Annu. Rev. Microbiol. 1992. 46:65–94

THE LIPOPHOSPHOGLYCAN OF *LEISHMANIA* PARASITES

Salvatore J. Turco and Albert Descoteaux

Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40536

KEY WORDS: glycolipid, glycoconjugates, glycosylphosphatidyl inositol, leishmaniasis, macrophages

CONTENTS

0066-4227/92/1001-0065$02.00

Abstract

Protozoan parasites of the genus *Leishmania* have the remarkable ability to avoid destruction in the hostile environments they encounter throughout their life cycle. The molecular details of how these pathogens persevere with impunity under harsh conditions are beginning to be understood. The fact that *Leishmania* parasites have adapted to not only survive, but to proliferate probably is due to the protection conferred by specialized molecules on the parasite's cell surface. One such macromolecule is a novel glycoconjugate called lipophosphoglycan. This heterogeneous, lipid-containing polysaccharide is the major surface molecule of the parasite and has been implicated in a surprisingly large number of functions that may contribute the the parasite's pathogenesis. This review emphasizes the structural aspects of lipophosphoglycan and its possible functions and biosynthesis.

INTRODUCTION

Leishmaniasis

Protozoan parasites of the genus *Leishmania* are the causative agents of leishmaniasis, a disease that annually afflicts millions of people world-wide. Depending on the pathogenic species involved, leishmaniasis is clinically divided into three types: cutaneous, mucocutaneous, and visceral. The etiologic agents of cutaneous disease are *L. major, L. tropica, L. mexicana,* and *L. aethiopica,* which restrict their infection to dermal tissues. Mucocutaneous disease (espundia) is attributed to *L. braziliensis,* which replicates primarily in mucous tissues and causes gross disfigurements. *L. donovani* is responsible for visceral leishmaniasis or kala-azar, a chronic and often fatal disease. Detailed descriptions of the clinical aspects and geographical distribution of the various leishmaniases are reviewed elsewhere (22, 52, 107).

Current treatment of leishmaniasis is rather crude and relies on chemotherapy. The various types of leishmaniases are treated with a repertoire of antimonial drugs (e.g. Pentostam) or diamidines (e.g. Pentamidine). Disadvantages of these antiquated drugs include their numerous side effects, the possibility of being mutagenic, the requirement of prolonged treatment, and the lack of knowledge about their precise mode of action. Because researchers are only now gaining fundamental knowledge of the molecular aspects that distinguish the parasites from their mammalian hosts, innovative drug design is in its infancy. Once the distinguishing features are elucidated, new and effective therapeutic protocols will undoubtedly evolve.

The Life Cycle of Leishmania *Parasites*

The life cycle of of *Leishmania* parasites consists of two stages. In one stage, the parasite lives as an extracellular, flagellate promastigote form in the

alimentary tract of its insect vector, the sandflies *Phlebotomus* and *Lutzomyia* spp. While attached to the epithelial cells lining the midgut of the sandfly, the promastigotes multiply and are avirulent. In a process called metacyclogenesis (127), promastigotes eventually cease dividing, detach from the epithelial cells, and migrate to the mouthparts of the insect. These metacyclic promastigotes are virulent. This sequential development of promastigotes from a dividing, noninfectious stage to a resting, infective stage has been observed for promastigotes growing in the sandfly alimentary tract and in axenic culture (127).

Metacyclic promastigotes are inoculated into the microscopic wound produced in a human or other suitable vertebrate when a sandfly feeds. The parasites then attach to the macrophages attracted to the wound and enter these host cells by a receptor-mediated process. Upon entry into the phagolysosome of the macrophage, promastigotes differentiate to nonflagellate amastigotes. Surprisingly, these amastigotes not only survive, they proliferate in this usually hostile environment. Ultimately, the infected macrophages lyse, releasing the amastigotes into the surrounding environment where they can infect other macrophages. The life cycle is completed when a feeding sandfly bites an infected host and the parasite is present in the bloodmeal.

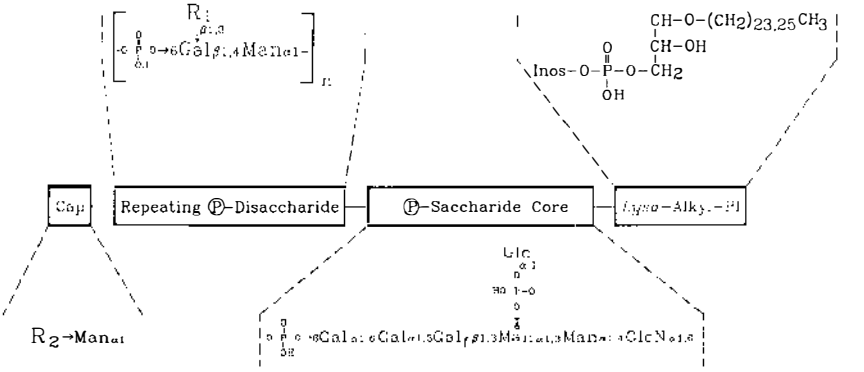
Importance of Cell-Surface Glycoconjugates

In order for a *Leishmania* parasite to propagate, it must avoid destruction (*a*) in the gut of the sandfly where the organism could be susceptible to digestive enzymes that degrade the bloodmeal, (*b*) in the bloodstream of the vertebrate host where the parasite transiently exists and would be exposed to the lytic complement pathway; and (*c*) in the phagolysosome of host macrophages where the parasite would be vulnerable to hydrolytic enzymes and the microbicidal oxidative burst. The molecular details of how this pathogen perseveres with impunity in obviously hostile conditions is beginning to be understood. Cell-surface glycoconjugates undoubtedly play a key role in the survival of the *Leishmania* parasite throughout its existence. One of the major glycoconjugates synthesized by the parasite is lipophosphoglycan (LPG), a heterogeneous, lipid-containing polysaccharide. This review emphasizes the structural aspects of LPG and its possible functions and biosynthesis.

THE STRUCTURE OF LPG

Lipophosphoglycan of Promastigotes

THE GENERALIZED STRUCTURE OF LPG The promastigote form of all *Leishmania* parasites synthesizes LPG. LPG is the major glycoconjugate of the promastigote and is localized over all its surface including the flagellum



	R_1	R_2
L. donovani	= H	= $Man_{\alpha 1,2}$–
(n_{avg}=16)		= $Gal_{\beta 1,4}$–
		= $Man_{\alpha 1,2}[Gal_{\beta 1,4}]$–
		= $Man_{\alpha 1,2}Man_{\alpha 1,2}$–
		= $Man_{\alpha 1,2}Man_{\alpha 1,2}[Gal_{\beta 1,4}]$–
L. major	= H	= $Man_{\alpha 1,2}$–
(n_{avg}=27)	= Gal	
	= $Ara_{\alpha 1,2}$Gal–	
	= $Gal_{\beta 1,3}$Gal–	
	= $Glc_{\beta 1,3}$Gal–	
	= $Ara_{\alpha 1,2}Gal_{\beta 1,3}$Gal–	
	= $Gal_{\beta 1,3}Gal_{\beta 1,3}$Gal–	
	= $Ara_{\alpha 1,2}Gal_{\beta 1,3}Gal_{\beta 1,3}$Gal–	
L. mexicana	= H	= $Man_{\alpha 1,2}$–
(n_{avg}=16)	= Glc	= $Man_{\alpha 1,2}Man_{\alpha 1,2}$–
		= $Man_{\alpha 1,2}[Gal_{\beta 1,4}]$–

Figure 1 Structures of LPG from three promastigote species of *Leishmania*, grown in log to late-log phase of growth.

(72, 109). Each parasite cell contains several million molecules of LPG (82, 84, 104). As shown in Figure 1, LPG consists of four domains: (*a*) a phosphatidylinositol lipid anchor, (*b*) a phosphosaccharide core, (*c*) a repeating phosphorylated saccharide region, and (*d*) a small oligosaccharide cap structure. Structural analyses of LPG from several species of *Leishmania* indicate complete conservation of the lipid anchor, extensive conservation of

the phosphosaccharide core, and variability of sugar composition and sequence in the repeating phosphorylated saccharide units and the cap structure (62a, 84, 104, 141a).

THE LIPID ANCHOR OF LPG The polysaccharide portion of LPG is anchored by the unusual phospholipid derivative 1-*O*-alkyl-2-*lyso*-phosphatidyl(*myo*)inositol. In the LPGs from *L. donovani* (104), *L. major* (81), and *L. mexicana* (62a), and probably in those from all species of *Leishmania*, the aliphatic chain consists of either a C_{24} or C_{26} saturated, unbranched hydrocarbon. As can many glycosylphosphatidylinositol (GPI)-anchored proteins (reviewed in 23), LPG can be hydrolyzed by bacterial phosphatidylinositol-specific phospholipase C, producing 1-*O*-alkylglycerol and the entire polysaccharide chain as products.

THE PHOSPHOSACCHARIDE CORE OF LPG Attached to the inositol of the lipid anchor of LPG is the phosphosaccharide core region. In *L. donovani* (147), *L. major* (84), and *L. mexicana* (62a), the glycan core consists of an unacetylated glucosamine, two mannoses, a galactose-6-phosphate, a galactopyranose, and a galactofuranose. The presence of the latter is extremely unusual in eukaryotic glycoconjugates, especially because the furanose is internal in a carbohydrate chain. As with all other reported glycosylphosphatidylinositol-anchored proteins reported thus far (23), LPG possesses the Man(α1,4)GlcN(α1,6)*myo*-inositol-1-PO_4 motif. The LPG cores of *L. donovani* (141a) and *L. mexicana* (62a) possess a glucosyl-α1-phosphate attached in phosphodiester linkage to the C6 hydroxyl of the proximal mannose residue. A substantial percentage of the *L. major* LPG also contains the identical glucosyl-α1-phosphate substitution, while the remainder does not (84). Another interesting sequence in the core region is the Gal(α1,3)Gal unit, which is reportedly the epitope for circulating antibodies in patients with leishmaniasis (3, 4, 144).

THE REPEATING UNITS OF LPG The salient feature of LPG is the repeating phosphorylated saccharide region. All LPG molecules reported thus far (62a, 82, 84, 146) contain multiple units of a backbone structure of PO_4-6Gal(β1,4)Man(α1). One of the noteworthy features of the backbone is the 4-*O*-substituted mannose residue, which is not present in any other known eukaryotic glycoconjugate. The *L. donovani* LPG (82, 146) contains no other substitutions of the backbone sequence, whereas in the *L. mexicana* LPG, approximately 25% of galactose residues are substituted at the C3 hydroxyl with βGlc residues (62a). The repeating units of the *L. major* LPG are the most complex in that approximately 87% of the galactose residues are further substituted with small saccharide side chains containing one to four residues

of galactose, glucose, or the pentose arabinose (84). The presence of the common Gal-Man disaccharide backbone and the species-specific substitutions on the galactose residue could account for common and species-specific epitopes reported in serological observations (135). The number of repeating units per LPG molecule directly depends on the growth stage of the promastigote and is discussed below.

THE CAPPING OLIGOSACCHARIDES OF LPG LPG is terminated at the nonreducing end with one of several small neutral oligosaccharides containing galactose or mannose. Although the LPG of *L. major* possesses the most complicated series of repeating units, it is capped with the simplest structure (84), consisting exclusively of the disaccharide Man(α1,2)Man(α1). The most abundant terminal oligosaccharide of the *L. donovani* LPG is the branched trisaccharide Gal(β1,4)[Man(α1,2)]Man(α1) (141a), which is also a capping structure of the LPG of *L. mexicana* (62a).

