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Leishmania braziliensis: a novel mechanism in the lipophosphoglycan regulation during metacyclogenesis

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Abstract

During metacyclogenesis of *Leishmania* in its sand fly vector, the parasite differentiates from a noninfective, procyclic form to an infective, metacyclic form, a process characterised by morphological changes of the parasite and also biochemical transformations in its major surface lipophosphoglycan (LPG). This lipid-anchored polysaccharide is polymorphic among species with variations in sugars that branch off the conserved Gal(β1,4)Man(α1)-PO_4 backbone of repeat units and the oligosaccharide cap. Lipophosphoglycan has been implicated as an adhesion molecule that mediates the interaction with the midgut epithelium of the sand fly in the subgenus *Leishmania*. This paper describes the LPG structure for the first time in a species from the subgenus *Viannia*, *Leishmania (Viannia) braziliensis*. The LPG from the procyclic form of *L. braziliensis* was found to lack side chain sugar substitutions. In contrast to other species from the subgenus *Leishmania*, metacyclic forms of *L. braziliensis* makes less LPG and add 1–2 (β1–3) glucose residues that branch off the disaccharide-phosphate repeat units of LPG. Thus, this represents a novel mechanism in the regulation of LPG structure during metacyclogenesis.

Keywords: Lipophosphoglycan; *Leishmania braziliensis*; Metacyclogenesis; Leishmaniasis

1. Introduction

Leishmania braziliensis (Vianna, 1911) is the etiological agent of the muco-cutaneous form of leishmaniasis in the New World, which causes facial disfigurement with high morbidity. The disease is very widespread, extending south from Belize and Panama throughout the forested parts of South America (Molyneux and Ashford, 1983). In general, the biological cycle of *Leishmania* parasites alternates between an aflagellated amastigote stage in the mammalian macrophage and a flagellated promastigote stage in the sand fly midgut. In the case of the members of the subgenus *Viannia*, such as *Leishmania (Viannia) braziliensis* (Rangel et al., 1992) and *Leishmania (Viannia) panamensis* (Walters et al., 1989), parasites are also seen attached to the hindgut lining by flagellar hemidesmosomes with later migration of flagellates to the midgut and foregut. In contrast, species from the subgenus *Leishmania* complete their life cycle in the midgut and foregut of the sand fly vector. Those behavioral differences resulted in the division of the genus *Leishmania* in two subgenera *Viannia* and *Leishmania* by Lainson and Shaw (1987).

Many aspects of the sand fly–parasite interactions have been carried out in the subgenus *Leishmania*, including Old World species such as *Leishmania major*, *Leishmania donovani* (from Sudan and India), *Leishmania tropica*, and *Leishmania infantum* (syn. *chagasi*). One important molecule involved in this interaction is dominant surface lipophosphoglycan (LPG) which has been biochemically characterised in all of the species mentioned above (Mahoney et al., 1999; McConville et al., 1992, 1995; Sacks et al., 1995; Soares et al., 2002). Attachment of the parasite to a microvillus receptor is a critical event for *Leishmania* survival. For instance, recognition of binding sites in the epithelium by the LPG is a crucial step preventing

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loss of the parasite during the excretion of the digested blood meal (Pimenta et al., 1992). LPG variations have also been implicated in the specificity of various *Leishmania* to different *Phlebotomus* species and thus promote vectorial competence to the invertebrate hosts (Kamhawi et al., 2000; Pimenta et al., 1994; Sacks et al., 1995).

Basically, all LPGs have a conserved glycan core region of galactose(α1,6)galactose(α1,3)galactofuranose(β1,3)[glucose(α1)-PO_4]mannose(α1,3)mannose(α1,4)*N*-acetylglucosamine(α1) linked to a 1-*O*-alkyl-2-*lyso*-phosphatidylinositol anchor. The distinguishing feature of LPGs, responsible for the polymorphisms among *Leishmania* species, is in the variability of sugar composition and sequence of branching sugars attached to the conserved repeat unit Gal(β1,4)Man(α1)-PO_4 backbone and in the cap structure (Turco and Descoteaux, 1992). The C3 hydroxyl of the repeat unit galactose is the site of most side chain modifications. For example, the LPG of *L. infantum* and *Leishmania mexicana* has β(1,3)glucose in the repeat units, the *L. major* LPG has β(1,3)galactose and is often terminated with β(1,2)arabinose, and the *L. tropica* LPG has the most complex side chain modifications with over 19 different types of glycans (Ilg et al., 1992; McConville et al., 1992; Soares et al., 2002, 2004). In the subgenus *Viannia*, preliminary observations on the repeat units of the LPG of *L. braziliensis*, *L. panamensis* and *Leishmania guyanensis* were reported (Muskus et al., 1997). In this paper, a more detailed structure of *L. braziliensis* procyclic and metacyclic LPGs is described with a distinguished feature in the regulation of side-chains during metacyclogenesis.

