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INTERACTION OF MYCOBACTERIAL GLYCOLIPIDS WITH HOST CELLS

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1. ABSTRACT

Mycobacteria elaborate a great variety of glycolipids of rather exotic structure. Some of these lipids are abundant cell envelope components and are exposed on the bacterial surface. These comprise the species-specific phenolic glycolipids, glycopeptidolipids, sulfatides, and lipooligosaccharides, and the ubiquitous phosphatidylinositolmannosides. Because pathogenic mycobacterial species are facultative intracellular parasites that infect and reside in host cells, some of them may represent potential virulent factors as they have been shown to inhibit both macrophage antimicrobial activities and lymphoproliferation. These biologic activities may derive, at least in part, from the modulation of the cell functions through the interactions between host membranes and these surface-exposed lipids whose structures are different from those of mammalian cell membrane components. In few cases purified glycolipids have been shown to profoundly affect the physical and functional properties of biologic membranes. Therefore, the enzymes involved in the biosynthesis of the biologically active glycolipids represent potential drug targets. However, definite proofs of their implication in the mycobacterial pathogenicity are lacking. Mutants unable to elaborate defined glycolipids are needed.

2. INTRODUCTION

The genus *Mycobacterium* is known for its high lipid content, constituting up to 40% of the dry weight (1). These are of two types: covalently-linked compounds and loosely-associated molecules that are readily extractable with organic solvents. The first class of lipid is composed of mycolic acids, high molecular weight (up to C90) alpha-branched, beta-hydroxylated fatty acids, that esterify the cell wall arabinogalactan; the second class is a mixture of ubiquitous, type-specific and species-specific lipids. The early observation showing that injection of delipidated tubercle bacilli to susceptible animals results in the formation of notably less tubercles than that of non-delipidated bacilli raised the possibility that the activity might resides in extracted lipids (1). This has initiated an intensive research program on

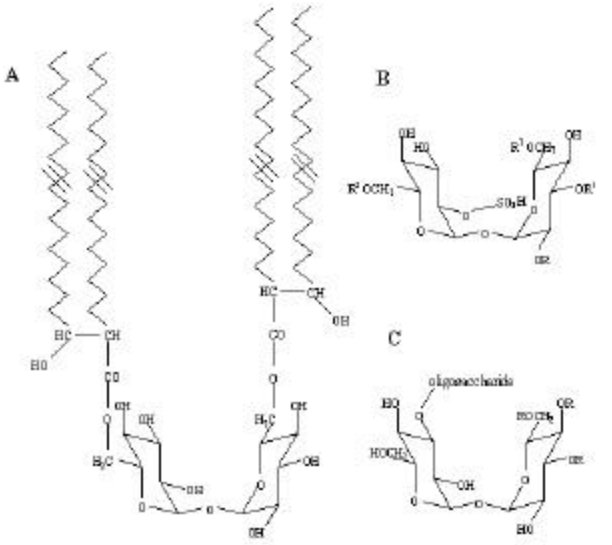

Figure 1: Trehalose-containing lipids. (A) Trehalose dimycolate from (3). (B) Sulfatide-I from (3). R: palmitoyl/stearoyl residues; R^1 phthioceranoyl residue; R^2 R^3 hydroxyphthioceranoyl residue. (C) Lipooligosaccharide from (11). R: fatty acyl.

mycobacterial lipids that has yielded a rich harvest of insight on their structures and possible functions (for recent reviews, see 2, 3, 4).

Mycobacterium tuberculosis, the causative agent of tuberculosis, *M. bovis* and *M. leprae* are apparent obligate pathogens. In addition, several other mycobacterial species, notably members of the *M. avium-intracellulare* complex, are opportunistic pathogens, *i.e.* they occur naturally in the environment but may cause serious disease in humans occasionally, especially in immunosuppressed individuals. In the course of the intracellular multiplication of the bacilli in the host, the non-covalently bound mycobacterial lipids which comprise many amphiphilic glycolipids that are abundantly produces by growing bacteria and located into the external layer of the bacterial envelope are free to diffuse and interact with host's membranes. Because these glycolipids are structurally different from eucaryotic membrane components, they may alter some of the biological functions associated to membranes such as cell/cell communication, signal transduction, membrane fusion (5). They may also act as bacterial ligands for cell receptors. Thus, through specific or non-specific interactions with host membranes mycobacterial glycolipids may contribute to the pathogenicity by modulating the activity of the infected cells.

A systematic description of the detailed structures and the numerous biologic activities attributed to mycobacterial lipids would be inappropriate in this review but the general structures, distribution of the glycolipids in the genus *Mycobacterium* and their localization in the cell envelope are summarized. Lipoarabinomannan and related mycobacterial lipopolysaccharides which are the subject of a dedicated chapter of this issue will not be considered in this chapter. We deliberately chose to focuse the review on the biological activities of the mycobacterial glycolipids that have been shown to interact with host immune cells other than B-cells, known to respond to almost all the glycolipids studied so far. When data are available, the contribution of the carbohydrate and the lipid moieties of a given glycolipid in the activity will be discussed.