THE TERTIARY STRUCTURE OF LPG The three-dimensional solution structure of the repeating PO_4-6Gal(β1,4)Man(α1) disaccharide units of the LPG derived from *L. donovani* has been recently determined by use of a combination of homo- and heteronuclear NMR spin coupling constant measurements together with restrained molecular mechanical minimization and molecular dynamics simulations (61). The repeating units, as expected, have limited mobility in solution about the Gal(β1,4)Man linkages. In contrast, a variety of stable rotamers exist about the Man(α1)-PO_4-6Gal linkages. An important feature of each of these low energy conformers is that the C3 hydroxyl of each galactose residue is exposed and freely accessible. This is the particular position for which glucose is substituted in the LPG from *L. mexicana* (62a), or for which galactose, glucose, and arabinose are substituted in the LPG from *L. major* (84). Thus, these additional units could be accommodated without major conformational changes to the repeat backbone.

Another intriguing finding from the molecular modeling studies (61) is that each of the stable conformers of the Man(α1)-PO_4-6Gal linkages may exist in a different configuration within the same LPG molecule. These torsional oscillations allow the LPG molecule to contract or expand in a manner reminiscent of a slinky spring, resulting in a molecule whose length can range from 90 Å when fully contracted to 160 Å when fully extended, assuming an average of 16 repeat units.

Developmental Modification of LPG During Promastigote Metacyclogenesis

A peculiar and significant observation of LPG is that modifications in its structure accompany the process of metacyclogenesis (127, 129, 130). These

changes in LPG were initially detected in studies using the peanut agglutinin lectin and using stage-specific monoclonal antibodies (129, 130). Recent investigations have focused on the structural comparison of the LPG isolated from *L. major* promastigotes grown logarithmically and from stationary phase of growth. Analysis of the LPG derived from these growth stages of *L. major* revealed conservation of the *lyso*-1-*O*-alkylphosphatidylinositol lipid anchor and the phosphosaccharide core. The most striking difference, however, was an approximate doubling in size displayed by the metacyclic form of LPG, resulting from an approximate doubling in the number of repeating phosphorylated saccharide units (128). The relative increase in size of metacyclic LPG is consistent with freeze-fracture electron microscopic studies of the surface of metacyclic promastigotes (108). Their cell surfaces contain densely packed filamentous structures not present on logarithmically grown, noninfectious promastigotes. Furthermore, a greater than twofold thickening of the surface glycocalyx could be specifically labeled with the monoclonal antibody directed against the metacyclic LPG.

A second and more subtle difference in the two versions of LPG from *L. major* have been noted in the repeating phosphorylated saccharide composition (128). The repeat units of LPG obtained from logarithmically grown *L. major* contain terminal β-galactose residues that branch off the disaccharide backbone as a side chain. These galactose residues account for the agglutinability of such parasites to peanut agglutinin (130). Upon differentiation to metacyclic promastigotes, the repeat units terminate predominately with α-arabinose and, to a lesser extent, β-glucose residues, which are not ligands for the lectin. These compositional differences undoubtedly explain the expression of a novel epitope on metacyclic LPG (129). Such changes in LPG structure may have profound implications on function and suggest that there are important points of regulation of the glycosyltransferases involved in LPG biosynthesis.

Similarly, the LPG of *L. donovani* also undergoes an approximate twofold elongation during differentiation of the parasite to the metacyclic state (D. L. Sacks & S. J. Turco, unpublished observations). A determination of an average number of 16 repeat units from LPG isolated from *L. donovani* grown for extended time in culture supports this observation (146). LPG prepared from promastigotes freshly differentiated from hamster-derived amastigotes has an average repeat unit number of approximately 30 (82). Furthermore, a compositional change occurs, but one unlike that observed with the *L. major* LPG. Although the repeating units of LPG remain as PO_4-6Gal (β1,4)Man(α1) in metacyclic *L. donovani,* alterations of the cap oligosaccharides emerge. The sole terminal galactose residue present in the cap oligosaccharide of LPG from noninfectious parasites is absent in metacyclic LPG. This absence of terminal galactose would explain the loss of the ability

of peanut agglutinin to agglutinate infectious *L. donovani* promastigotes (D. L. Sacks & S. J. Turco, unpublished observation).

LPG of Amastigotes

All of the structural information known about LPG has been obtained from LPG isolated from the *Leishmania* promastigotes. Until recently, there had been no information regarding the occurrence of LPG in amastigotes. The presence of LPG has been examined in the amastigotes of both *L. donovani* and *L. major,* and distinct results were obtained. *L. donovani* amastigotes apparently cannot synthesize LPG; at least a 10^4-fold down-regulation in LPG was found, corresponding to less than 100 molecules/cell (82). On the other hand, evidence has shown that *L. major* amastigotes express an LPG that is both biochemically (148) and antigenically (95) distinct from promastigotes. Although LPGs from both forms of *L. major* have much in common, the amastigote stage-specific LPG contains subtle carbohydrate differences that are not yet known (148). The temporal regulation of LPG expression during parasite differentiation was studied immunologically in vitro (45). During amastigote-to-promastigote transformation, the amastigote-specific form of LPG disappeared after subculture for 48 h, whereas during promastigote-to-amastigote transformation, the amastigote-specific form of LPG was detected in 12 h. The quantities of the *L. major* amastigote version of LPG appear to be much less than the promastigote counterpart. A stage-specific LPG has not been reported in other species of *Leishmania.*

Glycosylphosphoinositides

The various parts of LPG have been shown to exist in *Leishmania* promastigotes as components of proteins or as distinct entities. Regarding the latter, a family of molecules termed glycosylphosphatidylinositol antigens (GPIs) (119, 120, 137) or glycosylinositolphospholipids (GIPLs) (36, 79, 80, 82, 83) is abundant in *L. major* and *L. donovani*. Structural analyses of these glycolipids have indicated that they closely resemble the phosphosaccharide core–phosphatidylinositol region of LPG. In particular, the *L. major* GIPLs consist of a small mannose- and galactose-containing glycan that is glycosidically linked via an unacetylated glucosamine residue to either 1-*O*-alkyl-2-acyl-PI or *lyso*-1-*O*-alkyl-PI. The glycan parts of these molecules are completely identical to the analogous portions of LPG, including the salient Man(α1,4)GlcN(α1,6)*myo*-inositol and, in the larger GIPLs, the rare galactofuranose. Unlike those of *L. major,* the GIPLs of promastigotes of *L. donovani* are not galactosylated and synthesize abundant GIPLs containing one to four mannose residues (82, 137). Although *L. donovani* amastigotes do not synthesize LPG, they continue to synthesize GIPLs in quantities comparable to that reported in promastigotes (82). The *L. donovani* amastigote GIPLs,

containing one to three mannose residues, were structurally different from promastigote GIPLs and appear to be precursors to glycolipid anchors of proteins. In addition, ceramidephosphoinositides have been found in *L. donovani* (69). Whether these particular phosphosphingolipids can be further substituted with carbohydrate residues as observed in *Trypanosoma cruzi* (77, 110), yeast, fungi, and plants (75) has not been established.

Extracellular LPG-Like Glycoconjugates

LPG-like substances, collectively termed excreted factor (EF), are reportedly present in conditioned medium from *Leishmania* parasites (38, 72, 139). The components of the EF can be organized into three categories. In one, LPG can form very tight complexes with albumin in the medium (72). Analysis of this form of LPG in the medium indicates that it is identical in all respects to the cell-associated LPG (72). One probable interpretation is that the lipid portion of LPG interacts with the hydrophobic binding pocket of albumin, facilitating its release from the surface of the promastigote.

In a second category, the repeating phosphorylated saccharide units of LPG comprise a carbohydrate chain of an acid phosphatase secreted by *L. donovani* (6), *L. tropica* (64, 65), and *L. mexicana* (63). The number of repeating units per carbohydrate chain and the nature of the linkage to the polypeptide are unknown. The latter is not believed to involve a typical N-glycosidic linkage to an Asn residue because the repeating units were not removed from acid phosphatase following N-glycanase digestion (6).

The third category of LPG-like substances is an extracellular phosphoglycan (exPG), which was purified from conditioned medium of *L. donovani* and then characterized (51a). Structural analysis indicated that the exPG consisted of the following structure: (CAP)$\rightarrow$[PO_4-6Gal(β1,4)Man(α1)]$_{10-11}$. The cap was found to be one of several small neutral oligosaccharides; the most abundant was the branched trisaccharide Gal(β1,4)[Man(α1,2)]Man(α1). Thus, the exPG is identical to cell-derived LPG except that it lacks a lipid anchor, the phosphosaccharide core, and several repeating units. Results from surface labeling with galactose oxidase/Na[^{3}H]$_4$ led to the conclusion that the exPG originates from surface LPG. The mechanism of release of the exPG as well as its possible function, if any, are not known.

FUNCTIONS OF LPG

The uniqueness of the overall structure of LPG and its highly unusual domains indicates that LPG might have one, or possibly several, important functions for the *Leishmania* parasite in its life cycle. Indeed, evidence has been provided for a surprisingly large number of potential functions that enable the promastigote to survive in the hydrolytic environments it encounters.

Functions in Sandfly-Leishmania *Interactions*

Differentiation and multiplication of the promastigote form of *Leishmania* parasites take place in the midgut of the sandflies *Phlebotomus* and *Lutzomyia* spp. soon after the ingestion of amastigotes during a bloodmeal on an infected host (19). Metacyclogenesis, or development from dividing noninfective forms into a resting, infective form, is believed to take place temporally in the midgut of the invertebrate host as the parasites move toward the anterior end of the gut (19, 131, 132). As described above (Developmental Modification of LPG During Metacyclogenesis), one of the major physical changes that occurs during metacyclogenesis is a structural modification of LPG (130). Although the importance of these modifications with respect to the successful survival of the parasite within the mammalian host has been largely documented (127, 145), information is beginning to appear regarding their role during metacyclogenesis itself. The possibility that these developmentally regulated structural changes may control attachment and detachment of maturing promastigotes from midgut epithelial cells, and hence their migration toward the mouthparts (145), was recently substantiated (26). In this study, expression of both LPG and the major cell-surface glycoprotein gp63 during the development of *L. major* in the gut of the sandfly *Phlebotomus papatasi* was examined in situ. Large amounts of nonmetacyclic, parasite-free LPG were detected on the surface of epithelial cells from the sandfly gut wall. In contrast, despite its abundant expression on promastigotes, gp63 was not detected on gut cells. The absence of metacyclic LPG suggests that the modified version of the glycoconjugate does not bind to these cells and, consequently, would allow infective parasites to move forward as they mature.

LPG may also protect the promastigotes against hydrolytic activity within the sandfly gut. Indeed, Schlein et al (133) increased survival of a foreign *L. major* strain in the stomach of *P. papatasi* by supplementing infective bloodmeal with LPG derived from an indigenous *L. major* strain (133). Clearly, additional studies are required to understand the functions of LPG during the development of the parasite within the sandfly.

Functions in Bloodstream

COMPLEMENT ACTIVATION AND RESISTANCE TO COMPLEMENT-MEDIATED DAMAGES Between the time of inoculation and infection of macrophages, promastigotes are exposed to the potential lytic effects of normal serum. Several studies were aimed at understanding the mechanisms by which promastigotes avoid destruction by the host's complement system (reviewed in 68). It appears that the developmentally regulated modifications of LPG represent the major resistance mechanism.

