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Review

Glycoconjugates in *Leishmania* infectivity

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Abstract

Leishmaniasis is a major health problem to humans and is caused by one of the world's major pathogens, the *Leishmania* parasite. These protozoa have the remarkable ability to avoid destruction in hostile environments they encounter throughout their life cycle. That *Leishmania* parasites have adapted to not only survive, but to proliferate largely is due to the protection conferred by unique glycoconjugates that are either on the parasites' cell surface or secreted. Most of these specialized molecules are members of a family of phosphoglycans while others are a family of glycosylinositol phospholipids. Together they have been implicated in a surprisingly large number of functions for the parasites throughout their life cycle and, therefore, are key players in their pathogenesis. This review summarizes the biological roles of these glycoconjugates and how they are believed to contribute to *Leishmania* survival in destructive surroundings. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: *Leishmania*; Glycoconjugate; Macrophage; Parasite; Sand fly

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1. Leishmaniasis

Protozoan parasites of the genus *Leishmania* are members of the family Trypanosomatidae, which comprises unicellular organisms characterized by the presence of a single flagellum and of a DNA-rich, mitochondria-like organelle, the kinetoplast. The various species of the genus *Leishmania* infect millions of people worldwide, causing a wide spectrum of diseases collectively termed leishmaniases that vary in their clinical manifestations and symptoms. Cutaneous leishmaniasis, an infection characterized by the apparition of ulcerative lesions of the skin, is principally caused by *Leishmania major*, *Leishmania tropica*, and *Leishmania mexicana*. Although in some cases lesions will persist and disseminate, cutaneous leishmaniasis is generally a self-healing disease. A variant form of cutaneous leishmaniasis, called mucocutaneous leishmaniasis, is caused by *Leishmania braziliensis braziliensis*. This parasite has a tropism for macrophages of the oro-naso-pharyngeal region, were it produces a mucosal granuloma that eventually destroys the nose and mouth. Visceral leishmaniasis, also known as Kala-azar, represents the most severe clinical manifestation of *Leishmania* infection. The causative agent, *Leishmania donovanii*, disseminates and infects macrophages of the liver, the spleen, and the bone marrow. This infection is chronic and may be fatal in untreated cases. Efficient prophylactic measures, including safe vaccines, are currently not available and the success of chemotherapeutic treatments, which rely on toxic antimonial drugs or diamidine compounds, is threatened by the spread of drug resistance.

2. The life cycle of *Leishmania*

During its life cycle, the parasite *Leishmania* alternates between two distinct developmental stages. In the mammalian host, the parasite exists under the non-motile amastigote form, which proliferates within the acidic and hydrolase-rich phagolysosomal compartment of host macrophages [1]. Transmission of the parasite is mediated by the blood-sucking sand fly, of either the genus *Phlebotomus* (in the Old World) or the genus *Lutzomyia* (in the New World). When feeding on an infected mammal, the sand fly takes up amastigote-containing macrophages/monocytes. During digestion of the bloodmeal, amastigotes initiate their differentiation into the motile promastigote form, which will attach to the midgut epithelium to avoid being excreted together with the digested bloodmeal. Virulence is acquired during metacyclogenesis, a process by which dividing, non-infective promastigotes (procyclic) transform into a non-dividing infective form [2]. These metacyclic promastigotes detach from the gut epithelial cells and migrate towards the anterior end of the digestive tract. Upon the next bloodmeal, metacyclic promastigotes are inoculated into the mammalian host, where they must successfully evade and resist non-specific defense mechanisms such as complement-mediated lysis, to ultimately bind and enter mononuclear phagocytes by a receptor-mediated process.

Once inside a parasitophorous vacuole or phagosome, metacyclic promastigotes avoid degradation and establish conditions favorable to their proliferation. The increased temperature and the decreased phagosomal pH provide the signals required for the promastigote-to-amastigote differentiation [3]. Ultimately, infected macrophages rupture, releasing the amastigotes into the surrounding environment where they can infect neighboring macrophages.