3. STRUCTURE, DISTRIBUTION AND LOCALIZATION OF MYCOBACTERIAL GLYCOLIPIDS

The structures and distribution of the major glycolipids among the various mycobacterial species are summarized in table 1.

3.1. Acylatedtrehaloses

These molecules share a common alpha, alpha' trehalose unit, *i.e.* alpha-D-glucopyranosyl-alpha'-D-glucopyranoside and may be grouped into families (figure 1).

3.1.1. Trehalose dimycolate

The trehalose 6,6'-dimycolate (TDM, figure 1A), also named "Cord Factor", is found in all mycobacterial species examined with a notable exception, *M. leprae*. In this 'non-cultivable' leprosy bacillus, no TDM was detected; rather, a related compound, trehalose 6-monomycolate (TMM), compound widely distributed in the genus *Mycobacterium*, was the only trehalose ester characterized (26). Trehalose mycolates occur as a mixture, differing one from the other by the chemical groups present in the mycolic acid substituents (3).

In sharp contrast to the assumed surface location of TDM in mycobacteria, a recent study has demonstrated that in most mycobacterial species, including *M. tuberculosis*, TDM was found in peripheral layers of the cell envelope, but not on the bacterial surface (29).

3.1.2. Sulfatides

This family of five members is typified by a sulfate substituent on position 2' (figure 1B). They differ one from the other by the number and type of acyl substituents, and by their positions on trehalose. The fatty acyl substituents consist of straight chain ($C_{16:0}$ and $C_{18:0}$) and characteristic multimethyl-branched residues unique to sulfatides. The principal sulfatide, SL-I, is a 2,3,6,6'-tetraacyl-alpha,alpha'-D-trehalose 2' sulfate (30). Sulfatides have been characterized so far only from *M. tuberculosis.*

As sulfatides have been discovered in the search for compounds responsible for the unique ability of the tubercle bacillus to absorb the cationic phenazine dye, Neutral red, they are believed to be peripherally-located substances. Further data are needed to determine their precise location since many components of the tubercle bacillus may potentially react with this dye.

3.1.3. Miscellaneous trehalose esters

In addition to TDM and TMM, miscellaneous trehalose esters are also elaborated by some mycobacterial species; these include the fully acylated trehalose esters with highly unusual polyunsaturated acyl substituents (phleic acids) found in *M. phlei* and *M. smegmatis* (see 6), the polyphtheinoyl trehalose typifying *M. tuberculosis* (7), and the

Table 1. Distribution of glycolipids in the genus *Mycobacterium*

COMPOUNDS	DISTRIBUTION[a]	REFERENCES[b]
Trehalose dimycolate (TDM)	❑ *M. tuberculosis, M. bovis*	
	❑ *M. avium; M. kansasii*	
	❑ *M. phlei, M.smegmatis*	*6*
	❑ *M. parafortuitum*	
	❑ Other species[c]	
Sulfatides (SL)	❑ *M. tuberculosis*[d]	3
Polyphthienoyl trehalose	❑ *M. tuberculosis*	*7*
Polyphleoyl trehalose	❑ *M. phlei; M. smegmatis*	6
2,3 diacyltrehalose (DAT)	❑ *M. tuberculosis, M. africanum,*	*8*
	❑ *M. bovis*	*9*
	❑ *M. fortuitum*	*10*
Lipooligosaccharides (LOS)	❑ *M. kansasii, M. malmoense*	
	❑ *M. szulgaï, M. smegmatis*	*2*
	❑ *linda (M. paratuberculosis)*	
	❑ *M. tuberculosis*	*11*
	❑ *M. fortuitum* (3rd biovariant)	*12*
	❑ *M. gastri*	*13*
	❑ *M. gordonae*	*14*
	❑ *M. mucogenicum*	*15*
	❑ Other species[e]	
Glycopeptidolipids	❑ *M. avium intracellulare scrofulaceum*	*2*
(GPL)	❑ *M. paratuberculosis, M. simiae*	
C mycosides	❑ *M. chelonae, M. peregrinum*	*16,17,18,19*
	❑ *M. smegmatis (M. buryricum), M. farcinogenes, M.sp*	*20*
	❑ *M. lepraemurium*	*21*
	❑ *M. porcinum, M. senegalense*	*22*
	❑ *M. abscessus*	*19*
Phenolic glycolipids (PGL)	❑ *M. bovis, M. leprae, M. marinum, M. kansasii, M. gastri, M. tuberculosis*	*2,7,23*
	❑ *M. haemophilum*	*24*
	❑ *M. ulcerans*	*25*
Phosphatidylinositol mannosides (PIM)	❑ All mycobacterial species[f]	2