Promastigotes of all *Leishmania* species from log-phase cultures (noninfec-

tive) are extremely sensitive to fresh serum (43). On the other hand, stationary-phase cultures, which contain metacyclic promastigotes, display an increased resistance to lysis (43, 113). Activation of complement kills promastigotes because heat-inactivated serum and ethylenediaminetetraacetic acid (EDTA)-chelated serum fail to lyse promastigotes. Using the peanut agglutinin lectin, which does not agglutinate infective metacyclic promastigotes (130), Puentes et al (113) generated populations of pure log-phase and metacyclic *L. major* promastigotes and used them to further study complement resistance (113). Surprisingly, failure to bind the complement component C3 does not account for resistance to killing, inasmuch as both log-phase and metacyclic promastigotes activate complement rapidly and bind radiolabeled C3 after incubation in serum (113). These observations confirm the conclusions of an earlier study on resistance to complement-mediated lysis of amastigotes (98). The deposition of C3b on *L. major* metacyclics occurs through the classical pathway, and C3 is not covalently linked. On the other hand, C3 binding on log-phase promastigotes is mediated by efficient activation of the alternative pathway. Interestingly, LPG is the C3 acceptor molecule on both log-phase growth and metacyclic promastigotes, as determined by immunoprecipitation of LPG from promastigotes previously incubated with ^{125}I-C3. In contrast, regardless of the growth phase, *L. donovani* promastigotes bind C3 mainly as hemolytically inactive iC3b through activation of the alternative pathway (112). Half of the bound C3 is rapidly released from the parasite as a consequence of an unusual proteolytic cleavage, raising the possibility that C3 cleavage may be modulated by the major surface protease gp63. However, LPG does not mediate C3 binding on *L. mexicana* promastigotes, as it occurs solely on the gp63 (121).

The possibility that larger LPG molecules on metacyclic promastigotes are responsible for their resistance to complement-mediated lysis is supported by the observation that in *L. major,* most of C5b-9 complexes are spontaneously released from the metacyclic promastigote surface. This action precludes their insertion into the membrane and death of the parasite (111). Consistent with these observations, LPG may provide a barrier against the elevated titers of antileishmanial antibodies associated with kala-azar. Pooled kala-azar serum shows a strong reactivity with a LPG-deficient mutant of *L. donovani*. In contrast, little reactivity is observed with wild-type promastigotes (70). This observation is in agreement with the notion that the humoral response associated with kala-azar does not contribute to immunity. In addition, this masking effect of LPG may protect developing promastigotes in the insect's gut from antibodies present in the bloodmeal.

Collectively, these studies support the critical role of LPG, as well as the developmentally associated modifications, in the initial contact of *Leishmania* parasites with their mammalian hosts.

ATTACHMENT TO HOST MACROPHAGES Since *Leishmania* parasites infect primarily mononuclear phagocytic cells, attachment to potential host cells undoubtedly requires specific recognition molecules on the surface of both parasites and macrophages. Several *Leishmania* and macrophage cell-surface molecules have been implicated in the attachment of the parasite to its host cell (reviewed in 123). The glycoprotein gp63 present on all *Leishmania* species studied (9, 10, 40) and LPG can be considered parasite ligands (55, 118, 124, 125, 153), whereas CR1, CR3, and the mannose-fucose receptor represent the corresponding macrophage receptors (8, 25, 96, 141, 154, 155). Although binding occurs in the absence of serum, the presence of C3 dramatically increases the survival of metacyclic promastigotes (25, 97, 156). This phenomenon may be explained by the conversion of C3 into C3b through activation of the classical pathway and the subsequent CR1-mediated binding and internalization. The use of both CR1 and CR3 may favor the survival of *Leishmania* promastigotes because they reportedly promote phagocytosis without triggering the oxidative burst (157). Another report, however, stated that CR3, when suitably ligated, participates in macrophage activation (32).

Attachment of *L. major* but not *L. mexicana* promastigotes to macrophages is inhibited by the Fab fragment of an anti-*L. major* LPG antibody, suggesting that LPG is a parasite receptor for macrophages (55). Binding of purified *L. major* LPG to macrophage and nonmacrophage cells appears to be temperature dependent. Two mechanisms of binding may be involved: a specific mechanism, by which the carbohydrate part of the molecule binds to a macrophage receptor, and a nonspecific mechanism in which the lipid of LPG interacts with the membrane of the cells, probably through insertion into the lipid bilayer. In contrast, *L. donovani* LPG and its delipidated derivative bind to a variety of different cell types in a temperature-independent manner (143).

A study aimed at identifying the receptor(s) responsible for recognition of LPG on macrophages revealed that in the presence of serum, the binding site for *L. mexicana* LPG is contained, or conferred, by the α-chains of both CR3 and p150,95 (141), two members of the CD18 family of integrins. Competition experiments revealed that *L. mexicana* LPG and *Escherichia coli* lipopolysaccharide share the same binding site on the CD18 family of integrins. Interestingly, the binding site of LPG on CR3 is distinct from the binding site of C3bi.

These binding studies strongly suggest a role for LPG in the attachment of promastigotes to macrophages. However, in the absence of complement, phagocytosis of LPG-deficient mutants of *L. donovani* and *L. major* is similar or even superior to that of wild-type promastigotes (35, 57, 73, 88). LPG-deficient variants have exposed glycoproteins on their surface, such as gp63. Because these glycoproteins contain polymannose chains (89), the LPG-deficient variants may enter macrophages via a receptor not normally utilized.

Such a receptor may be the mannose-fucose receptor; the exact contribution of LPG in the recognition and attachment processes still remains to be determined.

Intracellular Functions

INTRACELLULAR SURVIVAL IN HOST PHAGOLYSOSOMES Subsequent to attachment of promastigotes to their receptors, internalization is achieved through the formation of a phagosome. Secondary lysosomes then fuse with the parasitophorous vacuole to form a phagolysosome in which the parasite transforms and multiplies as amastigotes (7, 18). This implies that *Leishmania* parasites have adapted to survive in the highly destructive environment of the phagolysosome, where they encounter degradative enzymes and toxic oxygen products.

Handman & Greenblatt (54) provided the first evidence that an excreted factor (presumably containing LPG) may be important for the intracellular survival of *Leishmania* parasites. They noticed that addition of concentrated excreted factor from *L. enrietti* promastigote cultures promoted the growth of this parasite in mouse peritoneal macrophages, which under normal circumstances are nonpermissive. Medium conditioned by *L. tropica* promastigotes was without effect. The possibility that the preparation of excreted factor contained other parasite molecules, however, cannot be excluded.

Use of *Leishmania* strains deficient in the biosynthesis of LPG clearly demonstrated that LPG is required for intracellular survival of promastigotes. An avirulent clone of *L. major* isolated from a rodent and lacking LPG was phagocytized by macrophages but was killed within 18 hours (57). Passive transfer of purified LPG from a virulent strain of *L. major* into the avirulent promastigotes confered the ability to survive in macrophages.

Several LPG-deficient variants of *L. donovani* selected for resistance to the ricin agglutinin lectin (73) are phagocytized but cannot survive in human monocytes. As in the avirulent strain of *L. major*, passive transfer of purified LPG significantly prolonged *L. donovani* mutant survival in monocytes (88). Because one particular ricin-resistant variant synthesizes lower amounts (20%) of a truncated form of LPG (86) and still could not sustain an infection (88), these results suggest that a minimum number of intact LPG molecules might be necessary for successful intracellular survival. Isolation and characterization of additional LPG-variants may be helpful to address this issue. Selection of ricin agglutinin-resistant mutants of *L. major* and determination of their infectivity both in vitro and in vivo (35) confirmed that LPG is a parasite factor determining infectivity and virulence, essential for intracellular survival. Interestingly, these *L. major* LPG-variants express normal or elevated amounts of glycosylinositolphospholipids, which are re-

lated to the phosphosaccharide core–phosphatidylinositol region of LPG (see Glycosylphosphoinositides).

The mechanisms by which LPG protects the parasite against the microbicidal activities displayed by the macrophage is an important area of research. Much hinges on the fate of LPG upon entry of the parasite into macrophages. LPG epitopes can be visualized by immunofluorescence with anti-LPG monoclonal antibodies on the surface of macrophages as early as five to ten min postinfection and are localized to the immediate area of internalization of the promastigote (143). The epitopes are evenly distributed over the entire macrophage surface by 25 min postinfection. The epitopes are maximally discernable one to two days postinfection, and by five to six days, the LPG epitopes can no longer be detected. Thus, intracellular functions for the promastigote form of LPG would have to be attributed within the first several days postinfection. The various properties of LPG with respect to intracellular survival are reviewed in the next sections.

INHIBITION OF HYDROLYTIC ENZYMES Adaptation to life in a phagolysosome requires the ability to resist, inhibit, or inactivate host hydrolytic enzymes. It has been suggested that survival of *Leishmania* parasites may depend on their ability to inhibit lysosomal enzymes (2). Resistance to lysosomal enzymic digestion was also suggested, based on surface properties of the parasite (18).

In an investigation (37) of the effect of partially purified excreted factor (LPG) upon the activity of four hydrolytic enzymes from peritoneal macrophages of mice, LPG did not affect acid phosphatase, β-glucuronidase, and N-acetyl-β-glucosaminidase. But β-galactosidase activity was highly inhibited after 3 h of incubation. The strong negative charge of the LPG molecule may account for the observed inhibitory effect. Competitive inhibition by the abundant phosphorylated disaccharide Gal(β1,4)Man in LPG provides an alternative explanation. Whether these in vitro results could be applied to the in vivo situation remains to be determined.

The role of LPG in protecting the parasite from digestion by lysosomal enzymes was further examined by measuring the rate of cytolysis of erythrocytes coated and uncoated with LPG (34). LPG coating significantly diminished the rate of cytolysis by macrophages, suggesting that this glycoconjugate may indeed enable *Leishmania* parasites to survive in the presence of hydrolytic enzymes.

CHELATOR OF CALCIUM Calcium plays an important role in the regulation of cellular functions, chiefly as an intracellular second messenger and as an enzyme cofactor. Interestingly, *L. major*-infected macrophages contain approximately 40% more exchangeable calcium than uninfected controls

(34). Similarly, macrophages engulfing LPG-coated erythrocytes have increased levels of calcium compared to macrophages engulfing control erythrocytes, possibly as a consequence of calcium binding by LPG (34). Recent studies using NMR examined the effect of calcium on the tertiary structure of the glycan moiety of LPG. The investigators concluded that calcium does not perturb the tridimensional structure of the glycan and that it binds to LPG in the vicinity of the phosphate groups (61). Therefore, the ability of LPG to chelate calcium may have important implications with respect to the ability of *Leishmania* parasites to survive within macrophages.

LPG might be a chelator of other important divalent cations, such as ferrous iron. Chelation of the latter metal presumably would prevent production, via the Fenton reaction, of the destructive hydroxyl radicals during activation of macrophages.