To understand the critical significance of glycoconjugates in *Leishmania* biology, it is important to investigate how the parasite is able to survive harsh environments in its life cycle. The parasite must avoid destruction in (i) the sand fly midgut where the parasite could be vulnerable to a variety of digestive enzymes, (ii) the bloodstream of the host where the organism transiently exists and would be exposed to the lytic complement pathway, and most spectacularly (iii) the phagolysosome of host macrophages where the parasite would be exposed to a number of hydrolytic enzymes, acidic pH, and the microbiocidal oxidative burst. Elucidating the molecular details of how this pathogen survives in obviously hostile environments involves a thorough understanding of the structure, biosynthesis, and function of glycoconjugates throughout its existence.

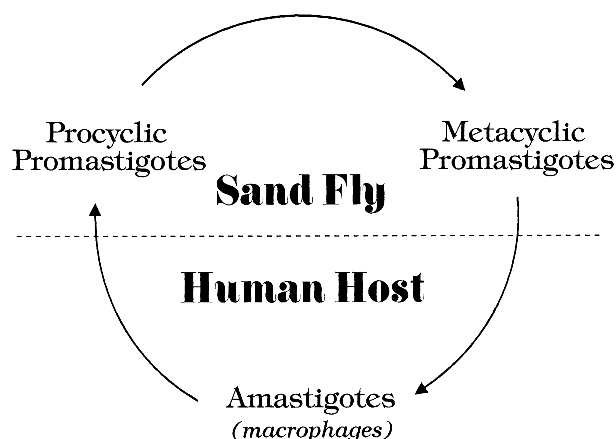

Fig. 1. Life cycle of *Leishmania*.

3. Major *Leishmania* glycoconjugates

3.1. Lipophosphoglycan

The major surface glycoconjugate of all *Leishmania* promastigotes is a unique molecule called lipophosphoglycan (LPG). The structure of LPG from diverse *Leishmania* species has been determined and the organization of its four domains is best illustrated by the prototypic *L. donovanii* LPG in Fig. 2. The four domains of the *L. donovanii* LPG are (i) a 1-*O*-alkyl-2-*lyso*-phosphatidyl(*myo*)inositol anchor, (ii) a glycan core, (iii) repeating disaccharide phosphate units, and (iv) a small oligosaccharide cap. LPG from all species of *Leishmania* have an identical lipid anchor and glycan core [4,5]. The Gal-Man-PO_4 backbone of the repeating units is also conserved, but the LPGs from other species of *Leishmania* can have additional sugars branching off the backbone sugars. There can also be minor variations in cap structure.

LPG undergoes several important modifications during the life cycle that are characteristic for each *Leishmania* species. During the process of metacyclogenesis [2,6] of *L. donovanii*, in which the promastigotes convert from non-infectious to highly infectious forms, LPG undergoes elongation due to an approximate doubling in the number of repeating units, from about 15 in procyclic promastigotes to about 30 in metacyclic forms [7]. In other species, substitutions to the repeating units often occur [8,9]. These changes play important roles in the bind-

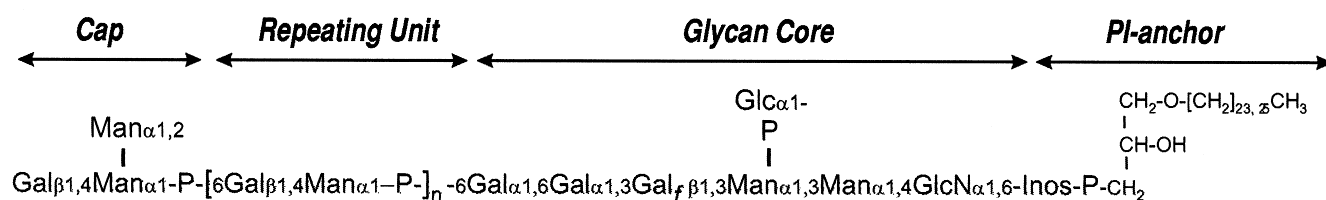

Fig. 2. Structure of LPG from *L. donovanii*. Gal_f, galactofuranose; P, phosphate.