a – Note that LOS, GPL and PGL are not found in all the strains of the same species.
b – reviews have been selected when too many references exist.
c – based on the authors unpublished data, DMT has been detected in all species examined so far; however, only monmycolyl trehalose has been found in *M. leprae* (26).
d – although ^{35}S labelled compounds have been detected in other species, it is very likely that the slufatides are confined in *M. tuberculosis* (3).
e – lipooligosaccharides have been partially characterized in *M. marinum* (27), some armadillo derived mycobacteria (28).
f – All species examined so far contain this class of phospholipids; the only exception, a strain of M. vaccae, contains phosphatidylserine not found in mycobacteria (see 2).

2,3-diacyl and 2,3,6-triacyl trehaloses recognized in *M. tuberculosis* (31, 32) and *M. fortuitum* (10); this latter species also elaborates the 2,3,4-triacyl trehalose antigen (10). As these glycolipids are easily extractable from the cells with petroleum ether, a procedure which does not affect the viability of the bacteria, trehalose esters are assumed to be peripherally-located compounds.

3.2. Lipooligosaccharides

Mycobacterial lipooligosaccharides (LOS, figure 1C) consist of a common core of polyacyl trehalose which may be *O*-methylated (11, 14); this core is further glycosylated by a mono- or more frequently an oligosaccharide unit (see 2). The structures of these alkali-labile glycolipids have been fully defined and have proved, in many cases, to be specific of the mycobacterial species due to the presence of exotic sugars such as 4,6-dideoxy-2-*O*-methyl-3-*C*-methyl-4-(2'-methoxypropionamido)-alpha-L-*manno*-hexapyranose (N-acylkanosamine). In the same mycobacterial species they generally occur as a mixture of compounds differing one from the other by both the composition of the oligosaccharide moiety and the number of fatty acyl substituents. In addition to straight chain fatty acyl residues, LOS may contain methyl-branched acyl substituents and pyruvate ketal units (2). The different species-and type- specific mycobacterial LOS differ mainly by their glycosyl composition (2).

LOS are exposed on the surface of the mycobacterial species which synthetize them (29, 33).

3.3. Mycosides

The term 'mycosides' was originally used to typify species- and type-specific glycolipids of mycobacterial origin which had been discovered by infrared spectroscopy of chromatographically fractionated ethanol/diethyl ether extracts (34). Further analyses of the purified compounds established

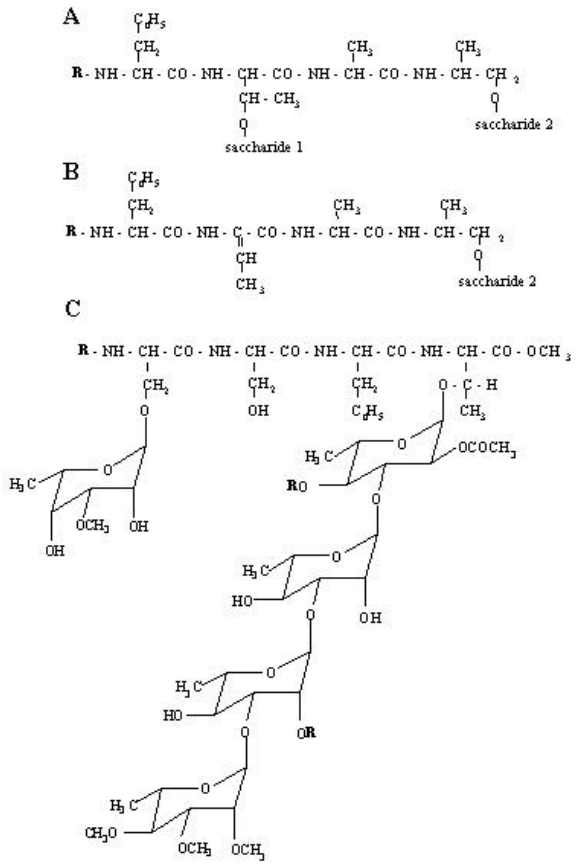

Figure 2: Glycopeptidolipids. (A) C-mycoside. R : C_{30} beta-methoxylated or beta-hydroxylated fatty acyl. (B) Beta-lipid. R: C_{30} beta-methoxylated or beta-hydroxylated fatty acyl. (C) Serine-containing glycopeptidolipid from (42). R: CH_3-$(CH2)_{10}$ CO.