INHIBITOR OF HOST PROTEIN KINASE C Protein kinase C (PKC) is a multifunctional protein kinase that specifically phosphorylates serine and threonine residues (reviewed in 101). This enzyme is characterized by a catalytic domain containing an ATP-binding site and a regulatory domain that contains the sites involved in calcium, diacylglycerol (the physiological activator), and phospholipid binding (102). By virtue of its pivotal role in transmembrane signaling (42, 62), PKC modulates a wide variety of cellular functions. In phagocytes, one of the events mediated by PKC is the initiation of the oxidative burst. Phosphorylation and membrane association of the NADPH oxidase complex components represent the first steps of this process (5, 30). The active NADPH oxidase complex catalyzes the one-electron transfer from NADPH to oxygen, generating superoxide anion released at the outer surface of the plasma membrane into the extracellular space or into phagocytic vacuoles. Further reductions of the superoxide anions results in formation of hydrogen peroxide, hydroxyl radicals, and singlet oxygen. These events are involved in one of the main physiological functions of macrophages, the elimination of microbes. Products of the oxidative burst are deleterious for *Leishmania* promastigotes, in particular hydrogen peroxide (100, 105, 116). Susceptibility of promastigotes to this oxygen product may be explained by a deficiency in catalase and glutathione peroxidase, which are both scavengers of hydrogen peroxide (100). In contrast, amastigotes display a superior ability to scavenge hydrogen peroxide (20), probably as a result of increased catalase production (21).

Attenuation or inhibition of the host cell's respiratory burst may represent a critical factor for the survival of *Leishmania* parasites. *Leishmania* parasites can impair the stimulation of the macrophage oxidative burst stimulated by zymosan, bacterial lipopolysaccharide, or lymphokines (14, 106). This impairment is parasite specific because inert latex beads do not block the

respiratory burst, and appears to be a function of the number of parasites per macrophage.

Inhibitor of the oxidative burst A role for LPG in the impairment of the respiratory burst was suggested by the demonstration that it is a potent inhibitor of purified rat-brain PKC activity in vitro (87). This study revealed that LPG is a competitive inhibitor ($K_I < 1$ μM) with respect to diolein (a diacylglycerol), and a noncompetitive inhibitor with respect to phosphatidylserine. Inhibition of PKC is selective, since LPG does not affect the catalytic fragment of PKC and the cAMP-dependent protein kinase. Additional studies revealed that the 1-*O*-alkylglycerol fragment exhibits the most potent inhibitory activity, although the phosphoglycan portion also significantly inhibits purified PKC activity (85). These results strongly suggest that LPG interacts with the regulatory domain of PKC, which contains the binding sites for diacylglycerol, calcium, and phospholipids. Decrease in the calcium-dependent activity of PKC was explained by chelation of calcium.

The inhibitory property of LPG on purified PKC may be of major significance, considering the role of PKC in the induction of the respiratory burst. In this regard, phagocytosis of LPG-coated beads inhibits oxygen consumption in monocytes stimulated with phorbol myristate acetate, a synthetic activator of PKC (88). Using indirect immunofluorescence, LPG or a LPG fragment was detected in the outer membrane of monocytes containing LPG-coated beads. Thus, LPG may effectively interact with the monocyte's PKC or another factor involved in production of the oxidative burst. Whether it is the intact LPG molecule or a processed form that mediates the inhibition of the respiratory burst is still unknown. Nevertheless, these findings are consistent with the hypothesis that inhibition of PKC activity represents an important, if not essential, event for successful establishment of *Leishmania* parasites within their host cell.

LPG may not be the only leishmanial molecule involved in suppressing the oxidative burst. A leishmanial-surface acid phosphatase inhibits the neutrophil-derived burst upon stimulation with a chemotactic peptide, but not with a phorbol ester (46).

Inhibitor of c-fos *gene expression* Activation of PKC also results in the expression of several genes, including several protooncogenes such as the c-*fos* gene (50, 74, 93). The Fos protein is a *trans*-acting transcription factor that forms a stable transcriptional complex with the product of another oncogene, c-*jun* (reviewed in 24). According to the prevailing hypothesis, Fos functions as a nuclear third messenger molecule that regulates gene expression in response to environmental signals. In macrophages, c-*fos* gene expression is inducible through a PKC- or a cAMP-dependent pathway.

Activation of PKC results in a rapid and transient increase in c-*fos* mRNA levels (114), whereas elevation of cAMP stimulates a stable and long-lasting expression of the c-*fos* gene (11).

Macrophages infected with *L. donovani* display an impaired ability to express the c-*fos* gene in response to lipopolysaccharide or diacylglycerol, suggesting that the parasite can interfere with a PKC-dependent gene expression pathway (28). Incubation of macrophages with LPG or its delipidated version resulted in the inhibition of PKC-dependent c-*fos* gene expression. In contrast, LPG did not impair the ability of macrophages to express the c-*fos* gene in response to cAMP (29). This observation is in agreement with the selective inhibitory effect of LPG on purified enzyme activity (87). However, the exact molecular mechanism by which LPG interacts with the components of this PKC-dependent signal transduction pathway is not known.

Because macrophage-activating cytokines such as interferon (IFN)-γ and TNF-α act through PKC-dependent signal transduction pathways (41, 53, 136), impairment of PKC-dependent gene expression would attenuate the impact of external activating signals and therefore be beneficial for intracellular *Leishmania* parasites.

Inhibitor of chemotaxis Rapid accumulation of blood monocytes at sites of inflammation represents an important step of the inflammatory response. Binding of a chemoattractant to its specific cell-surface receptor initiates a cascade of intracellular events (140, 151), which involves activation of PKC (76). *L. donovani* LPG and its delipidated counterpart are both potent inhibitors of monocyte and neutrophil chemotactic locomotion (44). Inhibition of inflammatory reactions in the lesion sites may contribute to the chronicity of the disease.

SCAVENGERS OF TOXIC OXYGEN METABOLITES In addition to inhibiting PKC-mediated enzymatic induction of the oxidative burst (88), LPG may protect promastigotes by scavenging toxic oxygen metabolites generated during the burst (17). Indeed, LPG is highly effective in scavenging hydroxyl radicals and superoxide anions, as determined in vitro using electron spin resonance spectroscopy and spin-trapping assays. The scavenging activity of LPG is largely conferred by the repeating phosphorylated disaccharide units. This property can be explained by oxidation of the hydroxyl groups of sugars to ketones (reviewed in 48).

Addition of LPG to monocytes following induction of the oxidative burst results in an immediate, dose-dependent reduction of chemiluminescence, suggesting an effective scavenging of the superoxide anions already secreted into the culture medium (88). Inhibition of chemiluminescence is also observed with monocytes treated with LPG but not with the glycosylinosi-

tolphospholipid antigens (44), which confirms the scavenging property of the repeating phosphorylated disaccharides. Incubation of *L. major* promastigotes and their excreted factors (which contain LPG) with polymorphonuclear leukocytes also inhibits chemiluminescence (39). Thus, LPG may protect the parasite from the damaging effects of the oxidative burst through at least two distinct mechanisms: (*a*) attenuation of the PKC-mediated induction of the burst, and (*b*) scavenging of the cytocidal products of the burst.

INHIBITOR OF IL-1 PRODUCTION Macrophages and other antigen-presenting cells play a crucial modulatory role during the establishment of an effective immune response through the activation of T lymphocytes (149). Two macrophage accessory functions regulate T helper lymphocyte activation: (*a*) the expression of major histocompatibility complex class II molecules and (*b*) the production of interleukin (IL)-1 (149). In *L. donovani*-infected macrophages, these two functions are defective (103, 115, 117). Interestingly, incubation of monocytes with purified *L. donovani* LPG inhibits lipopolysaccharide (LPS)-induced IL-1 secretion (44). Whether or not LPG is responsible for the inhibition of IL-1 in infected macrophages remains to be determined.

MODULATOR OF TNF RECEPTORS Tumor necrosis factor (TNF) is a cytokine that pleiotropically affects the immune response (138). Macrophages produce TNF in response to a broad range of stimuli, including several microorganisms and derived molecules. In turn, TNF activates macrophage cytotoxic functions and the subsequent destruction of the invader. *E. coli* LPS, a potent inducer of TNF, stimulates macrophages to rapidly internalize their TNF receptors (31). This phenomenon may reflect a microbial evasion strategy of host defense or a host strategy to prevent autotoxicity. As does bacterial LPS, LPG causes an important reduction in the ability of macrophages to bind TNF (29). Although the *Leishmania* parasite stimulates macrophages to secrete TNF (49), a down-regulation of TNF receptors may play a role in the survival of *Leishmania,* particularly during the initiation of infection. The fate of TNF receptors following phagocytosis of *Leishmania* parasites, however, is not known.

ANALOGOUS FUNCTIONS BY GLYCOLIPIDS FROM MYCOBACTERIA *Mycobacterium leprae* and *Mycobacterium tuberculosis,* the etiologic agents of leprosy and tuberculosis, respectively, survive and multiply in mononuclear phagocytes. Production of large amounts of cell wall–associated glycolipids is a feature common to both *M. leprae* and *M. tuberculosis* (12, 47). Recent studies indicate that these glycolipids may represent virulence factors that contribute to the intracellular survival of mycobacteria. Indeed, in a manner

similar to LPG, mycobacterial glycolipids effectively scavenge oxygen radicals (16, 17), suppress the oxidative burst in monocytes (13, 150), and inhibit PKC activity both in vitro and in vivo (13, 16) as well as PKC-dependent gene expression in macrophages (16). From an evolutionary point of view, it is striking that a protozoan and a bacterial intracellular parasite have both evolved structurally and functionally similar virulence factors to counter the microbicidal activities of macrophages.

BIOSYNTHESIS OF LPG

Because LPG is essential for the survival of *Leishmania* as it progresses through its life-cycle, chemotherapeutic drugs conceivably could be designed to inhibit LPG synthesis; the parasite should then be vulnerable to normal host defense mechanisms. Detailed knowledge of LPG biosynthesis might provide a specific target in the search for more efficaceous drugs against leishmaniasis.

Synthesis of the PI Anchor Region

Although LPG possesses a *lyso*-1-*O*-alkyl PI anchor, the synthesis probably involves formation of a 1-*O*-alkyl-2-acyl-PI precursor. *Leishmania* parasites possess significant amounts of 1-*O*-alkyl-2-acyl-phospholipids, but not *lyso*-alkylphospholipids (152). The latter are cytotoxic for *Leishmania* (1) as well as other eukcaryotic cells (60). Based on information from other eukaryotic systems, the possible pathway that leads to the assembly of the phosphatidylinositol anchor of LPG can be postulated. Ether phospholipid synthesis might be initiated by acylation of C1 of glycolytic intermediate dihydroxyacetonephosphate. A key enzyme believed to be involved in ether lipid synthesis (dihydroxyacetonephosphate acyltransferase) has been reported (58) in *Leishmania* glycosomes (organelles resembling peroxisomes). The acyl group may then be replaced with a fatty alcohol. *Leishmania* parasites incorporate fatty alcohols in ether phospholipids (59). The resultant alkyl dihydroxyacetonephosphate might then be reduced with NADPH and acylated at the *sn*-2 position forming 1-*O*-alkyl-2-acyl phosphatidic acid. The latter may be activated to the CDP-derivative by CTP and then condensed with *myo*-inositol to form 1-*O*-alkyl-2-acyl-PI. However, this pathway has not been established in *Leishmania* species, and another route of lipid anchor assembly for LPG may exist.

Assembly of the Core-PI Region

Predictions on the pathway of core-PI synthesis can be derived from the recently elucidated structures of the GIPLs isolated from *Leishmania* spp. (79, 80, 82, 83) and from details on GPI anchor assembly reported in African

trypanosomes (reviewed in 33). The biosynthetic pathway of the trypanosomal GPI anchor contains a transfer of N-acetylglucosamine from UDP-GlcNAc to PI forming GlcNAc-PI. Deacetylation of the product results in GlcN-PI. Mannose residues are then added by using mannosylphosphoryldolichol as the mannosyl donor (91). Analogous reactions in *Leishmania* spp. have not yet been demonstrated.

