanoyl)-6 alpha-D-trehalose, was obtained (31, 36). Avidin inhibited this condensation, suggesting the involvement of biotin, thus a substituted malonyl intermediate (formula XVI) was postulated.

Working with a 5000g supernatant of *Corynebacterium* (*Bacterionema*) *matruchotii* and 1-^{14}C-palmitate, Japanese workers detected a condensation, provided a fluffy layer was added. C_{14} or C_{16} acids were the best substrates; avidin showed no effect. The synthesized corynomycolic acid was obtained in a form linked by an alkali-labile bond to a molecule of alpha-D-trehalose (32, 37).

Recently, it has been shown that the alpha-proton (marked by * in formula XVI) of the attacking fatty acid did not disappear, a fact thought to eliminate the participation of a malonic condensation (38). However, a similar result was obtained in 1975 by Lynen and coll. in a study on the fatty acid elongation mechanism (39), who concluded in favor of a concerted process of decarboxylation and condensation of the malonyl thioester.

3.3.2. Elongation

Experiences done in that field were interpreted as the construction of a *mero*-mycolic acid from one fatty acid, by the usual additions of C2-units (elongation), and formation of the double bonds where it was required (40). It must be noted that in these experiences, the radioactive precursor (1-^{14}C-acetate) was incubated for more than fifteen days, but we know to-day that fifteen minutes are enough.

In the case of the biosynthesis of mycolic acids from *Nocardia asteroides*, data have been obtained which supported the omega-oxidation of a shorter mycolic acid (about C_{30}) into a carboxy group (in the *mero* part of the molecule) and its elongation (through this omega-carboxy group) by C_2-units to give the nocardo-mycolic acid chain (41). It may be reminded the isolation of both corynomycolic acid (C_{32}) and nocardo-mycolic acids (C_{50}-C_{60}) in some *Nocardia* species.

3.3.3. Biosynthesis from four fatty acid molecules

When studying the structure of mycolic acids, the fact that (16C x 2) + (26C x 2) = 84C was impressive enough to suggest that four acid molecules could be used to build the molecule of mycolic acid (42). The model postulating the

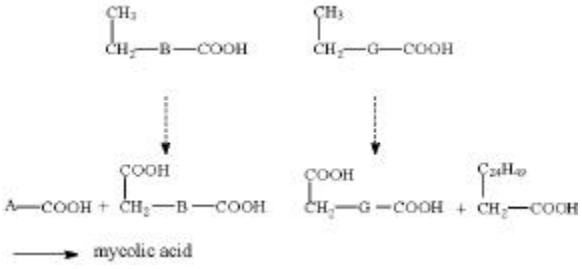

Figure 6. Mycolic acid biosynthesis. An alternative hypothesis postulating “head-to-tail” condensations of long chain fatty acids, with omega-oxidation steps

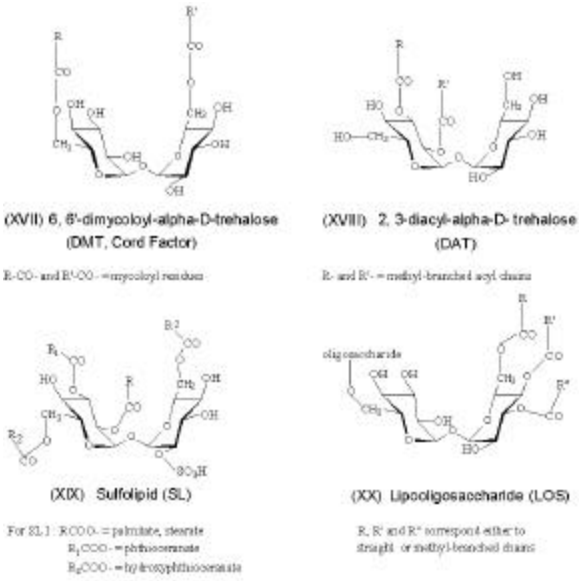

Figure 7. Trehalose-containing glycolipids

condensation of four molecules of fatty acids (figure 6) was recently re-examined (43). So far, it is still in competition with the elongation process. More information may be found in general articles (1, 2, 44, 45).