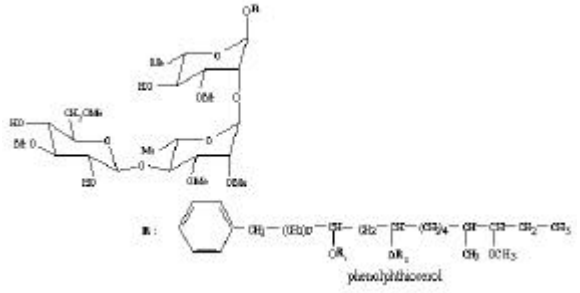

Figure 3: The major phenolic glycolipid from *M. leprae* (PGL-I) from 44 R_1; R_2 mycocerosic acyl (C_{30} C_{34}) residues.

the specificity, diversity and chemical structures of the various mycosides.

3.3.1. Glycopeptidolipids

Mycobacterial glycopeptidolipids (GPL) have been described so far in only non-tuberculous mycobacteria. The most encountered GPL, the so-called C-mycosides, are alkali-stable glycolipids, as opposed to LOS, and are produced by several mycobacterial species, including saprophytic (*M.smegmatis*), opportunistic pathogens for human (*M. avium*; *M. intracellulare*; *M. scrofulacerum*; *M. peregrinum*; *M. chelonae*; *M. abscessus*) and for animals (*M. lepraemurium*; *M. paratuberculosis*; *M. porcinum*; *M. senegalense*). All the molecules share a same lipopeptidyl core consisting of a long C_{30} acyl chain and a tripeptide composed of amino acids of the unusual D-series (D-phenyl alanine, D-*allo*-threonine and D-alanine) and terminated by L-alaninol (from alanine). They differ one from another by the number and the nature of the saccharidyl units linked to the hydroxyl group of *allo*-threonine and/or alaninol (figure 2A). In the most abundant molecular species, the 'apolar' GPL, the hydroxyl groups are substituted by a mono- or a di-saccharidyl unit composed of deoxysugar residue(s) that are usually *O*-methylated and/or *O*-acylated. In members of the *M. avium-intracellulare* complex an oligosacharidyl of unusual composition is linked to the 6-deoxytalosyl unit which in turn substitutes the *allo*-threonine residue, leading to the so-called 'polar' GPL (2). As in the case of LOS, the glycosyl units of 'polar' GPL contain unique sugar residues that are type- or species-specific. This structural variability and the antigenicity of GPL is the chemical basis of the identification, using seroagglutination assay, of the different serotypes (serovariants) within the *M. avium-intracellulare* complex.

Localization studies demonstrated that C-mycosides are exposed to the bacterial surface (29), in agreement with GPL being the Schaefer typing antigens (2) and their identification as the receptor of mycobacteriophage D4 (35, 36). *In vivo*, polar C-mycosides are poorly degraded after phagocytosis of *M. avium* by macrophages (37) and accumulate into the phagosome during the bacterial growth contributing to the formation of a capsule surrounding the bacteria (38, 39, 40)

More recently, a second family of GPL has been discovered in *M. xenopi* (41, 42). It consists of alkali-labile serine-containing glycopeptidolipid whose structures (figure 2C) differ greatly from those of C-mycosides.

3.3.2. Phenolic glycolipids

Phenolic glycolipids (PGL) are found in several obligate and opportunistic mycobacterial pathogens; these include members of the *M. tuberculosis* complex (*M. tuberculosis, M bovis* , *M. microti*), *M. leprae*, *M. kansasii*, *M. marinum* (7, 2), *M. haemophilum* (24), and *M. ulcerans* (25). The non-pathogenic species *M. gastri* , a species closely related to *M. kansasii*, also contain PGL (7). It is worthy of note that, to date, PGL and GPL, on the one hand, and LOS and GPL, on the other hand, have not been found in the same mycobacterial species.The lipid core of PGL is composed of phenol-phthiocerol and relatives (long chain C33-C41 beta-diols) which are esterified by two multimethyl-branched C27-C34 fatty acids (mycocerosic or phthioceranic acids). The sugar moiety of PGL is species-specific and consists of one to four *O*-methylated sugars (principally desoxysugars). The PGL of *M. leprae* (PGL-I, figure 3), constituting up to 2% of the mass of the bacillus, is a major component of the ultrastructurally observed electron-transparent zone surrounding the leprosy bacillus (43) and other pathogenic mycobacteria in infected cells and tissues; this zone may protect these bacteria from host's attacks. The oligosaccharide part of PGL-I consists of 3,6-di-*O*-methyl-beta-D-glucosyl (1->4) 2,3-di-*O*-methyl-alpha-L-rhamnosyl (1->2) 3-*O*-methyl-alpha-L-rhamnosyl (1-> (figure 3) (44).

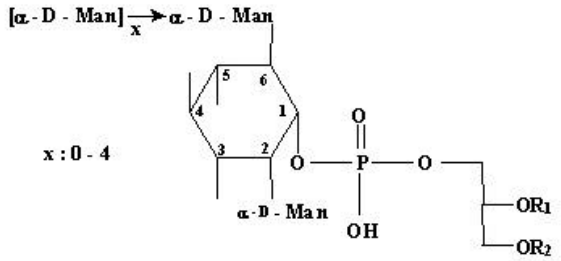

Figure 4: Phosphatidylinositolmannosides. R_1 and R_2: fatty acyl residues.