In *Leishmania* spp., addition of the first mannose residue to GlcN-PI presumably would yield Man(α1,4)GlcN-PI, a precursor to LPG and GPI-anchored proteins, such as the glycoprotein gp63 (134). The addition of the second mannose residue is at a branch point in the biosynthetic pathway of leishmanial GPI anchors. In the synthesis of the GPI anchor of gp63 (134), the second mannose would form Man(α1,6)Man(α1,4)GlcN-PI, whereas in LPG, it would yield Man(α1,3)Man(α1,4)GlcN-PI. In LPG biosynthesis, three galactose residues then would be added, one of which is the galactofuranose; the donor of this sugar is unknown. Three of the GIPLs that have been isolated and characterized from *L. major* (GIPLs 1–3) contain one to three galactose residues, respectively (83). Because their complete glycan structures are consistent with the LPG core structure including the galactofuranose residue, GIPLs 1–3 most likely are intermediates in LPG biosynthesis. Of significance, the lipid portions of GIPLs 1–3 are 1-*O*-alkyl-2-acyl-PI. Two other GIPLs have been isolated and are the *lyso*-derivatives of GIPLs 2 and 3. Thus, the pathway may involve assembly of the glycan core on 1-*O*-alkyl-2-acyl-PI, which is followed by deacylation of the fatty acid near completion of the core. The glucose(α1)-phosphate probably is added sometime after the deacylation step because, as reported so far, no GIPL contains this substituent.

As discussed above (LPG of Amastigotes), amastigotes of *L. donovani* do not synthesize LPG (82). These intracellular forms apparently do not synthesize Man(α1,3)Man(α1,4)GlcN-PI, which would be an intermediate in LPG biosynthesis. This observation led to the proposal that down-regulation of a putative α1,3-mannosyltransferase in amastigotes contributes to a lack of LPG expression (82).

Polymerization of Repeating Units

In contrast to the virtual lack of enzymological information regarding the synthesis of the other portions of LPG, data have been reported on repeating unit polymerization. An in vitro membrane system from *L. donovani* capable of synthesizing LPG repeating units has been developed (15). The galactose and mannose residues of the LPG repeating units of *L. donovani* are believed to be added from their respective nucleotide-sugar donors sequentially and directly to LPG (Figure 2). While mannosylphosphoryldolichol might participate as a mannosyl donor in core-PI synthesis, no evidence was found for the possible involvement of the mannolipid in repeating unit assembly. Addition-

al information indicates that guanosine diphosphate (GDP)-Man donates mannose-1-phosphate (15), thereby conserving the α-anomeric configuration of the mannosylphosphate bond. Thus, the repeating units of LPG appear to be polymerized by the individual alternating transfer of galactose and mannose-1-phosphate residues from their nucleotide derivatives. Experiments using monensin, an inhibitor of Golgi function, have produced evidence that repeating-unit assembly occurs in the Golgi (6). The addition of the hexoses that comprise side chains of the LPG repeating units of *L. major* and *L. mexicana* has not yet been examined. One of the key glycosyltransferases to be investigated is a putative arabinosyltransferase from *L. major*. As mentioned earlier (Developmental Modification of LPG During Promastigote Metacyclogenesis), the LPGs from metacyclic forms of *L. major* contain arabinose residues not contained in terminal side chains in the repeating units and in noninfectious forms. Thus, induction of the arabinosyltransferase in *L. major* may be an important regulatory enzyme in metacyclogenesis in that species.

Synthesis of Capping Oligosaccharides

All but one of the cap structures elucidated from various leishmanial LPGs contain a Man(α1,2)Man(α1) at the reducing end. It is, therefore, tempting to

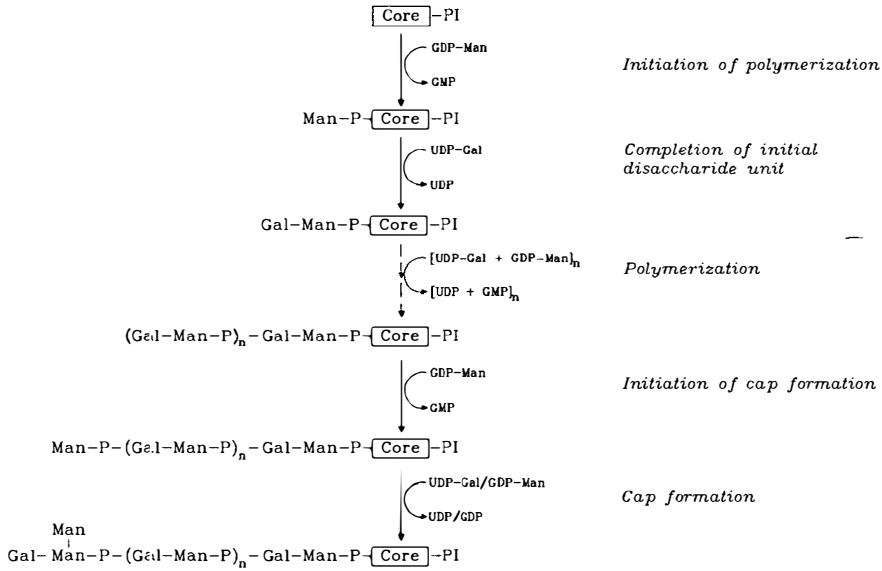


Figure 2 Proposed pathway of assembly of the repeating units and capping oligosaccharides of the *L. donovani* LPG. The core structure is Gal(α1,6)Gal(α1,3)Gal$_f$(β1,3)-Man-(α1,3)Man(α1,4)GlcN(α1,6), and PI is *lyso*-l-*O*-alkylphosphatidylinositol. The sequence of monosaccharide addition in cap formation is not yet known.

speculate on the existence of a Man(α1,2)mannosyltransferase. The activity of such a putative enzyme would result in the signal for cessation of LPG elongation with the formation of a chain-terminating Man(α1,2)Man-containing cap oligosaccharide. Because metacyclogenesis is accompanied by an approximate doubling in the size of LPG, a chain-terminating mannosyltransferase may prove to be one of the key regulatory enzymes in LPG biosynthesis. In the in vitro glycosylating system from *L. donovani* that can generate repeating units (15), several small neutral oligosaccharides were also observed upon fragmentation and analysis of the LPG product. These oligosaccharides may be capping structures, but are not characterized as yet.

IMMUNOLOGICAL ASPECTS OF LPG

LPG as a Vaccine Candidate

Because LPG is the most abundant surface molecule of *Leishmania* promastigotes, several studies have examined this molecule's potential as a chemically defined vaccine. When administered to genetically resistant mice, in which cutaneous lesions resolve spontaneously, purified *L. major* LPG induced full protection against a challenge with promastigotes, whereas partial protection was achieved in the susceptible mice (56). This observation suggested that LPG might be considered as a candidate vaccine antigen against cutaneous leishmaniasis. Immunization with the water-soluble carbohydrate portion (phosphoglycan), however, had no effect on the development of lesions (56). Further studies revealed that the phosphoglycan is in fact a disease-promoting antigen (92).

Incorporation of LPG into liposomes proved to be an efficient way to immunize mice against *L. mexicana* without causing any exacerbation of the disease (122). Adoptive transfer of T-cells isolated from immunized mice into syngeneic mice provided protection against a challenge with *L. mexicana* promastigotes, indicating that protection is a function of antigen-specific T-cells.

Activation of specific T helper subsets of lymphocytes represents one of the key cellular events in the establishment of an effective immune response. Several lines of evidence support the notion that T lymphocytes play a dominant role in the acquired resistance to *Leishmania* parasites (78, 99). It was therefore of interest to determine whether LPG can be recognized by T-cells. Mice vaccinated with *L. major* LPG contained an increased frequency of *L. major*-reactive T-cells (94). Furthermore, LPG induced a specific delayed-type hypersensitivity in *L. major*-infected mice, which also produced T-cell-dependent IgG to LPG. The authors suggested the possibility that T-cells can recognize and respond to LPG although the T-cells did not respond to LPG in vitro. Indeed, LPG stimulates a weak proliferation of T lymphocytes from patients with active cutaneous leishmaniasis caused by *L.*

major (66). Surprisingly, these T-cells did not respond to gp63, which is nonetheless considered as a potential vaccine candidate. Similarly, T lymphocytes from cured kala-azar patients proliferate and produce IFN-γ in response to *L. major* LPG (71).

In another observation, Mendonça and coworkers (90) reported that T lymphocytes from cutaneous leishmaniasis patients responded to highly purified *L. braziliensis* LPG, whereas proteinase K–treated LPG did not stimulate any response. This important observation indicates that stimulation of T-cell responses by LPG may, in fact, be induced by tightly associated protein contaminants.

Purification and partial characterization of the LPG-associated protein contaminants revealed the presence of several proteins. Subsequent T-cell proliferation studies showed that these peptides were potent stimulators of T-cells from leishmaniasis patients (126), as well as from mice immunized with protein-contaminated LPG (67), whereas protein-free LPG failed to stimulate any T-cell responses. While LPG does not appear to be able to elicit T-cell responses, the glycoconjugate may act as a natural adjuvant for the proteins with which it is tightly complexed.

Use for Serotyping Leishmania *Strains*

Several polyclonal and monoclonal antibodies generated against *Leishmania* strains react with epitopes present on LPG (27, 51, 135). These antibodies proved useful for serotyping *Leishmania* strains, with respect to their ability to selectively precipitate the excreted form of LPG. However, monoclonal antibodies that were initially believed to recognize the core region of *L. donovani* LPG (142) were subsequently found to react with protein contaminants (67). Therefore, we can infer that some of the monoclonal and polyclonal antibodies used for serotyping may, in fact, recognize LPG-associated proteins.

CONCLUDING REMARKS

The list of functions proposed for LPG is extensive and surprisingly large. However, the uniqueness of the overall structure of LPG with its unusual domains could account for the multifunctional aspects of the LPG molecule. Whether the structural polymorphisms of LPG contribute to the various pathologies associated with the different leishmaniases remains to be established.

ACKNOWLEDGMENTS

This laboratory is funded by the United Nations Development Program/World Bank/World Health Organization Special Program for Research and Training

in Tropical Diseases and by National Institutes of Health Grant AI20941. S. J. T. is a Burroughs Wellcome Scholar in Molecular Parasitology. A. D. is supported by a Postdoctoral fellowship from the Medical Research Council of Canada.