It is expected that this field will also benefit from the powerful tools of molecular genetics. At the present time, only a gene coding for an enoyl-reductase (46) and others coding for the introduction of cyclopropyl-, keto- and hydroxy- functions have been identified (47, 48).

4. ACYL TREHALOSES (FIGURE 7)

Trehalose-containing glycolipids represent the class of molecules that led to the most intense and extensive studies on mycobacterial lipids, and it still stimulates imagination of lipidologists and mycobacteriologists.

4.1. "Cord Factor"

The success story of trehalose dimycolate (TDM) is a good example of a simple, but unproven, idea that turned out a wealth of interesting works. H. Bloch (49) observed that extraction by petroleum ether of cells of a virulent strain of *M. tuberculosis* disorganized the cords they form at the culture medium surface, and that the resulting extract was toxic when injected to mouse. The toxic compound was shown to be 6,6'-dimycoloyl-alpha-D-trehalose (50, formula XVII). The idea that TDM is at the origin of "cord" formation is probably wrong, but TDM is actually toxic, in some experimental conditions.

A breakthrough on TDM activity came from M. Kato, who showed that, when injected to mouse, it induced symptoms of the dreadful 19th century "consumption" (51), and that this can be related to TDM activity on mouse liver mitochondria, *in vitro* and i*n vivo* (52, 53).

Another one came from A. Bekierkunst who showed that TDM induced lung granulomas (54), and has immunostimulating properties (55), that are probably at the origin of its antitumoral activity (56). TDM was at least one of the active compounds of the "Ribigens", patented and marketed by E. Ribi for veterinary application against some cancers. The above biological activities are fascinating, but they are even more poorly understood than the activation mechanism of immune cells, since toxicity as well as adjuvancy properties of TDM largely depend on the animal studied, the injection route chosen and on the characteristics of the TDM suspensions, as detailed in the excellent review of G. Lemaire *et al.* (57) on immunostimulation by TDM .

Takayama, Retzinger and collaborators coated polystyrene beads with mono- and multi-layers of TDM (58-60). One of the noted properties of this model was to form more or less linear aggregates (60). A model was proposed for TDM organization in monolayers, that could nicely explain bead aggregation, and "cord" formation by *M. tuberculosis*. However one can object that TDM is present in all mycobacteria, but only *M. tuberculosis* forms "cords". In addition, TDM has to be at the outer surface of cells to participate in aggregation, while a recent work suggests that TDM has a very superficial location in *M. aurum* (which is barely aggregated), and is much deeper in the envelope of *M. tuberculosis* (which does form cords) (61). The knot of mycobacterial cords is not yet untied .

Biosynthesis of TDM is not known. In intact cells, there is a slow and progressive labeling of the molecule in the presence of ^{14}C-acetate. In contrast, the monomycoloyl trehalose (MMT) is rapidly labeled, but it does not accumulate. This led to postulate that MMT is used to transfer mycolic acids towards molecules like the wall-linked arabinogalactan. In agreement with this hypothesis, a trehalose mycoloyltransferase was purified from *M. smegmatis* (62) and, in *M. tuberculosis*, this enzyme was recently identified with the antigens 85, which are excreted in the culture medium (63). A mannosyl isoprenoid compound has also been proposed by the same group as mycolic acid carrier (64).

4.2. Polyacyl trehaloses

This concerns two groups of molecules that draw much attention for their putative or demonstrated biological properties, namely sulfated trehaloses (sulfolipids, designated by SL), that are acylated by 2 to 4 highly branched fatty acids and the di- and tri-acyl trehaloses (DAT, TAT). In addition,

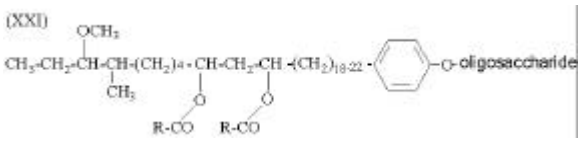

Figure 8. Pheolglycolipide (PGL). R=methyl-branched chains (mycocerosates)

trehaloses acylated by polyunsaturated fatty acids have been described in *M. phlei* (65).