Localization studies demonstrated that PGL are on the bacterial surface (45, 46, 29).

3.4. Phosphatidylinositolmannosides

Beside phosphatidylethanolamine, phosphatidyl inositolmannosides (PIM) are the major phospholipid component of the mycobacteria cell envelope, except for *M. vaccae* (2). PIM occur as a mixture of compounds differing one from the other by number of mannosyl residues. Their basic structure consists of a mannosyl unit attached to position 6 of the *myo*-inositol of a phosphatidylinositol (figure 4). Position 2 of inositol is further substituted by an alpha-D-mannosyl or a (1->6)-linked tetramannosyl unit, giving PIM_2 and PIM_5, the major PIM encountered in mycobacteria. PIM_5 may be further substituted at position 2 by an alpha-D-mannosyl, leading to PIM_6.

A recent study showed that PIM, which were known to be present in the plasma membrane, are also found on the bacterial surface of both pathogenic and non-pathogenic mycobacteria (29).

4. MODULATION OF HOST CELL RESPONSE BY MYCOBACTERIAL GLYCOLIPIDS

The principal biologic activities of mycobacterial glycolipids are summarized in table 2.

4.1. Inhibition of macrophage antimicrobial activity

4.1.1. Sulfatide

SL-I has been shown to inhibit the phagocytosis of IgG-opsonized sheep erythrocytes by lipopolysaccharides (LPS)-primed monocytes. SL-I also blocked the effect of several priming agents as interferon-gamma, LPS, interleukin-1-beta, tumor necrosis factor-alpha and muramyl dipeptide on macrophages, resulting in the inhibition of the release of O_2^- induced by phorbol 12-myristate 13-acetate (PMA) or formyl-methionyl-leucyl-phenylalanine (FMLP). This property was shown to be specific of sulfatide since dextran sulfate was inactive and trehalose dimycolate rather promoted macrophage priming. No effect of SL-I was observed on unprimed cells, suggesting that sulfatide altered the priming rather than the O_2^- release. Inhibition of the macrophage priming seems to occur indirectly by the inactivation of protein kinase C (49, 50). It is noteworthy, that sulfatide does not block the priming of neutrophils by LPS (51).

4.1.2 Phenolic glycolipids

Purified PGL-I from *M. leprae* can preclude *Staphylococci* killing by human monocyte-derived macrophages activated by interferon-gamma. This effect may result from its capacity to interfere with the system that produces reactive oxygen species (72). Launois *et al*. (73) have showed that PGL-I induces a decrease in the production of oxygen radicals by normal human mononuclear cells stimulated by *M. leprae, M. bovis* BCG and *M. kansasii* or by PMA. In contrast, PGL from *M. kansasii* can not decrease the chemiluminescence response of cells. Electron spin resonance studies on *in vitro* xanthine oxydase system demonstrated that deacylated PGL-I acts as a scavenger of $OH^{\cdot}$ or O_2^- and not as an inhibitor of the enzyme (71). This conclusion was questioned, however, by Vachula *et al*. (74, 75) who showed that PGL-I decreases the release of O_2^- by human monocyte-derived macrophages only in response to *M. leprae* but not to other stimuli as zymozan, PMA, *M. bov*is BCG and *M. kansasii*.

4.1.3. Glycopeptidolipids

The beta-lipid obtained by chemical means from C-mycoside GPL (figure 2B) affects the ability of human peripheral blood macrophages (HPBM) to control the growth of *M. avium* serovar 2. The lipid can also induce the release of high levels of prostaglandin E2 (PGE2) by the cells and some changes in the membrane ultrastructure (84), resulting in the alteration of macrophage functions (85). It is noteworthy that 'polar' GPL from serovar 8, but not those from serovars 4 and 20, exhibit similar properties, pointing out the probable role of the glycosyl composition of the sugar moiety in the biologic activity (86).

Glycolipid fractions extracted from *M. paratuberculosis* 18 inhibit the killing of *Candida albicans* by activated bovine peripheral-blood derived macrophages (87). As *M. paratuberculosis* is considered as a variant of *M. avium-intracellulare*, the active compounds may well correspond to GPL; however, further study are needed to elucidate the structure of these compounds.

4.2. Inhibition of lymphoproliferation

4.2.1. Phenolic glycolipids

In 1984 Mehra *et al.* published data supporting the view that PGL-I was able to selectively inhibit the concanavalin-A-induced proliferation of peripheral blood mononuclear cells from patients with lepromatous leprosy but neither from those with tuberculoid form of the disease nor those from healthy persons (68). Later studies indicated, however, that PGL-I induces a non-specific inhibition of the lymphoproliferation, irrespective to the donors (69, 70).