Literature Cited

1. Acterberg, V., Gercken, G. 1987. Cytotoxicity of ester and ether lysophospholipids on *Leishmania donovani* promastigotes. *Mol. Biochem. Parasitol.* 23:117–22
2. Alexander, J., Vickerman, K. 1975. Fusion of host cell secondary lysosomes with the parasitophorous vacuoles of *Leishmania mexicana* infected macrophages. *J Protozool.* 22:502–8
3. Avila, J. L., Rojas, M., Garcia, L. 1988. Persistence of elevated levels of galactosyl-α(1,3)galactose antibodies in sera from patients cured of visceral leishmaniasis. *J. Clin. Microbiol.* 26: 1842–47
4. Avila, J. L., Rojas, M., Towbin, H. 1988. Serological activity against galactosyl-α(1,3)galactose in sera from patients with several kinetoplastida infections. *J. Clin. Microbiol.* 26:126–32
5. Baggiolini, M., Wyman, M. P. 1990. Turning on the respiratory burst. *TIBS* 15:69–72
6. Bates, P. A., Hermes, I., Dwyer, D. M. 1990. Golgi-mediated posttranslational processing of secretary acid phosphatase by *Leishmania donovani* promastigotes. *Mol. Biochem. Parasitol.* 38:247–56
7. Berman, J. D., Dwyer, D. M., Wyler, D. J. 1979. Multiplication of *Leishmania* in human macrophages in vitro. *Infect. Immunol.* 26:375–79
8. Blackwell, J. M., Ezekowitz, R. A. B., Roberts, M. B., Channon, J. Y., Sim, R. B., et al. 1985. Macrophage complement and lectin-like receptors bind *Leishmania* in the absence of serum. *J. Exp. Med.* 162:324–31
9. Bouvier, J., Etges, R. J., Bordier, C. 1985. Identification and purification of membrane and soluble forms of the major surface protein of *Leishmania* promastigotes. *J. Biol. Chem.* 260:15504–9
10. Bouvier, J., Etges, R. J., Bordier, C. 1987. Identification of the promastigote surface protease in seven species of *Leishmania. Mol. Biochem. Parasitol.* 24:73–79
11. Bravo, R., Neuberg, M., Burckhardt, J., Almendral, J., Wallich, R., et al. 1987. Involvement of common and cell type–specific pathways in c-fos gene control: stable induction by cAMP in macrophages. *Cell* 48:251–60
12. Brennan, P. J. 1989. Structure of mycobacteria: recent developments in defining cell wall carbohydrates and proteins. *Rev. Infect. Dis.* 11:S420–30
13. Brozna, J. P., Horan, M., Rademacher, J. M., Pabst, K. M., Pabst, M. J. 1991. Monocyte responses to sulfatide from *Mycobacterium tuberculosis:* inhibition of priming for enhanced release of superoxide, associated with increased secretion of interleukin-1 and tumor necrosis factor alpha, and altered protein phosphorylation. *Infect. Immunol.* 59: 2542–48
14. Buchmuller-Rouiller, V., Mauël, J. 1987. Impairment of the oxidative metabolism of mouse peritoneal macrophages by intracellular *Leishmania* spp. *Infect. Immunol.* 55:587–93
15. Carver, M. A., Turco, S. J. 1991. Cell-free biosynthesis of lipophosphoglycan from *Leishmania donovani:* characterization of microsomal galactosyltransferase and mannosyltransferase activities. *J. Biol. Chem.* 266:10974–81
16. Chan, J., Fan, X., Hunter, S. W., Brennan, P. J., Bloom, B. R. 1991. Lipoarabinomannan, a possible virulence factor involved in persistence of *Mycobacterium tuberculosis* within macrophages. *Infect. Immunol.* 59:1755–61
17. Chan, J., Fujira, T., Brennan, P., McNeil, M., Turco, S. J., et al. 1989. Microbial glycolipids: possible virulence factors that scavenge oxygen radicals. *Proc. Natl. Acad. Sci. USA* 86:2453–57
18. Chang, K. P., Dwyer, D. M. 1976. Multiplication of a human parasite *Leishmania donovani* in phagolysosomes of hamster macrophages in vitro. *Science* 193:678–80
19. Chang, K. P., Fong, D., Bray, R. S. 1985. Biology of *Leishmania* and leishmaniasis. In *Leishmaniasis,* ed. K. P. Chang, R. S. Bray, pp. 1–30. New York: Elsevier
20. Channon, J. Y., Blackwell, J. M. 1985. A study of the sensitivity of *Leishmania donovani* promastigotes and amastigotes to hydrogen peroxide. I. Differences in

sensitivity correlate with parasite-mediated removal of hydrogen peroxide. *Parasitology* 91:197–206
21. Channon, J. Y., Blackwell, J. M. 1985. A study of the sensitivity of *Leishmania donovani* promastigotes and amastigotes to hydrogen peroxide. II. Possible mechanisms involved in protective H_2O_2 scavenging. *Parasitology* 91:207–17
22. Channon, J. Y., Blackwell, J. M. 1986. Molecular biology of *Leishmania*. *Parasitol. Today* 2:45–53
23. Cross, G. A. M. 1990. Glycolipid anchoring of plasma membrane proteins. *Annu. Rev. Cell. Biol.* 6:1–39
24. Curran, T., Franza, B. R. Jr. 1988. Fos and Jun: the AP-1 connection. *Cell* 55:395–97
25. Da Silva, R. P., Hall, B. F., Joiner, K. A., Sacks, D. L. 1989. CR1, the C3b receptor, mediates binding of infective *Leishmania major* metacyclic promastigotes to human macrophages. *J. Immunol.* 143:617–22
26. Davies, C. R., Cooper, A. M., Peacock, C., Lane, R. P., Blackwell, J. M. 1990. Expression of LPG and GP63 by different developmental stages of *Leishmania major* in the sandfly *Phlebotomus papatasi*. *Parasitology* 101:337–43
27. de Ibarra, A. A. L., Howard, J. G., Snary, D. 1982. Monoclonal antibodies to *Leishmania tropica major:* specificities and antigen location. *Parasitology* 85:523–31
28. Descoteaux, A., Matlashewski, G. 1989. C-*fos* and TNF gene expression in *Leishmania donovani*-infected macrophages. *Mol. Cell. Biol.* 9:5223–27
29. Descoteaux, A., Turco, S. J., Sacks, D. L., Matlashewski, G. 1991. *Leishmania donovani* lipophosphoglycan selectively inhibits signal transduction in macrophages. *J. Immunol.* 146:2747–53
30. Dewald, B., Thelen, M., Baggiolini, M. 1988. Two transduction sequences are necessary for neutrophil activation by receptor agonists. *J. Biol. Chem.* 263: 16179–84
31. Ding, A. H., Sanchez, E., Srimal, S., Nathan, C. F. 1989. Macrophages rapidly internalize their tumor necrosis factor receptors in response to bacterial lipopolysaccharide. *J Biol. Chem.* 264: 3924–29
32. Ding, A. H., Wright, S. D., Nathan, C. F. 1987. Activation of mouse peritoncal macrophages by monoclonal antibodies to MAC-1 (complement receptor type 2). *J. Exp. Med.* 165:733–49
33. Doering, T. L., Masterson, W. J., Hart, G. W., Englund, P. T. 1990. Biosynthesis of glycosyl phosphatidylinositol membrane anchors. *J. Biol. Chem.* 265: 611–14
34. Eilam, Y., El-On, J., Spira, D. T. 1985. *Leishmania major:* excreted factor, calcium ions, and the survival of amastigotes. *Exp. Parasitol.* 59:161–68
35. Elhay, M., Kelleher, M., Bacic, A., McConville, M. J., Tolson, D. L., et al. 1990. Lipophosphoglycan expression and virulence in ricin-resistant variants of *Leishmania major*. *Mol. Biochem. Parasitol.* 40:255–68
36. Elhay, M. J., McConville, M. J., Handman, E. 1988. Immunochemical characterization of a glycoinositol-phospholipid membrane antigen of *Leishmania major*. *J. Immunol.* 141:1326–31
37. El-On, J., Bradley, D. J., Freeman, J. C. 1980. *Leishmania donovani:* action of excreted factor on hydrolytic enzyme activity of macrophages from mice with genetically different resistance to infection. *Exp. Parasitol.* 49:167–74
38. El-On, J., Schnur, L. F., Greenblatt, C. L. 1979. *Leishmania donovani:* physiochemical, immunological and biological characterization of excreted factor from promastigotes. *Exp. Parasitol.* 47:254–69
39. El-On, J., Zvillich, M., Sarov, I. 1990. *Leishmania major:* inhibition of the chemiluminescent response of human polymorphonuclear leukocytes by promastigotes and their excreted factors. *Parasite Immunol.* 12:285–95
40. Etges, R. J., Bouvier, J., Hoffman, R., Bordier, C. 1985. Evidence that the major surface protein of three *Leishmania* species are structurally related. *Mol. Biochem. Parasitol.* 14:141–49
41. Fan, X.-D., Goldberg, M., Bloom, B. R. 1988. Interferon-γ-induced transcriptional activation is mediated by protein kinase C. *Proc. Natl. Acad. Sci. USA* 85:5122–25
42. Farago, A., Nishizuka, Y. 1990. Protein kinase C in transmembrane signalling. *FEBS Lett.* 268:350–54
43. Franke, E. D., McGreevy, P. B., Katz, S. P., Sacks, D. L. 1985. Growth cycle-dependent generation of complement-resistant *Leishmania* promastigotes. *J. Immunol.* 134:2713–18
44. Frankenburg, S., Leibovici, V., Mansbach, N., Turco, S. J., Rosen, G. 1990. Effect of glycolipids of *Leishmania* parasites on human monocyte activity. Inhibition by lipophosphoglycan. *J. Immunol.* 145:4284–89
45. Glaser, T. A., Moody, S. F., Handman, E., Bacic, A., Spithill, T. W. 1991. An antigenically distinct lipophosphoglycan

of *Leishmania major*. *Mol. Biochem. Parasitol.* 45:337–44
46. Glew, R. H., Saha, A. K., Das, S., Remaley, A. T. 1988. Biochemistry of *Leishmania* parasites. *Microbiol. Rev.* 52:412–32
47. Goren, M. B., Brokl, O., Roller, P., Fales, H. M., Das, B. C. 1976. Sulfatides of *Mycobacterium tuberculosis:* the structure of the principal sulfatide (SL-1). *Biochemistry* 15:2728–35
48. Green, J. W. 1980. Oxidative reactions and degradations. In *The Carbohydrates,* ed. W. Pigman, 1B:1101–66. New York: Academic
49. Green, S. I., Crawford, R. M., Hockmeyer, J. T., Meltzer, M. S., Nacy, C. A. 1990. *Leishmania major* amastigotes initiate the L-arginine-dependent killing mechanism in IFN-γ-stimulated macrophages by induction of tumor necrosis factor-α. *J. Immunol.* 145:4290–97
50. Greenberg, M. E., Ziff, E. B. 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. *Nature* 311:433–38
51. Greenblatt, C. L., Slutzky, G. M., de Ibarra, A. A. L., Snary, D. 1983. Monoclonal antibodies for serotyping of *Leishmania* strains. *J. Clin. Microbiol.* 18:191–93
51a. Greis, K. D., Turco, S. J., Thomas, J. R., McConville, M. J., Homans, S. W., Ferguson, M. A. J. 1992. Purification and characterization of an extracellular phosphoglycan from *Leishmania donovani*. *J. Biol. Chem.* In press
52. Grimaldi, G. Jr., Tesh, R. B., McMahon-Pratt, D. 1989. A review of the geographic distribution and epidemiology of leishmaniasis in the new world. *Am. J. Trop. Med. Hyg.* 41:687–725
53. Hamilton, T. A., Becton, D. L., Somers, S. D., Gray, P. W., Adams, D. O. 1985. Interferon-γ modulates protein kinase C activity in murine peritoneal macrophages. *J. Biol. Chem.* 260:1378–81
54. Handman, E., Greenblatt, C. L. 1977. Promotion of leishmanial infections in non-permissive host macrophages by conditioned medium. *Z. Parasitenkd.* 53:143–47
55. Handman, E., Goding, J. W. 1985. The *Leishmania* receptor for macrophages is a lipid-containing glycoconjugate. *EMBO J.* 4:329–36
56. Handman, E., Mitchell, G. F. 1985. Immunization with *Leishmania* receptors for macrophages protects mice against cutaneous leishmaniasis. *Proc. Natl. Acad. Sci. USA* 82:5910–14
57. Handman, E., Schnur, L. F., Spithill, T. W., Mitchell, G. F. 1986. Passive transfer of *Leishmania* lipopolysaccharide confers parasite survival in macrophages. *J. Immunol.* 137:3608–13
58. Hart, D. T., Opperdoes, F. R. 1984. The occurrence of glycosomes (microbodies) in the promastigote stage of four major *Leishmania* species. *Mol. Biochem. Parasitol.* 13:159–72
59. Herrmann, H., Gercken, G. 1980. Incorporation of octadecanol into the lipids of *Leishmania donovani*. *Lipids* 15:179–85
60. Hoffman, D. R., Hadju, J., Snyder, F. 1984. Cytotoxicity of platelet-activating factor and related alkyl-phospholipid analogs in human leukemia cells, polymorphonuclear neutrophils, and skin fibroblasts. *Blood* 63:545–52
61. Homans, S. W., Melhert, A., Turco, S. J. 1992. Solution structure of the lipophosphoglycan of *Leishmania donovani*. *Biochemistry*. 31:654–61
62. Houslay, M. D. 1991. Crosstalk: a pivotal role for protein kinase C in modulating relationships between signal transduction pathways. *Eur. J. Biochem.* 195:9–27
62a. Ilg, T., Etges, R., Overath, P., McConville, M. J., Thomas, J. R., et al. 1992. Structure of *Leishmania mexicana* lipophosphoglycan. *J. Biol. Chem.* In press
63. Ilg, T., Menz, B., Winter, G., Russell, D. G., Etges, R., et al. 1991. Monoclonal antibodies to *Leishmania mexicana* promastigote antigens. Secreted acid phosphatase and other proteins share epitopes with lipophosphoglycan. *J. Cell. Sci.* 99:175–80
64. Jaffe, C. L., Martinez, L. P., Sarfstein, R. 1989. *Leishmania tropica:* characterization of a lipophosphoglycan-like antigen recognized by species specific monoclonal antibodies. *Exp. Parasitol.* 70:12–24
65. Jaffe, C. L., Perez, L., Schnur, L. F. 1990. Lipophosphoglycan and secreted acid phosphatase of *Leishmania tropica* share species-specific epitopes. *Mol. Biochem. Parasitol.* 41:233–40
66. Jaffe, C. L., Shor, R., Trau, H., Passwell, J. H. 1990. Parasite antigens recognized by patients with cutaneous leishmaniasis. *Clin. Exp. Immunol.* 80: 77–82
67. Jardim, A., Tolson, D. L., Turco, S. J., Pearson, T. W., Olafson, R. W. 1991. The *Leishmania donovani* lipophosphoglycan T-lymphocyte reactive component is a tightly associated protein complex. *J. Immunol.* 147:3538–44
68. Joiner, K. A. 1988. Complement eva-