4.2.1. The sulfolipid family (SL I, II and III) of acyltrehaloses (Figure 7, formula XIX)

The sulfolipid family (SL I, II and III) of acyltrehaloses was detected while searching for the origin of adsorption of cationic neutral-red on *M. tuberculosis* (66). The structure and activity of this class of glycolipids were extensively studied by M. Goren (for a review see ref. 2).

Sulfate derivatives are few in natural substances, and the acyl chains of SLs are also not common, since they are mainly very long saturated and unsaturated (up to C64), highly branched in their carboxyl end. The sulfate moiety is on position 2 on one glucose residue of a trehalose residue bearing 3 or 4 acyl chains on various positions.

The sulfolipids attracted a great interest since it was shown that they seem to prevent phagosome-lysosome fusion in macrophages (67), a phenomenon that is observed after phagocytosis of virulent strains of *M. tuberculosis*. Unfortunately Goren showed years later in a rigorous set of experiments that the method he used introduced artifacts that forbid to conclude (68). However, inhibition of phagosome-lysosome fusion is still quoted as a known property of the sulfolipids This exemplifies the difficulty to give their right place to unproven ideas, especially when they are easy to understand .

4.2.2. Di- and tri-acyl trehaloses (DAT and TAT)

The search for ways to detect early and rapidly infections by *M. tuberculosis* led to recognize a diacyltrehalose as a potential probe, initially thought to be a sulfolipid (69). Actually, it is not a sulfolipid, but a 2,3-diacyltrehalose (DAT; figure 7, formula XVIII), the acyl groups of which are mainly polymethyl branched at their carboxyl end (70). DAT is interesting since it is present in a large number of clinical isolates (69, 71). IgG corresponding to DAT are detected with a high specificity (97%) and a good sensitivity (86%) in sera of infected persons (72). Triacyltrehaloses (TAT) were detected in *M. fortuitum* (73) and in *M. tuberculosis* (74)., and they can be used for the same purpose .

5. OLIGOSACCHARIDE-CONTAINING LIPIDS

This class of lipids can be divided in two groups : lipooligosaccharides, which are acylated by long chain fatty acids, and mycosides, in which oligosaccharide moieties are linked to unusual lipidic residues, specific of mycobacteria.

5.1. Lipooligosaccharides (LOS)

A polyacylated trehalose constitutes the common lipophilic moiety for lipooligosaccharides (75-77). It contains 2 or 3 straight or methyl-branched chains (figure 7). In some LOS, acyl residues can be distributed between the two glucose residues of the trehalose end of the polymer. Depending on the species, an oligosaccharide is linked either on carbon 3, 4 or 6 of the trehalose end, and this oligosaccharide has 2 to 6 sugar residues, some of which are new and specific carbohydrates (78, 79). Pyruvic acid residues (carboxyethylidene) can be present, giving an anionic character to the molecule.

LOS are likely to be the alkali-sensitive surface antigens detected by Schaefer in 1962. Immunochemichal experiments have shown that LOS are exposed at the mycobacterial surface, which location results in interesting properties : *i.* they are immunogenic ; *ii.* they are phage receptors (80). It was proposed that their presence in the outermost layer of the envelope could be related to the rough and smooth morphologies of colonies (81), but this was not confirmed (82).

5.2. Mycosides

In the fifties, Smith and coll. tried to characterize chemically immunizing fractions of extracts from *M. tuberculosis* by infrared spectroscopy (83). This original approach led to the detection of lipids specific for mycobacteria species (84). This led also to the discovery of the glycolipids called "mycosides" (85).

5.2.1. Phenolglycolipids (PGL ; mycosides A, B, G ; Figure 8, formula XXI)

. These glycolipids raised a great interest first for their unusual structure of their lipidic part, then as potential tools to detect infections by *M. tuberculosis.*

The structure of the lipidic aglycone moiety (phenolphthiocerol) was studied by Gastambide-Odier and coll. (86, 87). It is unusual by its terminal phenol moiety, deriving biosynthetically from tyrosine, bearing a long chain with 2 hydroxyl groups acylated by polymethyl-branched fatty acids. Detailed definition of the carbohydrate part needed modern NMR and mass spectrometry methods since, while some phenolglycolipids bears only one methyl rhamnoside residue (in *M. bovis* and *M. marinum*), others have oligosaccharides, one of them with a labile dideoxyhexose at its non-reducing end (in *M. kansasii*; 88).