The inhibitory effect was shown to be independent of the presence acyl chains, as deacylated PGL-I exhibited a comparative activity than the native PGL (68) whereas the relative importance of the oligosaccharide is not clear. While PGL from *M. kansasii* and *M. bovis* in liposomes at 0.5 micrograms/ml were found inactive (68), in dried state, Fournié *et al*., (70) found that these PGL at 25 micrograms/ml exhibit nearly the same inhibition effect as that of PGL-I.

4.2.2. Glycopeptidolipids

An intraperitoneal injection of GPL from *M. avium-intracellulare* serovars 4 and 20 causes the inhibition of the blastogenic response of murine splenic lymphocytes to

Table 2. Biologic activities of mycobacterial glycolipids

COMPOUNDS	BIOLOGIC ACTIVITIES[a]	REFERENCES[b]
	❑ Toxicity in mice[c]	
Trehalose dimycolate (DMT)	❑ Biochemical alterations of the mitochondrial phosphorlyation and respiration[d]	
	❑ Immunostimulation[e]	
	❑ Granulomagenic[f]	
	❑ Antitumor activity[g]	3
	❑ Stimulation of macrophages to secrete cachectintumor necrosis factor[h]	
	❑ Absorption of fibrinogen[i]	
	❑ Inhibition of fusion between phospholipid vesicles	47
	❑ *In vivo* induction of apoptosis in the thymus	48
Sulfatides (SL)	❑ Synergistic enhancement of TDM toxicity *in vivo*	3
	❑ Inhibition of macrophage priming	49-50
	❑ Activation of human neutrophils	51-52
	❑ Antigenic in tuberculosis	53
2,3diacyl and 2,3,6triacyl trehaloses	❑ Antigenic in tuberculosis and leprosy	32,54-56
Trehalose monomycolate	❑ Biochemical alterations of the mitochondrial phosphorylation and respiration	57
(TMM)	❑ Antigenic in mycobacterial infections	3
Lipooligosaccharides (LOS)	❑ Surface antigens of some nontuberculous mycobacterial species	58
Glycopeptidolipids (GPL)	❑ Antigens related to serotyping in the M. avium intracellulares crofulaceum complex	2
C mycoside	❑ Receptor of mycobacteriophage D4	59
	❑ Inhibition of mitochondrial oxidative phosphorylation and drastic increase of passive permeability	60-61
	❑ Inhibition of lymphoproliferative response	
	❑ Induction of TNFalpha release and PGE_2 production	
	❑ Increased survival of *M.avium* in macrophages	62
Phenolic glycolipids (PGL)	❑ Antigenic in leprosy and in tuberculosis	63-67
	❑ Inhibition of lymphoproliferative response	68-70
	❑ Scavenging of oxygen radicals	71-75
	❑ Suppression of monocyte oxidative responses	76
	❑ Activation of complement for the phagocytosis of *M. leprae* by human monocytes	77
Phosphatidylinositol mannosides	❑ Antigenic in mycobacterial infections	78
(PIM)	❑ Induction of cytokines to release: TNFalpha, IL6; GMCSF, IL8, IL10	79-80
	❑ Suppression of Ag induced T cell lymphoproliferation	79
	❑ Activation of mouse peritoneal macrophages	81
	❑ Adhesin mediating binding to nonphagocytic mammalian cells	82
	❑ Induction of NO synthase activity in mouse peritoneal macrophages primed either by IFNgamma or TDM	83

a – The biologic activities listed are those demonstrated or convincingly presumed.
b – Reviews have been selected rather than original publications when too many references exist.
c – Not only all the animal species other than mice are entirely resitant to TDM intoxication but various strains of mice are also resitant; these include nude mice ; of the succeptible mouse strains, some older mice are resistant (3).
d – in water dispersion
e – in small oil droplets or in water dispersion (3)
f – in large oil droplets emulsions (3)
g – in aqueous emulsion in oil; activity on intradermal syngeneic transplants of murine fibrosarcoma but ineffective in treatment of a variety of other tumors (3)
h – in oil solution
i – TDM absorbed on polystyrenebeads

nonspecific mitogens (concanavalin A, phytohemagglutinin and LPS) (88, 89). As the same effects were not observed *in vitro*, these data suggested a production of active GPL metabolites *in vivo* (88). Because the responsiveness of lymphocytes was down-regulated to a greater extent by the beta-lipid (obtained from the beta-elimination of GPL, figure 2B), the putative metabolites may be structurally related to the beta-lipid (84). The lymphoproliferative responses of human peripheral blood mononuclear cells to the stimulation by phytohemagglutinin was also affected in the same way by beta-lipid (85). This suppression of the lymphoproliferation seems to be mediated by soluble factors released by beta-lipid-treated macrophages which are different from prostaglandin E2 (84).