sion by bacteria and parasites. *Annu. Rev. Microbiol.* 42:201–30

69. Kaneshiro, E. S., Keka, J. A., Lester, R. L. 1986. Characterization of inositol lipids from *Leishmania donovani* promastigotes. Identification of inositol sphingo-phospholipids. *J. Lipid Res.* 27:1294–1303
70. Karp, C. L., Turco, S. J., Sacks, D. L. 1991. Lipophosphoglycan masks recognition of the *Leishmania donovani* promastigote surface by human kala-azar serum. *J. Immunol.* 147:680–84
71. Kemp, B. E., Theander, T. G., Handman, E., Hey, A. S., Kurtzhals, J. A. L., et al. 1991. Activation of human T lymphocytes by *Leishmania* lipophosphoglycan. *Scand. J. Immunol.* 33:219–24
72. King, D. L., Chang, Y. D., Turco, S. J. 1987. Cell surface lipophosphoglycan of *Leishmania donovani*. *Mol. Biochem. Parasitol.* 24:47–53
73. King, D. L., Turco, S. J. 1988. A ricin agglutinin-resistant clone of *Leishmania donovani*. *Mol. Biochem. Parasitol.* 28:285–94
74. Kruijer, W., Cooper, J. A., Hunter, T., Verma, I. 1984. Platelet-derived growth factor induces rapid but transient expression of the c-*fos* gene and protein. *Nature* 312:711–16
75. Laines, R. A., Hsieh, T. C. Y. 1987. Inositol-containing sphingolipids. *Methods Enzymol.* 138:186–95
76. Laskin, D. L., Gardner, C. R., Laskin, J. D. 1987. Induction of chemotaxis in mouse peritoneal macrophages by activators of protein kinase C. *J. Leuk. Biol.* 41:474–80
77. Lederkremer, R. M., Tanaka, C. T., Alves, M. J. M., Colli, W. 1977. Lipopeptidephosphoglycan from *Trypanosoma cruzi*. *Eur. J. Biochem.* 74:265–67
78. Louis, J., Milon, G. 1987. Immunobiology of experimental leishmaniasis. *Ann. Inst. Pasteur Immunol.* 138:737–95
79. McConville, M. J., Bacic, A. 1989. A family of glycoinositol phospholipids from *Leishmania major*. *J. Biol. Chem.* 264:757–66
80. McConville, M. J., Bacic, A. 1990. The glycoinositolphospholipids profiles of two *Leishmania major* strains that differ in lipophosphoglycan expression. *Mol. Biochem. Parasitol.* 38:57–68
81. McConville, M. J., Bacic, A., Mitchell, G. F., Handman, E. 1987. Lipophosphoglycan of *Leishmania major* that vaccinates against cutaneous leishmaniasis contains an alkylglycerophosphatidylinositol lipid anchor. *Proc. Natl. Acad. Sci. USA* 84:9841–45
82. McConville, M. J., Blackwell, J. M. 1991. Developmental changes in the glycosylated phosphatidylinositols of *Leishmania donovani*. *J. Biol. Chem.* 266:15170–79
83. McConville, M. J., Homans, S. W., Thomas-Oates, J. E., Dell, A., Bacic, A. 1990. Structures of the glycoinositolphospholipids from *Leishmania major:* a family of novel galactosylfuranose-containing glycolipids. *J. Biol. Chem.* 265:7385–94
84. McConville, M. J., Thomas-Oates, J. E., Ferguson, M. A. J., Homans, S. W. 1990. Structure of the lipophosphoglycan from *Leishmania major*. *J. Biol. Chem.* 265:19611–21
85. McNeely, T. B., Rosen, G., Londner, M. V., Turco, S. J. 1989. Inhibitory effects on protein kinase C by lipophosphoglycan and glycosyl-phosphatidylinositol antigens of *Leishmania*. *Biochem. J.* 259:601–4
86. McNeely, T. B., Tolson, D. L., Pearson, T. W., Turco, S. J. 1990. Characterization of *Leishmania donovani* variant clones using anti-lipophosphoglycan monoclonal antibodies. *Glycobiology* 1:63–69
87. McNeely, T. B., Turco, S. J. 1987. Inhibition of protein kinase C by the *Leishmania donovani* lipophosphoglycan. *Biochem. Biophys. Res. Commun.* 148:653–57
88. McNeely, T. B., Turco, S. J. 1990. Requirement of lipophosphoglycan for intracellular survival of *Leishmania donovani* within human monocytes. *J. Immunol.* 144:2745–50
89. Mendelzon, D. H., Previato, J. O., Parodi, A. J. 1986. Characterization of protein-linked oligosaccharides in trypanosomatid flagellates. *Mol. Biochem. Parasitol.* 18:355–67
90. Mendonça, S. C. F., Russell, D. G., Coutinho, S. G. 1991. Analysis of the human T cell responsiveness to purified antigens of *Leishmania:* lipophosphoglycan (LPG) and glycoprotein 63 (gp63). *Clin. Exp. Immunol.* 83:472–78
91. Menon, A., Mayor, S., Schwarz, R. T. 1990. Biosynthesis of glycosyl-phosphatidylinositol lipids in *Trypanosoma brucei:* involvement of mannosylphosphoryldolichol as the mannose donor. *EMBO J.* 9:4249–58
92. Mitchell, G. F., Handman, E. 1986. The glycoconjugate derived from a *Leishmania major* receptor for macrophages is a suppressogenic, disease-

promoting antigen in murine cutaneous leishmaniasis. *Parasite Immunol.* 8: 255–63

93. Mitchell, R. L., Zokas, L., Schreiber, R. D., Verma, I. M. 1985. Rapid induction of the expression of proto-oncogene fos during human monocytic differentiation. *Cell* 40:209–17
94. Moll, H., Mitchell, G. F., McConville, M. J., Handman, E. 1989. Evidence for T-cell recognition in mice of a purified lipophosphoglycan from *Leishmania major*. *Infect. Immunol.* 57:3349–56
95. Moody, S. F., Handman, E., Bacic, A. 1991. Structure and antigenicity of the lipophosphoglycan from *Leishmania major* amastigotes. *Glycobiology* 1:419–24
96. Mosser, D. M., Edelson, P. J. 1985. The mouse macrophage receptor for C3bi (CR3) is a major mechanism in the phagocytosis of *Leishmania* promastigotes. *J. Immunol.* 135:2785–89
97. Mosser, D. M., Edelson, P. J. 1987. The third component of complement (C3) is responsible for the intracellular survival of *Leishmania major*. *Nature* 327:329–31
98. Mosser, D. M., Wedgwood, J. F., Edelson, P. J. 1985. *Leishmania* amastigotes: resistance to complement-mediated lysis is not due to a failure to fix C3. *J. Immunol.* 134:4128–31
99. Müller, I., Pedrazzini, T., Farrell, J. P., Louis, J. 1989. T-cell responses and immunity to experimental infection with *Leishmania major*. *Annu. Rev. Immunol.* 7:561–78
100. Murray, H. W. 1981. Susceptibility of *Leishmania* to oxygen intermediates and killing by normal macrophages. *J. Exp. Med.* 153:1302–15
101. Nishizuka, Y. 1986. Studies and perspectives of protein kinase C. *Science* 233:305–12
102. Nishizuka, Y. 1988. The molecular heterogeneity of protein kinase C and its implication for cellular regulation. *Nature* 334:661–65
103. Olivier, M., Tanner, C. E. 1989. The effect of cyclosporin A in murine visceral leishmaniasis. *Trop. Med. Parasitol.* 40:32–38
104. Orlandi, P. A. Turco, S. J. 1987. Structure of the lipid moiety of the *Leishmania donovani* lipophosphoglycan. *J. Biol. Chem.* 262:10384–91
105. Pearson, R. D., Harcus, J. L., Roberts, D., Donowitz, G. R. 1983. Differential survival of *Leishmania donovani* amastigotes in human monocytes. *J. Immunol.* 131:1994–99
106. Pearson, R. D., Harcus, J. L., Symes, P. H., Romito, R., Donowitz, G. R. 1982. Failure of the phagocytic oxidative response to protect human monocyte-derived macrophages from infection by *Leishmania donovani*. *J. Immunol.* 129:1282–86
107. Pearson, R. D., Sousa, A. Q. 1985. *Leishmania* species. (Kala-azar, cutaneous, and mucocutaneous leishmaniasis). In *Principles and Practice of Infectious Diseases*, ed. G. L. Mandell, R. G. Douglas Jr., J. E. Bennett, pp. 1522–31. New York: Wiley
108. Pimenta, P. F. P., da Silva, R. P., Sacks, D. L., da Silva, P. 1989. Cell surface nanoanatomy of *Leishmania major* as revealed by fracture flip. A surface meshwork of 44 nm fusiform filaments identifies infective developmental stage promastigotes. *Eur. J. Cell Biol.* 48:180–90
109. Pimenta, P. F. P., Saraiva, E. M. B., Sacks, D. L. 1991. The comparative fine structure and surface glycoconjugate expression of three life stages of *Leishmania major*. *Exp. Parasitol.* 72: 191–204
110. Previato, J. O., Gorin, P. A. J., Mazurek, M., Xavier, M. T., Fournet, B., et al. 1990. Primary structure of the oligosaccharide chain of lipopeptidophosphoglycan of epimastigote forms of *Trypanosoma cruzi*. *J. Biol. Chem.* 265:2518–26
111. Puentes, S. M., da Silva, R. P., Sacks, D. L., Hammer, C. H., Joiner, K. A. 1990. Serum resistance of metacyclic stage *Leishmania* major promastigotes is due to release of C5b-9. *J. Immunol.* 145:4311–16
112. Puentes, S. M., Dwyer, D. M., Bates, P. A., Joiner, K. A. 1989. Binding and release of C3 from *Leishmania donovani* promastigotes during incubation in normal human serum. *J. Immunol.* 143: 3743–49
113. Puentes, S. M., Sacks, D. L., da Silva, R. P., Joiner, K. A. 1988. Complement binding by two developmental stages of *Leishmania major* promastigotes varying in expression of a surface lipophosphoglycan. *J. Exp. Med.* 167: 887–902
114. Radzioch, D., Bottazzi, B., Varesio, L. 1987. Augmentation of c-*fos* mRNA expression by activators of protein kinase C in fresh, terminally differentiated resting macrophages. *Mol. Cell. Biol.* 7: 595–99
115. Reiner, N. E. 1987. Parasite-accessory cell interactions in murine leishmaniasis.

