Targeted Gene Deletion of *Leishmania major* UDP-galactopyranose Mutase Leads to Attenuated Virulence*

Received for publication, January 2, 2007, and in revised form, February 6, 2007 Published, JBC Papers in Press, February 6, 2007 DOI 10.1074/jbc.M700023200

Barbara Kleczka1, Anne-Christin Lamerz1, Ger van Zandbergen5, Alexander Wenzel1, Rita Gerardy-Schah2, Martin Wiese4, and Françoise H. Routier1‡

From the 1Medizinische Hochschule Hannover, Carl-Neuberg Strasse 1, 30625 Hannover, Germany, 5Institute for Medical Microbiology and Hygiene, Ratzeburger Allee 160, 23538 Luebeck, Germany, and 2Bernhard Nocht Institute for Tropical Medicine, Bernhard Nocht Strasse 74, 20359 Hamburg, Germany

Protozoan parasites of the genus *Leishmania* are the etiologic agents of leishmaniasis, a widespread group of diseases that affect millions of people. These diseases encountered in tropical and subtropical areas of the world range from self-healing cutaneous leishmaniasis to lethal visceral leishmaniasis. Throughout the world, pentavalent antimonials have been the treatment of choice for more than 50 years. However, increasing drug resistance as well as the high cost and toxicity of these drugs considerably limit their use. Nowadays, other medications such as amphotericin B, liposomal amphotericin B, pentamidine, or miltefosine offer an alternative for treatment, but like antimonials they are toxic and/or expensive. In addition, the emergence of resistant strains, which already seriously compromise the efficacy of pentamidine, is also expected with the latest antileishmanial drug miltefosine because of its long half-life (1). Efforts to develop new effective treatments have thus to be pursued.

The promastigote form of *Leishmania* parasites transmitted to human and other mammalian reservoirs by a sandfly bite is coated by molecules of the glycosylphosphatidylinositol (GPI) family comprising lipophosphoglycan (LPG), GPI-anchored proteins such as membrane proteophosphoglycans (mPPG) or the metalloprotease gp63 and a heterogeneous group of glycosinositolphospholipids (GIPLs) (2). Once in the mammalian host, the promastigotes enter hematopoietic cells of the monocyte/macrophage lineage by phagocytosis (3) and differentiate into amastigotes responsible for disease propagation. This latter form is covered by a thinner glyocalyx mainly composed of GIPLs and glycosphingolipids acquired from the host (4, 5) because both LPG and GPI-anchored proteins are strongly down-regulated at this stage.

As the major macromolecule present on the promastigote, LPG has received much attention and its contribution to *Leishmania* pathogenesis has been critically defined. LPG is essential for the binding and detachment of parasites to the midgut of the insect vector and therefore for the transmission of the parasites to the mammalian host (6). It also protects the parasite from hydrolytic enzymes, oxidants, and human complement (7). LPG is hence crucial for *Leishmania major* virulence as demonstrated using a mutant exclusively deficient in LPG obtained by targeted gene replacement of the putative galactofuranosyltransferase *LPG1* involved in the anchor synthesis (8). Interestingly, LPG and some other related glycoconjugates are dispensable for *L. mexicana* virulence that seems to have evolved different mechanisms of host cell manipulation (9–13).

In contrast, the role of GIPLs is still controversial. GIPLs are the predominant glycoconjugates of the intracellular amastigote stage and might thus play important roles in macrophage invasion and parasite survival within phagocytes. Although several studies support such roles (14–18), the decisive contribution of GIPLs in these processes was recently called into question by a *L. major* mutant deficient in all ether lipids including LPG and GIPLs (19).

Interestingly, LPG membrane anchor and GIPLs of *L. major* are structurally related molecules that contain notably a galactofuranose residue (Galp) (2, 20). This uncommon monosaccharide is highly immunogenic and present in the sur-

*The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed. Tel: 49-511-5329807; Fax: 49-511-5323956; E-mail: Routier.Francoise@mh-hannover.de.