4.2.3. Phospholipids

Mycobacterial phospholipids may be also implicated in the modulation of the host immune system. Phospholipid fractions from *M. intracellulare* have been shown to inhibit the concanavalin-induced proliferative response of spleen cells by reducing the ability of cells to produce interleukin 2 (90). Blood monocytes which had phagocytosed *M. tuberculosis* were shown to release phosphatidylethanolamine and phosphatidylinositol of

mycobacterial origin which exhibited a suppressor T cell-inducing ability (91). Finally, PIM were shown to inhibit Ag-induced proliferation peripheral blood monocyte cells (79).

5. INTERACTION OF MYCOBACTERIAL GLYCOLIPIDS WITH BIOLOGIC MEMBRANES

5.1. Trehalose dimycolate

Different preparations of TDM have been shown to alter the integrity of natural membranes. For instance, as a molecular monolayer at the surface of a hydrophobic particle, TDM can kill mouse peritoneal macrophages and induce hemolysis of red cells by disrupting the cellular membranes. These effects require direct contact between TDM and cells and involve the formation of hydrogen bonds (92); thus, a model of organization of TDM molecules has been elaborated to explain the action of the glycolipid. In the model, TDM is presented as a oriented two-dimensional crystalline surface monolayer composed of arrays of trehalose molecules separated by mycolic acids (93). Although such a damage of membranes could explain the much debated observation of McDonough *et al*. (94) who reported the escape of the tubercle bacillus from the phagolysosome to the cytoplasm after phagocytosis of bacteria, in most mycobacteria, including *M. tuberculosis*, TDM is not found on the bacterial cell surface (29).

A suspension of TDM in water can induce the swelling and fragmention of isolated mitochondrial membranes and the detachment of inner-membrane spheres (95). These effects led to the loss of the respiratory control and to the inhibition of the oxidative phosphorylation observed on liver mitochondria. In spite of an apparently coarse alteration of membranes, the inhibition is site II-specific (96) and is widely sensitive to the configuration of both sugar units and the nature of the acyl substitutents and to organization state of suspensions (97, 98). Surprisingly, however, a suspension of TDM in water or TDM inserted in membranes have no effect on the integrity of phospholipid bilayers (60, 47).

The mechanism of action of TDM on mitochondria and on cellular membranes in general is still hazy. Syed &Hunter (92) hypothesize that the alteration of natural membranes by the glycolipid is due to the adhesive properties of TDM monolayers; other authors explain the effects of TDM by the insertion of TDM molecules in membranes; non-specific interaction with membrane proteins is evoked.

One of the key stages of the mycobacterial infection is the survival and the multiplication of bacilli in macrophages. These result, at least in part, from the inhibition of the fusion between mycobacteria-containing vacuoles and lysosomes. As TDM was thought to be surface-exposed until recently, the potential implication of TDM in this phenomenon has been tested *in vitro*. Trehalose dimycolate from *Nocardia asteroïdes* (strain GUH-2) inserted into phospholipid vesicles was shown to block Ca^{2+}-induced fusion between vesicles. It has to be noticed that no TDM of mycobacterial source has been tested in these experiments. The different portion of TDM from *Nocardia*, trehalose and mycolic acids, alone are inefficient in blocking the fusion; it seems that the whole TDM is needed for the activity. The inhibition may be carried out by increasing either the membrane fluidity or the amount of water closely associated with phospholipids, thus thwarting the effect of Ca^{2+} (47, 99). The possibility that TDM plays a similar role *in vivo* is difficult to conceive, primarily because TDM is not surface-exposed in the pathogenic mycobacterial species examined so far (29). In addition, in the above experiments, TDM was inserted in all vesicles and, *in vivo*, it is hard to imagine that TDM will be present both in phagosomal and lysosomal membranes.

5.2. Glycopeptidolipids

In contrast to TDM, the surface-exposed diglycosylpeptidolipid (GPL2) from *M. smegmatis* causes the decrease of the phosphorylation efficiency (ADP/O ratio) of mitochondria isolated from rat liver without modifying the active respiration (60). The oxidative phosphorylation uncoupling resulted from the decrease of the transmembrane electrical potential. GPL2 increases the permeability of liposomes to carboxyfluorescein, suggesting that GPL could act on mitochondria by enhancing the passive permeability of the inner membrane to proton (61). Several GPL, differing one from the other by the number of saccharide units on the peptide moiety have been tested both on mitochondria and on carboxyfluorescein-containing liposomes. All the molecules exhibit the same type of activity but the monoglycosyl beta-lipid (GPL1) was more active than GPL2 and the triglycosyl molecule (GPL3); the aglycosylated substance (GPL0) was poorly active (61).