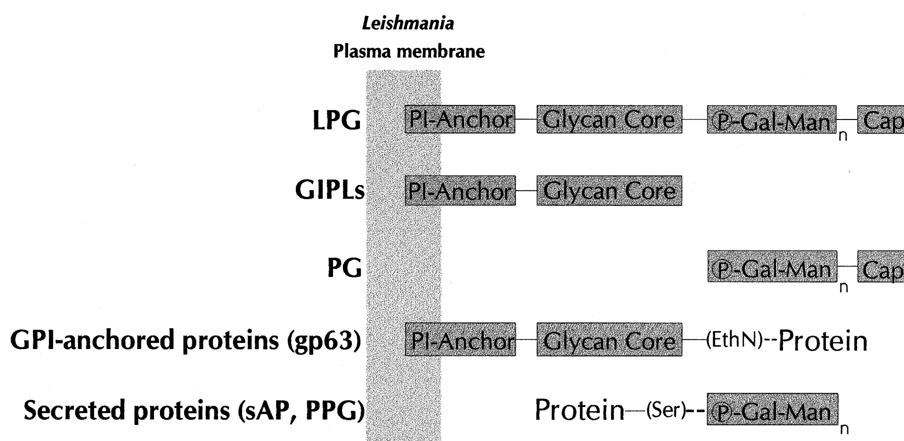

Fig. 3. Types of *Leishmania* molecules containing LPG domains. PI, phosphatidylinositol; GIPL, glycosylinositol phospholipids; PG, extracellular phosphoglycan; sAP, secreted phosphoglycan; PPG, proteophosphoglycan; EthN, phosphoethanolamine. In LPG the anchor is 1-*O*-alkyl-2-*lyso*-PI compared to 1-*O*-alkyl-2-acyl-PI in GIPLs and GPI-anchored proteins. The glycan cores in LPG, GIPLs and GPI-anchored proteins vary in structure, but all have the conserved Man(α1,4)GlcN(α1,6)-*myo*-inositol motif that is characteristic of GPI-anchored molecules.

ing and release of the parasite from the sand fly midgut (discussed below) [9,10]. While LPG is abundantly present on the surface of promastigotes, it is down-regulated in the amastigote stage by at least three orders of magnitude [11,12]. Additionally, the LPG synthesized in amastigotes frequently differs in structure from that of the promastigote [11,13].

3.2. *Surface or secreted molecules bearing domains related to LPG*

All of the domains of LPG appear as components of other parasite glycoconjugates [14] (Fig. 3). The surface protease gp63 is anchored by a GPI anchor [15]. The secreted acid phosphatase (sAP) and a secreted mucin-like proteophosphoglycan (PPG) both bear disaccharide phosphate repeating units and capping structures [16–19]; sAP additionally bears typical N-linked oligosaccharides. The secreted extracellular phosphoglycan (PG, formerly 'excreted factor') consists exclusively of a polymer of disaccharide phosphate repeating units bearing LPG-like capping sugars [20]. Lastly, a family of small and structurally related glycosylinositol phospholipids (GIPLs) is present at high levels in both promastigotes and amastigotes. GIPLs vary in sugar and lipid compositions; some GIPLs are precursors of LPG or protein GPI anchors, whereas others are distinct surface entities [5,21].

Thus, virtually every known surface molecule as well as some secreted glycoconjugates of this parasite show some intersection with the LPG biosynthetic pathway, especially in the synthesis of the repeating units and GPI anchors. This underscores the importance of these structures to the parasite's survival.

4. Glycoconjugates in sand fly-*Leishmania* interactions

4.1. *Overview*

Procyclic promastigotes exhibit an inherent capacity to attach to midgut epithelial cells, which enables the parasite to persist in the gut during excretion of the digested bloodmeal (Fig. 1). In contrast, metacyclic promastigotes lose this capacity, thereby permitting the detachment and anterior migration of infective forms so that they can be transmitted during a subsequent bloodmeal. Metacyclic promastigotes are well adapted for both arrival at and survival in the vertebrate host. Thus, the ability of *Leishmania* to bind to the midgut of their phlebotomine vectors during excretion of the digested bloodmeal is essential for the development of transmissible infections. Understanding the basis of these molecular interactions is fundamental to investigating vector

competence and disease transmission. Sand fly vectors can, in some instances, transmit only certain species of *Leishmania*. Such species-specific differences in vectorial competence have been directly correlated with the ability of promastigotes to attach to the sand fly midgut, the variable outcomes of which are controlled by structural polymorphisms in LPG [10].