2 The abbreviations used are: GPI, glycosylphosphatidylinositol; PI, phosphatidylinositol; Galp, galactopyranose; Galf, galactofuranose; GPI, glycosinositolphospholipid; LPG, lipophosphoglycan; PPg, proteophosphoglycan; UGM, UDP-galactopyranose mutase; SAP, secreted acid phosphatase; GFP, green fluorescent protein; PBS, phosphate-buffered saline.
face glycoconjugates of many pathogenic bacteria, fungi, and protozoan parasites (21). It originates from the action of the UDP-galactopyranose mutase (UGM) that catalyzes the interconversion of UDP-galactopyranose (UDP-Galp) into UDP-galactofuranose (UDP-Galf) (22–26). Because Galp is essential for the survival or virulence of various pathogenic bacteria (27) but is absent from higher eukaryotes, UGM is a recognized drug target and has been extensively studied in prokaryotes (28–33). Interestingly, the phylogenetic distribution of the GLF gene (encoding UGM) suggests that Galp is more widespread in lower eukaryotes than previously thought. Nevertheless its role in lower eukaryotes is still undefined.

In this study we address the role of Galp in L. major pathogenesis by targeted replacement of GLF. Absence of UGM is expected to affect not only the synthesis of the virulence factor LPG but also that of GPIs. Moreover, the L. major genome encodes at least six putative galactofuranosyltransferases homologous to LPG1 whose functions are still undefined (34). Therefore, targeting a central enzyme of Galp metabolism such as UGM rather than individual galactofuranosyltransferases could have a more pronounced effect on Leishmania pathogenesis.

**EXPERIMENTAL PROCEDURES**

**Materials**—Vectors pXG(NEO) (B1288), pXG-GFP+2 (B2952), pXG-GFP+ (B2799), and pXG-LmexSAP1 (B3092) were kind gifts from Stephen M. Beverley (St. Louis, MO). Antibody gp63–235 (35) was generously provided by W. Robert McMaster (Vancouver, Canada).

**Parasite Culture**—Promastigotes of L. major MHOM/SU/73/5ASKH were grown at 27 °C in M199 medium supplemented with 10% fetal bovine serum, 40 mM Hepes, pH 7.5, 0.1 mM adenine, 0.0005% hemin, 0.0002% biotin, and 50 units/ml penicillin. As needed, G418, hygromycin B, and phleomycin were added at 30, 50, and 5 μg/ml, respectively. Selection of individual clones after transfection was done on semi-solid M199 medium containing 1% agar.

**Targeted Gene Replacement of L. major GLF**—A 1.5-kb region directly upstream of the start codon of GLF and a 1.5-kb region directly downstream of the stop codon were amplified by PCR from L. major genomic DNA using the primer pairs CATCGGAAACGCAGGACACACACGGCAGCAAATCCATG (in the sense orientation into the BamHI site of pXG-GFP) and CTGCTTGCACTGCTGCGTGTG (in the final constructs). The latter was made by amplification of GLF with the primers ATATCCCGGGATGAGCGCTGACAAGGTGGTCATA and ATATCCCGGGATGAGCGCTGACAAGGTGGTCATA and subsequently introducing the start and stop codons of the antibiotic resistance genes replace those of GLF in the final constructs. GLF::HYG and GLF::PHLEO were digested with XhoI and SacI and the desired fragments purified from agarose gel and precipitated by ethanol. The heterozygous mutant ∆glf::PHLEO/GLF was first obtained by electroporation of 2 μg of GLF::PHLEO fragment in L. major as described previously (36). A second targeting round with the GLF::HYG fragment resulted in ∆glf::HYG/∆glf::PHLEO, referred to as *glf* mutant.

GLF expression was restored in several lines of the transfection with 10 μg of pXG-GLF construct. The latter was made by amplification of GLF with the primers GTCTGGATCCAGCGCTGACAAGGTGGTC and GCCGTGGAATCCTICTACGAGCCGTCGACGAC and insertion in the Smal and BamHI sites of pXG(NEO) (37). For reason of simplicity, the transfectants ∆glf::HYG/∆glf::PHLEO [pXG GLF] are designated ∆*glf*+/+ GLF.

**Subcellular Localization of L. major UGM**—To generate an N-terminal GFP-tagged UGM, the full-length open reading frame lacking the start codon was amplified using the primers GTCTGGATCCAGCGCTGACAAGGTGGTC and GCCGTGGAATCCTICTACGAGCCGTCGACGAC and insertion in the sense orientation into the BamHI site of pXG-GLF+2 (37). 10 μg of the resulting plasmid were transfected in wild-type L. major and in the *glf* mutant. Cells were immobilized to poly-L-lysine-coated coverslips and fixed in 4% paraformaldehyde in PBS for 15 min at room temperature. 4′,6-Diamidino-2-phenylindole staining (8 μg/ml in PBS) was performed to visualize the nucleus and kinetoplast. GFP encoded by pXG-GFP+ was used as control.