Insertion experiments of GPL into phospholipid monolayers at the air-water interface has suggested that the poor activity of GPL0 is probably due to its unability to insert into membranes. On the other hand, the insertion of GPL3, which was less active than GPL1, into membranes was better than that of GPL1 (100). Determination of compression isotherms and surface potentials of the various GPL led the authors to propose a model in which the glycopeptid moiety of GPL3 dips into the water phase and, consequently, hinders the molecule to induce any alteration of the phospholipid organization; in contrast, peptidic moiety of GPL1 lains at the interface, rendering the molecule more disturbing (61, 100). Recent data, however, contradict this attractive model. Analysis of the compression isotherms and Fourier-transformed infrared spectroscopy (FTIR) studies of GPL-phospholipid preformed monolayers indicated that almost all the GPL molecules have their glycopeptid moiety parallel to the interface. Interestingly, the lateral organization of molecules in GPL3-phospholipid mixed monolayers differ significantly from that of GPL1. The former GPL, *i.e*. the triglycosylated molecules, segregate from the phospholipid phase and self-associate to form intermolecular beta-sheets. GPL1 are miscible with phospholipid molecules and increase disorder of the phospholipid acyl chains, thus explaining the great alteration of the permeability of liposomes observed with GPL1 (101 & unpublished data).

Although the carbohydrate moiety of GPL helps the insertion of the molecule into membranes, in polar GPL it also has the tendency to favor the large segregation. Thus, the most efficient glycopeptidolipid in the membrane impairment is the one possessing a moderate number of saccharide units.

5.3. Phosphatidylinositolmannosides

Besides their properties as B-cell antigens (102) and their other biologic activities cited above, PIM are involved in the

interactions of whole mycobacteria with host cells. They can act as an adhesin, allowing the binding of *M. tuberculosis* (strain H37Rv) and *M. smegmatis* to Chinese Hamster Ovary fibroblasts and porcine aortic endothelial cells, either directly or after opsonization with serum proteins such as the mannose-binding protein (MBP). Although the mode of association of PIM with these non-phagocytic mammalian cells is not yet elucidated, the cell specificity and the inhibiting effect of periodate treatment strongly suggest the participation of a lectin-like receptor in these interactions (82).

Previous nonopsonic binding experiments using murine macrophages have showed that PIM, as well as LAM, inhibit the binding of *M. tuberculosis* and that the phosphatidylinositol moiety was important in the abrogation of the binding (103). This work also suggests that a competitive inhibition of a receptor/ligand interaction is probably not the cause of the observed phenomenon. Two investigations have brought insights into the mechanisms by which PIM could interact with cell membranes and induce macrophage responses. Firstly, Barratt *et al.* (104) have shown that PIM-containing liposomes inhibit the uptake of mannosylated-bovine serum albumin (BSA) by mouse inflammatory macrophages, suggesting an interaction with the mannose receptor. Indeed, the presence of PIM on mycobacterial cell surface (29) is in accordance with the possible implication of PIM in the binding and phagocytosis of mycobacteria to macrophages*via* the mannose receptor. Secondly, PIM may be anchored in lymphomonocytic cell plasma membrane via the glycosylphosphatidylinositol (GPI) moiety, as does LAM, and specially into glycosylinositol-rich domains. This insertion may modulate the signal transduction used by GPI-linked proteins of host cells (105).

6. CONCLUSIONS AND PERSPECTIVES

Although the biologic activities of some of the isolated mycobacterial glycolipids on host's cells have been clearly demonstrated, suggesting that the enzymes involved in their biosynthesis represent potential targets for new drugs, the roles of these molecules in the pathogenesis remain hypothetical. Several reasons may be evoked. First, to achieve their potential roles glycolipids need to be inserted into membranes, implying that the molecules can be transferred from an organized complex structure to cellular membranes. Furthermore, biological activities of lipids are highly dependent of supramolecular structure of molecules (106) which may be different between the artificial structural models composed of purified glycolipid and their organization in the mycobacterial cell envelope. Second, glycolipids are not abundant components of the outermost layer, representing only 1 to 6% of the dried cell surface material (107); some classes of lipids such as trehalose dimycolate are absent from the bacterial surface of most of the mycobacterial species examined (29). It remains, however, that during infection this situation may be quite different from the *in vitro*-grown bacilli. Finally, clinical isolates of pathogenic species may be devoid of some biologically active glycolipids such as PGL whereas other active glycolipids are found both in pathogenic and saprophytic mycobacteria. Nevertheless, subtile variations in their structures such as in the length of acyl chains or the composition and architecture of the envelope could modulate the properties of various glycolipids in their interactions with eucaryotic cells.

With the development of molecular biology of mycobacteria it is hope that the isolation of mutants devoid of a specific glycolipid and the study of their fate in infected animals will establish the importance of mycobacterial glycolipids in the pathogenicity of the organisms producing them. Another challenge to investigators is the elucidation of the molecular mechanisms by which mycobacterial glycolipids modulate the activities of host immune system.

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