4.2. *L. major*-sand fly interactions

Evidence that LPG plays a substantial role in metacyclic virulence was first described based on a series of studies with *L. major* [22]. Procyclic *L. major* promastigotes express an LPG that is characterized by mono- or di-β1,4-galactose side chain substitutions that branch off the 3-OH of the galactose residues in the repeating units [23,24] (Fig. 1). A β1,3-galactosyltransferase involved in synthesis of these side chain galactose residues has recently been described [24]. As in all procyclic forms, the number of repeating units in the *L. major* LPG is approx. 15 [23]. Studies have shown that the natural vector, *Phlebotomine papatasi*, possesses a β-galactose-binding lectin in its midgut that recognizes the terminal β-linked galactose residues of the *L. major* LPG [9,25,26]. LPG enables the attachment of the parasite to the midgut lectin after a few days following ingestion of a bloodmeal and also confers protection from digestive enzymes. While the parasite is attached, the digested blood meal is excreted. Gut-associated lectins or lectin-like molecules have been described in sand flies [27]. During metacyclogenesis of *L. major* promastigotes, the LPG undergoes extensive modifications. These include an elongation of the molecule due to an approximate doubling in the number of oligosaccharide phosphate units expressed, and a down-regulation in the number of side chain substitutions expressing the terminal β1,3-linked galactose in favor of side chains terminating in β1,3-linked arabinopyranose [9,25]. The latter accounts for the diminished binding of the lectins peanut agglutinin and ricinus agglutinin to metacyclic promastigotes previously observed [28,29]. More importantly, the switch from terminal β1,3-linked galactose to β1,3-linked arabinopyranose in the metacyclic LPG allows the parasite to detach from the midgut and migrate towards the insect's mouthparts [9,25].

4.3. *L. donovanii*-sand fly interactions

Procyclic *L. donovanii* promastigotes express an LPG (shown in Fig. 1) that differs from *L. major* by having no side chain substitutions [30]. The natural vector for *L. donovanii* is *P. argentipes*. It has recently been found that *L. donovanii* attaches to *P. argentipes* midguts via LPG cap structures which terminate in β-linked galactose and α-linked mannose residues [7]. While both of these non-reducing sugars are required for high affinity binding to the midguts, there are very few details available about the putative lectin(s). Similar to *L. major*, after several days in which the bloodmeal is digested and excreted, *L. donovanii* differentiate to metacyclic forms and migrate to the mouth parts of the insect. The molecular basis for the detachment is accomplished by an alteration in LPG structure. The metacyclic version of LPG is approx. 2–2.5 times larger than the procyclic form due to an approximate doubling in the number of Galβ1,4Manα1-PO_4 repeat units [7]. However, this is the only difference that has been detected in the primary structure of LPG, including the identical β-linked galactose and α-linked mannose residues in the terminal capping structure. So how is detachment of the metacyclic *L. donovanii* achieved? The explanation appears to be a masking of the terminal cap sequence such that the β-linked galactose and α-linked mannose residues are cryptic in metacyclic LPG of *L. donovanii* [7]. Although the biochemical reason for the cryptic nature of the cap is unknown, the changes in the overall appearance of LPG can account for the attachment/detachment process.

In summary, despite their considerable differences in structure, the LPGs from both *L. major* and *L. donovanii* each undergo modifications in size and expression of terminally exposed sugars. This evidently may reflect common molecular strategies for transmissible infections in the sand fly.