**Western Blot Analysis**—Total cell lysates of Log phase promastigotes were separated by SDS-PAGE and transferred to nitrocellulose membranes (Whatman Schleicher & Schuell). The monoclonal antibody WIC79.3 (38) and a secondary anti-mouse antibody coupled to horseradish peroxidase (Dianova) were used at a dilution of 1:4,000 and 1:50,000, respectively. Monoclonal antibody gp63–235 was used at a dilution of 1:50 of a hybridoma culture supernatant. Detection was performed with an enhanced chemiluminescence system (Pierce) followed by autoradiography.

**Immunofluorescence Microscopy—Leishmania** wild-type parasites, *glf*− and *glf*+/+ GLF promastigotes were washed in PBS, immobilized onto poly-L-lysine-coated coverslips, and fixed in 2% paraformaldehyde for 15 min at room temperature. Cells were washed and incubated with 50 mM NH₄Cl for 15 min and eventually permeabilized with 0.1% saponin. Preincubation, antibody incubation, and washes were conducted in PBS buffer containing 2% bovine serum albumin. The monoclonal antibody WIC79.3 and the secondary antibody goat anti-mouse

---

3 M. Wiese, unpublished data.

---

*Leishmania major UDP-galactopyranose Mutase*
Leishmania major UDP-galactopyranose Mutase

Cy3 (Sigma) were used at a dilution of 1:1000 and 1:500, respectively. Monoclonal antibody gp63–235 was used at a dilution of 1:10 of a hybridoma culture supernatant. Cells were stained with 8 μg/ml 4′,6-diamidino-2-phenylindole for 30 min in the dark before embedding in Mowiol and inspection by fluorescence microscopy.

Purification and Analysis of PPGs—PPGs were partially purified from cell lysates of stationary phase promastigotes by Triton-X114 partitioning and analyzed by Western blotting with WIC79.3 at a 1:4000 dilution. L. mexicana secreted acid phosphatase (SAP) was heterologously expressed in the different cell lines by transfection with pXG-LmexSAP1 and immunoprecipitated with the anti-SAP monoclonal antibody LT8.2. Phosphoglycosylation was analyzed by Western blot analysis with WIC79.3 and LT8.2 using the SuperSignal West Femto ECL substrate (Pierce).

Purification and Analysis of GIPLs—A pellet of 4 × 10⁶ parasites washed with PBS was disrupted by 5 × 30 s of sonication in 500 μl of chloroform/methanol/water (1:2:0.8) and centrifuged at 11,000 × g for 10 min. The supernatants obtained from two successive extractions were dried under a nitrogen stream, resuspended in 1.5 ml of H₂O, and applied to a C₁₈/SepPak® Plus column (Waters) pre-equilibrated with 5 ml of methanol and 20 ml of H₂O. After washing the column with 10 ml of H₂O, GIPLs were eluted in 1.5 ml of methanol, dried under a nitrogen stream, and dissolved in 200 μl of chloroform/methanol/water (3:6:0.8). For mass spectrometry measurement, 1 μl of 6-aza-2-thiothymine matrix (5 μg/μl), 5 μl of sample, and 1 μl of matrix were successively deposited on a metal target plate under a hot air stream. Mass spectra were recorded on a Bruker ULTRAFLEX™ matrix-assisted laser desorption ionization time-of-flight/time-of-flight spectrometer in the negative mode. The instrument was used at a maximum accelerating potential of 20 kV.

Mouse Infection Studies—Parasites were first passed through BALB/c mice (Charles River) and maintained for less than three passages in vitro. Then 2 × 10⁶ stationary phase promastigotes were injected subcutaneously into the footpad of BALB/c mice (5 mice/group), and lesions were monitored by measuring the thickness of the infected footpad with a Vernier caliper and comparing it to the non-infected footpad. Mice were sacrificed when necrosis appeared in the group. Lesion-derived parasites were enumerated by limiting dilution assay (39). Amastigotes recovered from infected animals were differentiated into promastigotes, and LPG expression was checked by immunofluorescence as described above.