Biosynthesis of phenolphthiocerol was explored early (89). A biosynthetic pathway, and the corresponding genes involved, were recently postulated (90).

Phenolglycolipids are immunogenic with their carbohydrate at the non-reducing end as recognized epitope. It was found that *M. leprae* produces such glycolipid (91), which was detected in the liver of infected armadillos (92). Leprosy patients have antibodies against it, thus it seemed it could be used for serodiagnosis of leprosy (93, 94). Then, a phenolglycolipid (PGL-Tb) was found in the Canetti strain of *M. tuberculosis* (95). This arose the expectation of obtaining a serodiagnosis of tuberculosis, efficient and specific since few mycobacterial species produce phenolglycolipids (96). However it appeared that there were large variations among tuberculous patients in the response to this antigen (97-99). This is likely due to large differences in the amounts of phenolglycolipid produced by different strains (100).

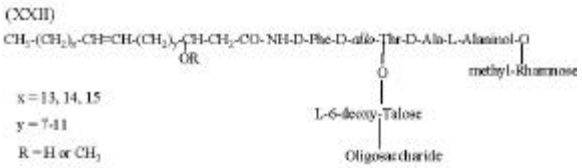

Figure 9. Glycopeptidolipid (GPL, mycoside C)

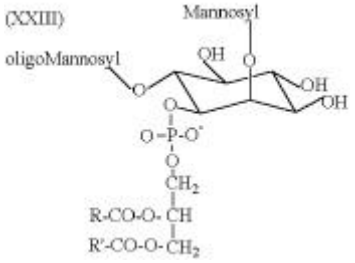

Figure 10. Phosphatidylinositomannoside (PIM)

5.2.2. Glycopeptidolipids (GPL ; mycoside C; Figure 9, formula XXII)

Non-polar mycosides C have been discovered in the course of a systematic study of mycosides, and have been mainly found in the *Mycobacterium avium - intracellulare - scrofulaceum* (MAIS) complex. The first complete structure of a mycoside C isolated from the strain 1217 has been proposed in 1968 (101), in which the three amino acids were of the D series, linked by the N-terminal group to 3-hydroxy-C_{28} fatty acid, and by the C-terminal group, to alaninol. A diacetylated 6-deoxy-L-talose was glycosidically linked to the hydroxyl of the residue of D-*allo*-threonine and a 2,3,4-tri-O-methyl L-rhamnose, to that of alaninol. In the fatty acid, the hydroxyl group may be free or methylated, a double bond may or not be present in the middle of the chain. In the rhamnosyl group, a 3,4-di-O-methyl can replace the 2,3,4-tri-O-methyl.

Besides these non-polar mycosides C, a group of polar mycosides C has been detected, following the work of Schaefer on the antigens of MAIS complex and that of Marks and Jenkins on glycolipids of the same mycobacterial strains. Brennan and Goren (102) demonstrated that oligosaccharidic chains can be linked to the hydroxyl-2 of the 6-deoxy-talose. Several polar mycosides C have been isolated from different serovars of the MAIS complex (1), the oligosacharidic chains of which had 3 to 6 sugar residues. The oligosaccharide linked to the *allo*threonine moiety can be split by alkaline treatment (beta-elimination).

A variant in the structure of GPL was discovered in the lipids of *M. fortuitum*. The peptidolipid structure was the same as before, but a disaccharide made of 2 rhamnose residues more or less O-methylated was linked to alaninol, whereas a 3-O-methyl rhamnose was linked to *allo*Thr (103). No 6-deoxy-talose was present. A small amount of a 2-O-sulfate ester of a PGL, with one rhamnosyl linked to *allo*Thr and one to alaninol has been found; the sulfate group was linked to the sugar born by alaninol (104). Working on the lipids of *M. xenopi*, a family of GPL variants were isolated. All of them had the same peptide skeleton H_2N-L-Ser-L-Ser-L-Phe-D-*allo*Thr-COOH, with lauric acid linked to the free amino group (105, 106).