5. Glycoconjugates in vertebrate host-*Leishmania* interactions

5.1. Interactions with host serum components

5.1.1. The complement system

Serum components, including molecules of the

complement system, represent the first vertebrate host defense molecules encountered by metacyclic promastigotes following their inoculation into the bloodstream. To succeed within the mammalian host, *Leishmania* have evolved strategies both to resist the lytic action of the complement system and use it to gain access to a safe heaven, the inside of a macrophage [31–33]. In this regard, numerous studies have highlighted the importance of glycoconjugates in the *Leishmania*-complement interaction. Sacks and colleagues evidenced a marked difference between non-infective (procyclic) and infective (metacyclic) promastigotes with respect to serum sensitivity [34]. Indeed, non-infective (procyclic) promastigotes of all *Leishmania* species are extremely sensitive to fresh serum, whereas infectious (metacyclic) promastigotes display an increased resistance to lysis. *L. major* procyclic promastigotes rapidly activate the complement cascade via the alternative pathway, with deposition of covalently bound C3b on the parasite surface. Deposition of C3b also occurs on *L. major* metacyclic promastigotes, but these infective forms are unable to activate the alternative pathway in non-immune serum [35]. Further analyses of this phenomenon revealed that resistance to lysis is partly related to the developmentally regulated modifications of LPG [2,34]. The longer LPG molecules expressed on metacyclic promastigotes may contribute to their resistance to serum by preventing access of the C5b-9 membrane attack complex to the promastigote membrane. In addition, most of C5b-9 complexes are spontaneously released from the surface of *L. major* metacyclic promastigotes, thereby precluding their insertion into the membrane and the subsequent death of the parasite [34]. *L. donovanii* promastigotes appear to avoid C5b-9 formation and lysis by a distinct mechanism since C3bi, the major form of C3 deposited on their surface, cannot participate in the formation of a C5 convertase [36].

Analysis of lesion-derived *L. major* amastigotes revealed that they activate complement and fix C3 [37], which may facilitate their re-entry into macrophages [38]. Interestingly, whereas *L. mexicana* amastigotes can activate the alternative complement pathway and fix C3 in vitro [39], *L. mexicana* amastigotes isolated from lesions contain no C3 at their surface [40]. These observations suggest that in lesions, complement fixation is prevented [40]. This phenomenon might be related to the abundance of proteophosphoglycan (PPG) which acts as a potent activator of the complement cascade [41]. The numerous *O*-linked phosphooligosaccharides capped by mannooligosaccharides on the PPG provide a ligand for the mannan-binding protein (MBP), which initiates one of the pathways leading to complement activation. The large quantities of PPG secreted by *L. mexicana* amastigotes within the phagolysosomal compartment may be released in the lesion when infected macrophages rupture [17], thereby leading to a local depletion of complement. Complement activation by PPG, away from the amastigote cell surface, may be necessary for the pathology of *L. mexicana* infection, including prevention of amastigote lysis and recruitment of safe targets at the site of infection [41].

5.1.2. Other serum proteins

In addition to components of the complement system, *Leishmania* promastigotes interact and bind to other serum proteins to promote uptake by host macrophages. Hence, the mannan-binding protein (MBP) binds to mannose-terminating oligosaccharides present in the cap structure of LPG and acts as an activator of the complement cascade. Therefore, binding of MBP at the surface of *Leishmania* promastigotes provides an additional mechanism for the formation of a C3 convertase and the subsequent formation of C3b which participates in the attachment to macrophages [42]. Phagocytosis of *L. donovanii* promastigotes by human macrophages is also enhanced following opsonization by the C-reactive protein. This opsonin is a major acute phase protein present in the serum during inflammation, and specifically binds to the Gal(β1,4)Man(α1-PO_4) repeating units of *L. donovanii* LPG [43].

In summary, it is clear that both cell surface (LPG) and secreted (PPG) glycoconjugates play a key role in protecting *Leishmania* against non-specific host defense and in interacting with host serum components.

6. Interactions with macrophages

6.1. Attachment

Because *Leishmania* infects primarily mononuclear

phagocytic cells, attachment requires specific recognition molecules on the surfaces of both the parasite and the host cell. Several *Leishmania* and macrophage cell surface molecules have been implicated in the attachment process [44,45]. Although promastigotes may bind to macrophages in the absence of serum, presumably through the mannose receptor, attachment normally occurs through the CR1, CR3 (Mac-1), and p150,95 [44]. Surface glycoconjugates including LPG and gp63 play a major role in the attachment process, as they represent the major acceptors for C3b and C3bi. Indeed, attachment of *L. major* promastigotes to macrophages is inhibited by the Fab fragment of an anti-*L. major* LPG antibody [46]. However, phagocytosis of LPG-defective mutants is similar or even superior to that of wild type promastigotes [46–48]. It is possible that these LPG-defective mutants enter macrophages via the mannose/fucose receptor. Therefore, although the exact contribution of LPG and gp63 in the attachment process remains to be determined, it is clear that using both CR1 and CR3 may favor the survival of *Leishmania* promastigotes [32] since these receptors promote phagocytosis without triggering the oxidative burst [49]. Moreover, ligation of CR3 results in an inhibition of IL-12 production, a key mediator of cell-mediated immunity [50,51].