RESULTS

L. major UGM Is Localized in the Cytoplasm—The protein UGM encoded by GLF was described previously (22, 23). There is no signal in the polypeptide sequence of UGM typical of membrane association or anchorage suggesting that this protein is soluble. However, there is precedent in trypanosomatids and other organisms for the compartmentalization of proteins involved in nucleotide sugar interconversion (40–42). The cellular localization of UGM was thus examined using episcopal expression of N-terminal GFP-tagged protein (GFP::GLF) in wild type L. major and glf– mutant (see below). Fluorescence microscopy showed that the GFP-tagged UGM is distributed throughout the whole cell within L. major wild-type (Fig. 1), supporting the cytoplasmic location of UGM. The cytoplasmic localization is consistent with the absence of peroxisomal targeting sequence type 1 or type 2 in UGM amino acid sequence. As a control, LPG that is absent from the glf– mutant was re-expressed after transfection, demonstrating that the GFP-tagged protein is enzymatically active (data not shown).

Generation of L. major glf Null Mutant—Southern blot analysis indicated that GLF is a single copy gene (data not shown), which was confirmed by targeted gene replacement. Moreover, L. major genome sequencing was recently completed (43) and revealed a single homologue of GLF present on chromosome 18. Because there is evidence that gene expression in Leishmania is directed by sequences present in the 3′- and 5′-flanking regions, the targeting constructs were prepared by homologous recombination to allow a precise replacement of GLF by the antibiotic resistance genes and thus ensure correct expression of these markers. Two successive rounds of gene replacement were required to obtain a glf null mutant (Δglf::HYG/Δglf::BLE) because Leishmania has a diploid genome. Southern blots of AccI-digested genomic DNA from wild-type, single, and double targeted parasites were hybridized with a digoxigenin-labeled GLF probe (Fig. 2). Bands corresponding to the predicted size of AccI fragments were observed in wild-type and GLF::BLE heterozygous mutant. In contrast, no hybridization was observed in the glf– mutant, confirming successful deletion of both GLF alleles. Integration of the BLE and HYG markers at the correct locus was confirmed by an additional Southern blot hybridized with a digoxigenin-labeled probe situated in the GLF 5′-flanking region outside the region used for homologous recombination (Fig. 2). Further Southern blots using digoxigenin-labeled probes specific for BLE and HYG excluded random integration of the markers (data not shown).

All glf– clones appeared morphologically normal and grew in culture as well as the parental wild-type strain. As a control for subsequent analysis, several lines of glf– mutant were transfected with an GLF expression plasmid, yielding transfectants designated glf–/+GLF (Δglf::HYG/Δglf::BLE [pXG-GLF]). In all analyses, sibling clonal lines behaved similarly, and thus results from a single glf– and glf–/+GLF mutant will be shown.

The glf– Mutant Lacks LPG—Presence of phosphoglycans in L. major wild-type, glf–, and glf–/+GLF mutants was first analyzed by immunofluorescence microscopy using the monoclonal antibody WIC79.3. This antibody specifically recognizes the galactose-substituted repeat units present in L. major LPG and PPGs and strongly labels the cell surface of wild-type and glf–/+GLF parasites. In contrast, no fluorescent signal was discernable at the cell surface of the glf– mutant, indicating the...
absence of phosphoglycans (Fig. 3A). Lack of LPG was confirmed by Western blotting of total cell extracts labeled with WIC79.3. A characteristic smear of LPG migrating around 30–80 kDa is observed with wild-type *L. major* but not with the glf− mutant (Fig. 3B). As expected, re-expression of UGM restores LPG synthesis (Fig. 3).

The **glf**− mutant lacks LPG. A, indirect immunofluorescence microscopy of fixed permeabilized promastigotes with the monoclonal antibody WIC79.3. B, Western blot of wild-type, glf−, and glf+/+GLF cell extracts detected with WIC79.3 and ECL.

**Phosphoglycosylation of Reporter Secreted Acid Phosphatase** —Secreted and cell-associated PPGs were partially purified from culture supernatants and cell extracts by Triton X114 partitioning and analyzed by Western blotting with WIC79.3. Surprisingly, only very low levels of PPGs were detectable in the fractions obtained from *L. major* MHOM/SU/73/5ASKH and derivative cell lines. In contrast, PPGs were clearly visible in the stacking gel in control experiments with *L. major* strain MHOM/IL/80/Friedlin (data not shown). These results suggest the low abundance of PPGs in *L. major* MHOM/SU/73/5ASKH and are consistent with the absence of immunolabeling of permeabilized glf− parasites with WIC79.3 (Fig. 3).

To study phosphoglycosylation, *L. mexicana* secreted acid phosphatase (SAP) was heterologously expressed in wild-type *L. major* and the glf− mutant, immunoprecipitated with the anti-SAP monoclonal antibody LT8.2, and analyzed by Western blotting using the antibody WIC79.3. Untransfected wild-type parasites were used as control (lane 3). Equal loading of samples was confirmed by staining with LT8.2.