Free GPLs are serologically active; however, they require a protein carrier to show immunogenicity (107). The lipopeptide fragment of mycosides C modifies the lymphocyte response to mitogens (108).

GPL suspensions uncouple oxidative phosphorylation of isolated mitochondria and increase passive permeability of liposomes (109). Highly glycosylated GPL are less active (110). Monolayer studies suggested a first explanatory model (111), but IR-spectroscopy on monolayers indicated that GPL molecules with 1 to 3 sugar residues are randomly inserted in the phospholipidic layer and deorganize it, while highly glycosylated GPLs seggregate in the layer without disturbing it significantly (112).

6. GLYCOSYL DERIVATIVES OF PHOPHATIDYLINOSITOL

6.1. Phosphatidylinositomannosides (PIM ; figure 10, formula XXIII)

As early as 1930, it was recognized by Anderson that the phospholipidic fraction extracted from the tubercle bacillus and related mycobacteria contained inositol and mannose. Studies by Lederer and coll. (113), and by Lee and Ballou (114), revealed the structure of a family of molecules in which 1 to 5 mannose residues are linked to phosphatidylinositol (PIM_1 to PIM_5).

6.2. Lipomannans (LM) and Lipoarabinomannans (LAM)

Arabinomannans and mannans were known for several years (115) when it was shown that at least some of these polysaccharides can be linked to a phosphatidyl residue (116), which was firmly identified (117). Additional inositol residues linked by a phosphodiester to the mannose core are present.

It is still a matter of debate to know whether or not all these polysaccharides molecules are synthesized initially with a lipidic moiety. It was proposed that the biosynthesis of LM and LAM proceeds by successive additions of mannose or arabinose residues on a PIM_5, through a polyprenyl carrier (118).

These anionic polysaccharides can be considered as « atypical lipoteichoic acids » (119), and one can attribute to them all the ultrastructural and physiological functions postulated for teichoic acids. They also share some structural features with lipopolysaccharides of gram-negative bacteria, due to the anionic reducing end of the polysaccharide and to their negatively charged lipidic anchor. It was shown that they also share some biological properties with the LPS, since LAM, but not LM, are endowed with many activities on immune cells. These properties are influenced by the presence of a mannose « capping » on the non-reducing arabinan end of some LAM molecules (Man-LAM). For a detailed discussion on structure and properties of LAM, see the article by Vercellone *et al* in this issue.

PIM, Mannans, Arabinomannans, LM and Man-LAM have mannose residues at their non-reducing ends. As the mannose receptor is currently considered of major

importance for the entry of *M. tuberculosis* in macrophages, it will be interesting to know which of these molecules are involved in the interaction of the tubercle bacillus with macrophages. The beginning of an answer could come from the location of these molecules in the outer envelope of the bacillus. On this aspect, see the article by Draper in this issue.

7. CODA

The structural side of the Anderson's target is nearly reached, since it is likely that the development of powerful chromatographic tools, and the recent emergence of a great variety of techniques in NMR and mass spectrometry analysis, led to the structural elucidation of most of the molecules of the envelope of mycobacteria. Among the unsolved problems, one can point to the fine structure of large amphiphilic molecules, which are not easily obtained as pure molecular species. It could also be interesting to look for very minor compounds, especially if their synthesis is linked to the interaction of mycobacteria with host cells.

Concerning the biological activity of mycobacterial lipids, a « trial and error » approach is still common. A more rational way to explore this field will surely derive from molecular genetics : inactivation of the coding genes, or their expression in other organisms, will permit a more safe and systematic analysis of the effective role of lipids in pathogenicity.

After that, the next frontier is likely to be more difficult to cross : lipids are amphiphilic molecules that cannot be present in the envelope as isolated molecules. They participate to supramolecular structures, and it is known that the physical organization is of major importance for the biological activity of lipids; this was clearly shown for the activities of dimycoloyltrehalose (cord factor). Thus, the next challenge will be the determination of the physical organization of lipids, together with polysaccharides and proteins, in the envelope of mycobacteria, and the correlation of this state to the activity. This way is not yet paved.

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