6.2. Inside the macrophage

Subsequent to their attachment to macrophage receptors, promastigotes are internalized in a phagosome. By interacting with various endocytic organelles through a series of fusion and fission events [52], the parasitophorous vacuole matures into a phagolysosome in which the promastigote transforms and multiplies as amastigote [53,54]. This implies that in the mammalian host, the amastigote stage is adapted to proliferate within the acidic and hydrolase-rich environment of the phagolysosome. The molecular mechanisms by which promastigotes efficiently initiate infection, however, are poorly understood. The requirement for LPG repeating units in this process was shown by the demonstration that LPG repeating unit-defective mutants are rapidly destroyed following phagocytosis [46–48,55]. Without LPG repeating units, promastigotes are thus unable to withstand the conditions prevailing inside the maturing parasitophorous vacuole. Because LPG repeating units are also present on several secreted glycoconjugates, including the secreted acid phosphatase (sAP), the phosphoglycan (PG), and the proteophosphoglycan (PPG) (see above), a role for all of these phosphoglycan-containing glycoconjugates must be considered in the *Leishmania*-macrophage interaction. In this regard, amastigotes proliferate inside acidic, hydrolase-rich vacuoles, despite the fact that they synthesize little or no detectable cell surface LPG [56,57]. Secretion of large amounts of PPG inside the phagolysosome by *L. mexicana* amastigotes indicates that other phosphoglycan-containing glycoconjugates may play a role during the intracellular life cycle of the parasite [58].

The mechanisms by which the various virulence-associated glycoconjugates enable *Leishmania* to either withstand or turn off the macrophage anti-microbicidal arsenal is an important area of research. The contribution of LPG during the establishment of infection by promastigotes has received a great deal of attention. In this regard, LPG repeating units epitopes are present within minutes on the macrophage surface at the immediate area of internalization of the promastigote [61]. The epitopes are maximally present in the macrophage membrane 1–2 days post infection, and by 5–6 days, they are no longer detectable [59]. Thus, intracellular functions of LPG would have to be attributed within the first several days post infection when the parasite is most vulnerable. The various properties of LPG and the other glycoconjugates with respect to intracellular parasitism are reviewed in the next sections.

6.3. Inhibition of phagosome-endosome fusion by LPG

Whereas amastigotes reside inside acidic, hydrolase-rich phagolysosomes [1,53,54,60], *L. donovanii* promastigotes inhibit phagosome-endosome fusion during the early phase of infection [61]. The role of LPG in this inhibition was evidenced by the observation that mutants defective in the synthesis of LPG repeating units induced phagosomes that fuse extensively with endocytic organelles, whereas wild type *L. donovanii* promastigotes were present in non-fusogenic phagosomes [61]. Recent studies revealed that while phagosomes containing wild type *L. donovanii* promastigotes fail to acquire the late endocytic and

lysosomal markers rab7 and LAMP1, LPG repeating unit-defective mutant-containing phagosomes mature rapidly into phagolysosomes by acquiring rab7 and LAMP1 (Scianimanico et al. submitted for publication).

The mechanism by which LPG repeating units inhibit phagosome-endosome fusion may be related to the demonstration that insertion of LPG in lipid bilayer membranes stabilizes the bilayer against the formation of an inverted hexagonal structure, resulting in reduced fusogenic properties [62]. As a consequence, LPG would give rise to an effective steric repulsion between phagosomal and endosomal membranes or reduce the negative curvature strain in bilayers, increasing the energy barrier for forming highly curved fusion intermediates, thereby preventing fusion. Truncated forms of LPG containing few repeating units are ineffective in modifying the fusogenic properties of membranes [62]. This was confirmed recently when a mutant expressing truncated LPG with three to five repeating units, compared to the normal 15–30 units, was unable to inhibit phagosome-endosome fusion [61].

The extent to which the inhibition of phagosome-endosome fusion contributes to the establishment of infection by promastigotes remains to be determined. Obviously, an impaired maturation of the parasitophorous vacuole may protect the promastigotes from hydrolytic degradation and provide an environment propitious for their differentiation into amastigotes.