**Leishmania major UDP-galactopyranose Mutase**

**FIGURE 2.** Targeted gene replacement of GLF alleles. A, schematic representation of *L. major* GLF locus in wild-type and *glf*− replacement lines indicating AccI and ApaI fragments expected after hybridization with a GLF probe (upper lines) or a 5′-flanking probe (lower lines), respectively. B, Southern blot analysis of genomic DNA from wild-type (+/−), heterozygous GLF::glf::BLE mutant (+/−), and homozygous *glf*− mutant (−/−). DNA was digested with either AccI (left panel) or ApaI (right panel), separated on agarose gel, blotted onto nylon membrane, and hybridized with digoxigenin-labeled GLF (left panel) or a digoxigenin-labeled 5′-flanking probe (right panel).

**FIGURE 3.** The *glf*− mutant lacks LPG. A, indirect immunofluorescence microscopy of fixed permeabilized promastigotes with the monoclonal antibody WIC79.3. B, Western blot of wild-type, *glf*−, and *glf*+/+GLF cell extracts detected with WIC79.3 and ECL.

**FIGURE 4.** Phosphoglycosylation of reporter secreted acid phosphatase is more extensive in the *glf*− mutant. A, SAP expressed in wild-type (lane 1) and *glf*− (lane 2) parasites was immunoprecipitated with the monoclonal antibody LT8.2 and analyzed by Western blotting using the antibody WIC79.3. Untransfected wild-type parasites were used as control (lane 3). B, equal loading of samples was confirmed by staining with LT8.2.
Leishmania major UDP-galactopyranose Mutase

residues that they contain (20). Because of additional heterogeneity in the lipid part, 10 distinct glycolipids ions can be observed in the negative ion spectrum of L. major MHOM/SU/73/5ASKH GIPLs (Fig. 5). The ions at m/z 1498.8, 1526.9, 1660.9, 1688.9, 1822.9, and 1851.0 represent GIPL-1, GIPL-2, and GIPL-3 with C24:0 alkyl and C12:0 or C14:0 acyl chains. Additionally, the spectrum presents ions at m/z 1414.7, 1442.8, and 1470.8 corresponding to GIPL-1 with C18:0 alkyl and C12:0, C14:0, or C16:0 acyl chains, respectively. These assignments were confirmed by tandem mass spectrometry and are in agreement with the structures previously described in L. major V121 (20, 44). Interestingly GIPL-1 containing a sn1-alkyl-2-lyso-PI with C24:0 alkyl chain (m/z 1316.7) is also present in the wild-type spectrum. The latter was not reported in L. major V121 but seems to be present in L. major Friedlin V1 (19). The negative ion spectrum of GIPLs extracted from GLF/Δglf::BLE heterozygous mutant was identical to the wild-type spectrum (data not shown). In contrast, analysis of glf− mutant revealed the presence of truncated GIPLs (Fig. 5). As expected the biosynthesis of GIPLs seems to stop after the addition of the second mannose, giving rise to GIPLs having the basic structure Man(α1-3)Man(α1-4)GlcN-PI known as iM2. The same lipid heterogeneity as seen in wild-type GIPLs leads to the observation of six different iM2 peaks at m/z 1154.6, 1252.7, 1280.7, 1308.7, 1336.8, and 1364.8. As a result of UDP-Gal, absence, LPG biosynthesis is also prematurely stopped, leading to the accumulation of a residual LPG anchor with the structure Glcα1-HPO4-6-Man(α1-3)Man(α1-4)GlcN-srl1-alkyl-2-lyso-PI. The spectrum is therefore dominated by an ion at m/z 1418.6 corresponding to a sodium adduct ([M-2H+Na]+) of this residual LPG anchor with a C24:0 alkyl chain. The formation of a sodium adduct is enabled by the presence of two phosphate groups in this glycolipid. The molecular ion [M-H]− at m/z 1396.6 corresponds to the same molecule. Additionally, a small amount of truncated LPG anchor with a C26:0 alkyl chain can be observed (sodium adduct at m/z 1446.6). All assignments were confirmed by tandem mass spectrometry.

