LEISHMANIA AND THE LEISHMANIASES

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Lipophosphoglycan of the protozoan parasite Leishmania: stage- and species-specific importance for colonization of the sandfly vector, transmission and virulence to mammals

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Abstract Leishmaniasis is a major health problem to the human population of the tropics, subtropics and Mediterranean regions. This disease is caused by the parasitic protozoa Leishmania, which have adapted to survive in several hostile environments such as the vector insect midgut, blood and the mammalian macrophage phagolysosome. Several Leishmania glycoconjugates have been implicated as key molecules for these remarkable capabilities. This review summarizes the current knowledge on potential and proven functions of the most prominent of the Leishmania glycoconjugates, the lipophosphoglycan.

Introduction

Leishmania are protozoan parasites that are the causative agents of a variety of human and animal diseases called Leishmaniasis, which are divided clinically in cutaneous, diffuse cutaneous, mucocutaneous or visceral forms. Leishmaniasis occur worldwide in 88 countries, especially in the tropics, the subtropics and the Mediterranean regions, where they affect 12 million and threaten more than 350 million people [1]. Leishmania have a digenetic life cycle that alternates between the colonization of an insect vector by extracellular, flagellated and motile promastigotes, and of a mammalian host by obligatory intracellular, nonflagellated amastigotes (Fig. 1) [2]. The natural vectors of all Leishmania are about 30 species of phlebotomine sandflies. After a sandfly bites a Leishmania-infected mammal, amastigotes in the ingested blood transform and develop to various forms of free-swimming and attached promastigotes, culminating in the highly motile, non-dividing metacyclic promastigotes, which are mainly present in the foregut (Fig. 1A) [3]. This latter stage is transmitted into the skin of a mammalian host during the bite of an infected sandfly (Fig. 1B) and is pre-adapted for this radical change of environment [4]. In the mammalian skin, the metacyclic promastigotes bind to receptors on the surface of macrophages and are subsequently taken up by phagocytosis. Inside the host cell, the promastigotes rapidly transform within 12–24 h to amastigotes (Fig. 1C), while the initial phagosome matures to a phagolysosome by acidification and acquisition of lysosomal hydrolases. Within this hostile environment the amastigotes multiply and, eventually, the host cell lyses by an unknown mechanism (Fig. 1D). The released amastigotes spread by invasion and colonization of other macrophages. A key question in the biology of Leishmania is how these parasites survive and thrive in several different hostile environments like the sandfly digestive tract, human blood and tissue fluid, and the macrophage phagolysosome. A large body of evidence suggests that several types of glycoconjugates are key molecules for these unique capabilities of Leishmania parasites [5]. The best-studied Leishmania glycoconjugate to date is lipophosphoglycan (LPG), and a large number of potential functions in the sandfly vector, during transmission and for disease formation in mammalian hosts including humans have been described for this parasite glycolipid. These potential functions of LPG are the topic of this paper.

LPG structure

LPG is a complex cell surface glycolipid first described in 1984 [6, 7] and appears to be synthesized by promastigotes of all Leishmania species investigated to date. The structure of LPG from five different species has been elucidated, including life stage- and strain-specific modifications [8, 9]. The glycolipid anchor of LPG consists of 2-lyso-1-alkylphosphatidylmyo-inositol that
Fig. 1A–D Scheme of the developmental stages of *Leishmania*. (A) In the abdominal midgut of a *Leishmania*-infected sandfly, many nectomonad promastigotes are attached via their flagella to epithelial microvilli. Metacyclic promastigotes detach from the gut wall and migrate to the foregut [10]. (B) During blood feeding of a *Leishmania*-infected sandfly, few (<100) metacyclic promastigotes are injected into a pool of blood within the mammalian skin [10]. (C) Metacyclic promastigotes bind to skin macrophages, are taken up into a phagolysosome and transform to amastigotes. (D) Amastigotes multiply within the macrophage phagolysosome, are released from ruptured host cells and spread the infection by invading other macrophages. LPG is crucial for the processes depicted in (A) and probably for those shown in (B). For *L. mexicana* promastigotes, LPG expression is dispensable for binding and uptake by macrophages, intracellular survival and transformation into amastigotes, as depicted in (C) [17], while *L. major* promastigotes lacking LPG are less successful than LPG-expressing forms in surviving these processes [16]. Neither *L. mexicana* nor *L. major* amastigotes require the capacity for LPG expression to infect and multiply within mammalian macrophages as depicted in (D) and thereby causing disease [16, 17] (*N* nucleus)

is linked to a diphosphoheptasaccharide core structure. The terminal z-Gal of the LPG core is linked via a phosphodiester to a linear phosphoglycan (PG) chain consisting of repetitive (up to 40) phosphodiester-linked PO₄-6Gal/β1-4Man units that terminates with neutral cap oligosaccharides (Fig. 2). While these basic structural elements are conserved in all *Leishmania* LPGs, species- and stage-specific variations do occur as subtle differences in (1) the cap structures, (2) the number of repeat units per LPG molecule, and (3) the nature and frequency of side chain modifications on the basic PO₄-6Gal/β1-4Man repeat units (Fig. 2). LPG covers the entire promastigote surface where it may form a shielding glycoalyx. Continuous release from the parasite cell surface leads to very fast turnover (<12 h) of this molecule [8].

**LPG functions in the sandfly**

While residing in the sandfly’s digestive tract, the LPG glycoalyx may protect the *Leishmania* promastigotes against hydrolactic enzymes. Furthermore, it has been shown that the stage-specific LPG of nectomonad promastigotes serves as a ligand for the attachment to the sandfly midgut lining (Fig. 1A). In *Phlebotomus papatasi*, which is only permissive for *L. major*, species-specific LPG modifications are required for vector competence of the parasites. This effect is mediated by specific midgut receptors recognizing preferentially the galactose-rich side chains of *L. major* nectomonad LPG [10]. Similar observations in other natural *Leishmania* sandfly vector combinations provide further evidence for the hypothesis that LPG structure polymorphisms between *Leishmania* species may be adaptations to midgut lectins of their respective vectors [11]. While these earlier studies provided circumstantial evidence for the importance of LPG in the sandfly, a recent report demonstrated directly and unequivocally, by a gene deletion approach, that LPG is essential for the parasites to successfully colonize the insect vector: while *L. major* promastigotes specifically defective in LPG synthesis were able to initiate an infection in *Phlebotomus*...