2. Materials and methods

2.1. Materials

Materials were obtained as follows: Medium 199 from Gibco Life Technologies; Bacto-Brain Heart Infusion from Difco Laboratories (Mauston, WI); FCS from Atlanta Biologicals; AG50W-X12 cation-exchange resin, AG1-X8 anion-exchange resin from Bio-Rad (Hercules, CA); phenyl-Sepharose CL-4B, octyl-sepharose, adenosine, fast garnet GBC, α-naphthyl phosphate, PI-specific phospholipase C from *Bacillus cereus*, alkaline phosphatase (*Escherichia coli*), β-glucosidase (sweet almond), β-galactosidase (*E. coli*, positionally specific for β1,4 linkages), and α-mannosidase (jack bean) from Sigma; peanut agglutinin (PNA) from E Y Laboratories, Inc. (San Mateo, CA) and D-[6-^{3}H]galactose (1 mCi/ml) from American Radiolabeled Chemicals (St Louis, MO).

2.2. Parasites

Leishmania braziliensis World Health Organisation reference strain (MHOM/BR/75/M2903) was used. *Leishmania infantum* (syn. *chagasi*) (MHOM/BR/74/PP75), *L. donovani* Sudan strain (MHOM/SD/00/1S-2D) and Indian strain (MHOM/IN/83/Mongi-142) were used as controls. Identity of the strains was established by polymerase chain reaction amplification of mini-exon intragenic regions (Fernandes et al., 1994). Starter cultures of promastigotes were grown in Medium 199 supplemented with 10% heat-inactivated FBS, penicillin (100 units/ml), streptomycin (50 μg/ml), 12.5 mM glutamine, 0.1 M adenine, 0.0005% hemin, and 40 mM Hepes, pH 7.4 at 25 °C. For isolation and purification of large amounts of LPG, 1 l cultures of Brain Heart Infusion supplemented with adenosine (27 mg/l) and hemin (5 mg/l) were seeded with 100 ml of starter cultures. Cells were grown at room temperature in an incubator shaker to a density of 1–1.2 × 10^7 cells/ml (Orlandi and Turco, 1987).

2.3. Purification of PNA negative cells

Procyclic forms of *L. braziliensis* were grown in Schneider's media with a low pH 6.5 according to Zakai et al. (1998). Parasites from stationary phase (1.0 × 10^7 cells/ml) were harvested and resuspended in Medium 199 containing PNA (*Arachis hypogaea*) at a final concentration of 35 μg/ml. This lectin recognises terminal βGal and was used for purification of metacyclic promastigotes by negative selection (Sacks and Silva, 1987). After a 30 min incubation at room temperature, agglutinated parasites (PNA+) were removed by low speed centrifugation (150 × *g*, 5 min), and metacyclic cells remaining in the supernatant (PNA−) were washed two times by centrifugation with 5 ml of phosphate-buffered saline (PBS) at 2100 × *g* for 15 min at 4 °C.

2.4. Scanning electron microscopy

Procyclic and metacyclic (PNA−) parasites were harvested by centrifugation at 4000 × *g* for 10 min at 4 °C. Cell pellets were resuspended in ice-cold PBS, pH 7.2, and centrifuged again. Washed parasites were resuspended and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, containing 0.146 M sucrose and 5 mM $CaCl_2$ for 1 h at room temperature. Parasites were allowed to adhere on cover slips previously coated with 0.1% aqueous poly-L-lysine (Sigma) for 30 min at 37 °C. Subsequently, cover slips were washed with PBS, dehydrated through an ascending acetone series and two changes of absolute acetone. Samples were critically point dried using liquid CO_2 in EMITECH K850 apparatus and coated with gold particles in an EMITECH K550. Samples were observed and image photographed using a JEOL JSM-5600 scanning electron microscope.

2.5. Metabolic labeling of LPG

Promastigotes (log, stationary and PNA−) of *L. braziliensis* were harvested and radiolabeled at