I. Evasion and stimulus-dependent suppression of the macrophage interleukin I response by *Leishmania donovani*. *J. Immunol.* 138:1919–25
116. Reiner, N. E., Kazura, J. W. 1982. Oxidant-mediated damage of *Leishmania donovani* promastigotes. *Infect. Immunol.* 36:1023–27
117. Reiner, N. E., Ng, W., McMaster, W. R. 1987. Parasite-accessory cell interactions in murine leishmaniasis. II. *Leishmania donovani* suppresses macrophage expression of class I and II major histocompatibility complex gene products. *J. Immunol.* 138:1926–32
118. Rizvi, F. S., Ouaissi, M. A., Marty, B., Santoro, F., Capron, A. 1988. The major surface protein of *Leishmania* promastigotes is a fibronectin-like molecule. *Eur. J. Immunol.* 18:473–78
119. Rosen, G., Londner, M. V., Sevlever, D., Greenblatt, C. L. 1988. *Leishmania major:* glycolipid antigens recognized by immune human sera. *Mol. Biochem. Parasitol.* 27:93–100
120. Rosen, G., Pahlsson, P., Londner, M. V., Westerman, M. E., Nilsson, B. 1989. Structural analysis of glycosylphosphatidylinositol antigens of *Leishmania major*. *J. Biol. Chem.* 264: 10457–63
121. Russell, D. G. 1987. The macrophage-attachment glycoprotein gp63 is the predominant C3-acceptor site on *Leishmania mexicana* promastigotes. *Eur. J. Biochem.* 164:213–21
122. Russell, D. G., Alexander, J. 1988. Effective immunization against cutaneous leishmaniasis with defined membrane antigens reconstituted into liposomes. *J. Immunol.* 140:1274–79
123. Russell, D. G., Talamas-Rohana, P. 1989. *Leishmania* and the macrophage: a marriage of inconvenience. *Immunol. Today* 10:328–33
124. Russell, D. G., Wilhelm, H. 1986. The involvement of the major surface glycoprotein (gp63) of *Leishmania* promastigotes in attachment to macrophages. *J. Immunol.* 136:2613–20
125. Russell, D. G., Wright, S. D. 1988. Complement receptor type 3 (CR3) binds to an Arg-Gly-Asp-containing region of the major surface glycoprotein, gp63, of *Leishmania* promastigotes. *J. Exp. Med.* 168:279–92
126. Russo, D. M., Turco, S. J., Burns, J. M. Jr., Reed, S. G. 1992. Stimulation of human T lymphocytes by *Leishmania* lipophosphoglycan-associated proteins. *J. Immunol.* 148:202–7
127. Sacks, D. L. 1989. Metacyclogenesis in *Leishmania* promastigotes. *Exp. Parasitol.* 69:100–3
128. Sacks, D. L., Brodin, T., Turco, S. J. 1990. Developmental modification of the lipophosphoglycan from *Leishmania major* promastigotes during metacyclogenesis. *Mol. Biochem. Parasitol.* 42:225–34
129. Sacks, D. L., da Silva, R. P. 1987. The generation of infective stage *Leishmania major* promastigotes is associated with the cell-surface expression and release of a developmentally regulated glycolipid. *J. Immunol.* 139:3099–3106
130. Sacks, D. L., Hieny, S., Sher, A. 1985. Identification of cell surface carbohydrate and antigenic changes between noninfective and infective developmental stages of *Leishmania major* promastigotes. *J. Immunol.* 135:564–69
131. Sacks, D. L., Perkins, P. V. 1984. Identification of an infective stage of *Leishmania* promastigotes. *Science* 223: 1417–19
132. Sacks, D. L., Perkins, P. V. 1985. Development of infective stage *Leishmania* promastigotes within phlebotomine sandflies. *Am. J. Trop. Med. Hyg.* 34: 456–59
133. Schlein, Y., Schnur, L. F., Jacobson, R. L. 1990. Released glycoconjugate of indigenous *Leishmania major* enhances survival of a foreign *L. major* in *Phlebotomus papatasi*. *Trans. R. Soc. Trop. Med. Hyg.* 84:353–55
134. Schneider, P., Ferguson, M. A. J., McConville, M. J., Mehlert, A., Homans, S. W., et al. 1990. Structure of the glycosyl-phosphatidylinositol membrane anchor of the *Leishmania major* promastigote surface protease. *J. Biol. Chem.* 265:16955–64
135. Schnur, L. F., Zuckerman, A., Greenblatt, C. L. 1972. Leishmanial serotypes as distinguished by the gel diffusion of factors excreted *in vitro* and *in vivo*. *Isr. J. Med. Sci.* 8:932–42
136. Schutze, S., Nottrott, S., Pfizenmaier, K., Kronke, M. 1990. Tumor necrosis factor signal transduction. Cell-type-specific activation and translocation of protein kinase C. *J. Immunol.* 144: 2604–8
137. Sevlever, D., Pahlsson, P., Rosen, G., Nilsson, B., Londner, M. V. 1991. Structural analysis of a glycosylphosphatidylinositol glycolipid of *Leishmania donovani*. *Glycoconjug. J.* 8: 321–29
138. Sherry, B., Cerami, A. 1988. Cachectin/tumor necrosis factor exerts endocrine, paracrine, and autocrine control

of inflammatory responses. *J. Cell Biol.* 107:1269–77
139. Slutzky, G. M., El-On, J., Greenblatt, C. L. 1979. Leishmanial excreted factor: protein-bound and free forms from promastigote cultures of *Leishmania tropica* and *Leishmania donovani*. *Infect. Immunol.* 26:916–24
140. Snyderman, R., Pike, M. C. 1984. Chemoattractant receptors on phagocytic cells. *Annu. Rev. Immunol.* 2:257–81
141. Talamàs-Rohana, P., Wright, S. D., Lennartz, M. R., Russell, D. G. 1990. Lipophosphoglycan from *Leishmania mexicana* promastigotes binds to members of the CR3, p150,95, and LFA-1 family of leukocyte integrins. *J. Immunol.* 144:4817–24
141a. Thomas, J. R., McConville, M. J., Thomas-Oates, J. E., Homans, S. W., Ferguson, M. A. J., et al. 1992. Refined structure of the lipophosphoglycan of *Leishmania donovani*. *J. Biol. Chem.* In press
142. Tolson, D. L., Turco, S. J., Beecroft, R. P., Pearson, T. W. 1989. Immunochemical and cell surface arrangement of the *Leishmania donovani* lipophosphoglycan determined using monoclonal antibodies. *Mol. Biochem. Parasitol.* 35:109–18
143. Tolson, D. L., Turco, S. J., Pearson, T. W. 1990. Expression of a repeating phosphorylated disaccharide lipophosphoglycan epitope on the surface of macrophages infected with *Leishmania donovani*. *Infect. Immunol.* 58:3500–7
144. Towbin, H. Rosenfelder, G., Wieslander, J., Avila, J. L., Rojas, M., et al. 1987. Circulating antibodies to mouse laminin in Chagas disease, American cutaneous leishmaniasis, and normal individuals recognize terminal galactosyl(α1,3)galactose epitopes. *J. Exp. Med.* 166:419–32
145. Turco, S. J. 1990. The leishmanial lipophosphoglycan: a multifunctional molecule. *Exp. Parasitol.* 70:241–45
146. Turco, S. J., Hull, S. R., Orlandi, P. A. Jr., Shepherd, S. D., Homans, S. W., et al. 1987. Structure of the major carbohydrate fragment of the *Leishmania donovani* lipophosphoglycan. *Biochemistry* 26:6233–38
147. Turco, S. J., Orlandi, P. A. Jr., Homans, S. W., Ferguson, M. A. J., Dwek, R. A., et al. 1989. Structure of the phosphosaccharide-inositol core of the *Leishmania donovani* lipophosphoglycan. *J. Biol. Chem.* 264:6711–15
148. Turco, S. J., Sacks, D. L. 1991. Expression of a stage-specific lipophosphoglycan in *Leishmania major* amastigotes. *Mol. Biochem. Parasitol.* 45:91–100
149. Unanue, E. R., Allen, P. M. 1987. The basis for the immunoregulatory role of macrophages and other accessory cells. *Science* 236:551–57
150. Vachula, M., Holzer, T. J., Andersen, B. R. 1989. Suppression of monocyte oxidative response by phenolic glycolipid I of Mycobacterium leprae. *J. Immunol.* 142:1696–1701
151. Verghese, M. W., Smith, C. D., Charles, L. A., Jakoi, L., Snyderman, R. 1986. A guanine nucleotide regulatory protein controls polyphosphoinositide metabolism, Ca^{2+} mobilization, and cellular responses to chemoattractants in human monocytes. *J. Immunol.* 137:271–75
152. Wassef, M. K., Fioretti, T. B., Dwyer, D. M. 1985. Lipid analysis of isolated surface membranes of *Leishmania donovani* promastigotes. *Lipids* 20:108–15
153. Wilson, M. E., Hardin, K. K. 1988. The major concanavalin A-binding surface glycoprotein of *Leishmania donovani chagasi* promastigotes is involved in attachment to human macrophages. *J. Immunol.* 141:265–72
154. Wilson, M. E., Pearson, R. D. 1986. Evidence that *Leishmania donovani* utilizes a mannose receptor on human mononuclear phagocytes to establish intracellular parasitism. *J. Immunol.* 136:4681–88
155. Wilson, M. E., Pearson, R. D. 1988. Roles of CR3 and mannose receptors in the attachment and ingestion of *Leishmania donovani* by human mononuclear phagocytes. *Infect. Immunol.* 56:363–69
156. Wozencraft, A. O., Blackwell, J. M. 1987. Increased infectivity of stationary phase promastigotes of *Leishmania donovani:* correlation with enhanced C3 binding capacity and CR3 mediated attachment to host macrophages. *Immunology* 60:559–66
157. Wright, S. D., Silverstein, S. C. 1983. Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes. *J. Exp. Med.* 158:2016–23