6.4. Expansion of L. mexicana phagolysosomes by PPG

In contrast to *L. donovanii*, *L. mexicana* amastigotes are taken up in a phagolysosome that expands into a very large parasitophorous vacuole that can occupy up to 70% of the host cell [63]. This phenomenon is still poorly understood, but may be related to the abundant secretion of PPG into the parasitophorous vacuole. This hypothesis is based on the demonstration that addition of purified amastigote-derived PPG is highly effective in inducing vacuolization of macrophages in vitro [58]. The role of such a modification of the parasitophorous vacuole by PPG remains to be elucidated.

6.5. Inhibition of hydrolytic enzymes by LPG

Proliferation within a phagolysosome entails the ability to resist, inhibit, or inactivate host hydrolytic enzymes. A role for LPG in protecting the parasite from digestion by lysosomal enzymes was suggested based on the observation that LPG coating of erythrocytes significantly diminished their rate of cytolysis by macrophages [64]. Although LPG may have provided a physical barrier against the hydrolytic enzymes present in the phagolysosome, the possibility exists that LPG-coated erythrocytes prevented phagosome maturation, and hence, the acquisition of hydrolytic enzymes from endocytic organelles. Nevertheless, it is quite conceivable that the highly anionic nature of LPG along with its unique Galβ1,4Man linkages in the repeating units may afford protection against degradative attack.

6.6. Chelation of calcium by LPG

Phagocytosis of LPG-coated erythrocytes caused an increased in intracellular calcium levels, possibly a consequence of calcium binding by LPG [64]. Indeed, NMR studies revealed that calcium binds to the LPG repeating units in the vicinity of the phosphate groups without perturbing the tridimensional structure of the glycan [65]. Inasmuch as calcium plays an important role in the regulation of various cellular functions, its chelation by LPG may have important implications with respect to the ability of *Leishmania* parasites to survive within macrophages.

6.7. Modulation of macrophage signaling pathways

The expression of macrophage accessory and effector functions is stringently regulated by multiple intracellular signal transduction pathways. For an intracellular parasite such as *Leishmania*, impairment of host macrophage signaling pathways may represent a logical strategy to turn off key microbicidal functions. Indeed, *Leishmania*-infected macrophages display an impaired responsiveness to interferon (IFN)-γ, lipopolysaccharide and activators of protein kinase C (PKC) [66,67].

PKC was first characterized as a Ca^{2+}-dependent and phospholipid-dependent protein serine/threonine kinase that requires diacylglycerol (DAG) for activ-

ity. Subsequently, it has been established that PKC is not a single entity, but rather a family of closely related isoenzymes comprising at least 12 different members. LPG is a potent inhibitor of purified rat brain PKC activity in vitro ($K_i < 1$ μM) [68]. The inhibition is selective, since LPG displays no effect on 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 causes significant inhibition of purified PKC activity [69]. These results suggest that LPG interacts with the regulatory domain of PKC, which contains the binding sites for diacylglycerol, calcium, and phospholipids. Another interpretation of these in vitro data is that LPG perturbs the insertion of PKC within the membrane, thereby precluding its activation.

During infection, the parasite is present inside a phagosome while PKC binds to the cytoplasmic side of the plasma membrane. Despite the positions of LPG and PKC on opposite sides of the membrane, inhibition of PKC activity by LPG is still observed [70]. Moreover, while 1 or 2% LPG modestly inhibited binding of PKC to sucrose-loaded vesicles, the presence of 5% LPG completely prevented binding. A full-length LPG molecule is necessary for maximal inhibition of PKC, which may be the consequence of alterations in the physical properties of the membrane. Indeed, insertion of LPG in lipid bilayers raises the T_H (transition temperature in which a bilayer forms hexagonal phase) of the membrane and makes the rearrangement of proteins in membranes more difficult [62,70]. GIPLs, which represent the most abundant glycoconjugates of the amastigote stage, also display an inhibitory activity towards PKC [69]. The absence of repeating units suggests that GIPLs and LPG use different mechanisms to inhibit PKC activity.

By virtue of its pivotal role in transmembrane signaling, PKC modulates a wide variety of cellular functions. Consequently, treatment of macrophages with purified LPG inhibited several PKC-dependent events including induction of the respiratory burst, c-*fos* gene expression, and chemotaxis [4,66]. Thus, since macrophage-activating cytokines such as interferon-γ and TNF-α act through PKC-dependent signal transduction pathways, impairment of PKC-dependent gene expression would attenuate the impact of external activating signals and therefore be beneficial for intracellular *Leishmania*.

6.8. Modulation of nitric oxide production

Macrophages express the inducible nitric oxide synthase (iNOS) in response to various extracellular signals, including IFN-γ, and bacterial lipopolysaccharide (LPS) [71]. The iNOS enzyme is responsible for the production of nitric oxide (NO), a molecule with potent microbicidal activity, and is required for resistance to *Leishmania* infection in mice [72]. Treatment of macrophages with GIPLs inhibited synthesis of NO in a time- and dose-dependent manner [73]. Consistently, leishmanicidal activity was reduced in these GIPL-treated macrophages. While the whole LPG molecule had no effect on NO production, preincubation of macrophages with the phosphoglycan moiety (PG) of LPG potently inhibited iNOS expression [74]. Simultaneous addition of PG and interferon-γ to macrophages, however, induced leishmanicidal activity and NO secretion [74]. Thus, the production of NO, a key host defense molecule, can be modulated by *Leishmania* glycoconjugates.

6.9. Scavenging of toxic oxygen metabolites

Because LPG repeating units are highly effective in scavenging hydroxyl radicals and superoxide anions, it has been proposed that they may protect promastigotes from these toxic oxygen metabolites generated during the oxidative burst [47,75,76]. Thus, repeating disaccharide phosphate unit-containing glycoconjugates may protect *Leishmania* from the oxidative burst by at least two distinct mechanisms: (i) attenuation of the PKC-mediated induction of the burst, and (ii) scavenging of the cytocidal products of the burst.

6.10. Suppression of macrophage IL-1β expression by LPG

Among the consequences of *Leishmania* infection on macrophage function, it has been reported that agonist-induced production of IL-1, a key mediator of immunity and inflammation, is impaired [77,78]. Although the amastigotes molecule(s) responsible for

this impairment remain to be identified, evidence was provided that preincubation of macrophages with LPG potently inhibited LPS-induced IL-1 production [79]. Further analysis of this phenomenon revealed that LPG inhibits IL-1β gene expression by suppressing transcriptional activity, and involves a unique sequence within the IL-1β promoter that acts as a gene silencer. Interestingly, the whole LPG molecule is required for this inhibitory activity, which is agonist-specific [80]. Suppression of transcriptional activity by LPG may represent the first example of a pathogen-derived molecule that mediates its action by a unique promoter sequence acting as a gene silencer [80].

6.11. LPG in HIV-1 pathogenesis

Epidemiological studies indicate that *Leishmania* is an opportunistic pathogen in immunocompromised, HIV-1-infected individuals [81,82]. The observation that incubation of a human T cell line with purified LPG induced HIV-1 LTR activity led to the suggestion that *Leishmania* infection may contribute to the pathogenesis of HIV infection [83]. On the other hand, it has been demonstrated that LPG, a potent inhibitor of viral membrane fusion [62], inhibited in a dose-dependent manner HIV-1-induced syncytia formation in $CD4^+$ T cells infected with syncytia-inducing isolates of HIV-1, as well as viral replication in $CD4^+$ T cells [84]. Thus, to reconcile these apparently contradictory data, it can be envisioned that while LPG may induce HIV-1 LTR activity, it may also inhibit the subsequent steps leading to the spread of the virus. Clearly, additional studies will be necessary to determine to which extent LPG plays a role in the pathogenesis of HIV-1 in *Leishmania*-infected individuals.

6.12. Concluding remark

The remarkably large number of functions that have been proposed for LPG and related glycoconjugates is truly astounding. These functions can be attributed to the uniqueness of the distinct carbohydrate and lipid domains of these glycoconjugates. Since many of these proposed functions contribute to the *Leishmania* pathogenesis, LPG and related glycoconjugates may prove ultimately to be ideal targets of chemotherapeutic intervention.

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