Interestingly, iM2 molecules with a C24:0 alkyl and a C12:0 or C14:0 acyl chain (ions at m/z 1336.8 and 1364.8) are not abundant in the glf− mutant, although these two PIs are the main lipids found in wild-type GIPL-1 (ions at m/z 1498.8 and 1526.9), GIPL-2 (ions at m/z 1660.9 and 1688.9), and GIPL-3 (ions at m/z 1822.9 and 1851.0). As remodeling of the acyl chain, but not of the alkyl chain, has previously been described (45), this suggests that these two iM2 molecules can be directed toward LPG biosynthesis by deacylation and Glc-1-P addition.

The glf− Mutant Synthesizes Normal Amount of GPI-anchored Proteins—The synthesis of the abundant GPI-anchored metalloprotease gp63 was examined by immunofluorescence and Western blot analysis with the L. major-specific monoclonal antibody gp63–235. Coomassie blue staining was used to confirm equal loading of samples. As expected, loss of GLF did not affect the expression or localization of gp63 (data not shown).

Galactofuranose Biosynthesis Is Important for Establishment of Infections in Mice—The virulence of the glf− mutant was assessed by infection of susceptible BALB/c mice. The results presented in Fig. 6 are the average of two individual experiments. When inoculated with wild-type or glf−/+ GLF−stationary phase promastigotes, mice presented lesions at the site of infection after ~3 to 4 weeks. In contrast, disease appearance was delayed to 9 weeks in mice infected with the glf− mutant. In both cases, lesions developed progressively thereafter and the lesion size correlated with parasite burden. Importantly glf−
parasites recovered from infected animals did not express any LPG, thus excluding the presence of contaminants.

DISCUSSION

In recent years, different enzymes involved in Galφ metabolism have been characterized in bacteria and revealed the crucial role of Galφ for survival or virulence of several pathogenic bacteria (e.g. Mycobacteria) (21). In contrast, the role of Galφ in eukaryotes is still undefined. In this work, the role of Galφ for L. major virulence was investigated by targeted deletion of the GLF gene encoding UGM. We and others recently identified and partially characterized this central enzyme in Galφ biosynthesis that converts UDP-Galφ into UDP-Galf (22, 23). L. major UGM was localized in the cytoplasm as predicted from the lack of polyepitope sequences typical of peroxisomal targeting, membrane association, or anchorage. Interestingly, the UDP-glucose 4′-epimerase of Trypanosoma brucei has been localized in the glycosome (42) and several other trypanosomatid enzymes involved in UDP-Gal production are predicted to be in this microbody (46). The cytoplasmic localization of UGM implies thus the existence of a UDP-Gal transporter exporting UDP-Gal from the glycosome into the cytoplasm and of a Golgi-localized UDP-Gal transporter because the galactofuranosyltransferase LPG1 is situated in this organelle (37). The cytoplasmic localization of UGM would be consistent with this hypothesis. To date it is still unclear whether different galactofuranosyltransferases are involved in the biosynthesis of GIPLsC24:0 and LPG (8) and thus whether the biosynthetic pathway of these molecules diverges after the assembly of iM2. Additionally, the accumulation of residual LPG with a Glc-1-P residue and a lyso-alkyl in the glf mutant indicate that the enzymes involved in these modifications do not require the prior addition of Galφ. As expected, GIPLsC18:0 are not processed by these enzymes either because of their specificity or because of compartmentalization of the pathway.

Using genetic inactivation of the putative galactofuranosyltransferase LPG1 that results in the exclusive loss of LPG, it was clearly established that LPG is a virulence factor in L. major (8). Thus, the reduction of L. major glf virulence observed in a mouse model was anticipated. The role of GIPLs, on the other hand, is more disputed. Unlike LPG and the major surface proteins, they are abundant in both promastigote and amastigote developmental stages. The 4 × 10^6 GIPL molecules present at the surface of each amastigote are thought to cover 45–60% of the parasite plasma membrane (48, 49) and play a protective role against the immune system of the host (50).

4. F. H. Routier and W. Vervecken, unpublished results.
role. Importantly, the terminal Gal residue present in GIPL-1 was proposed to participate in the interaction of the parasites with macrophage and their internalization (50). Gal would indeed be exposed at the apex of GIPLs and thus easily accessible (49). More recently, however, the \(ads 1^- \) mutant that is deficient in all ether phospholipids including LPG and GIPLs opppugned the importance of GIPLs for Leishmania pathogenesis (19). In agreement with this last study, we found that the complete lack of Gal, reflected by the absence of LPG backbone and truncation of the GIPLs results in a delay of disease appearance comparable with that obtained when LPG alone is deficient (8). Thus, the additional truncation of GIPLs had no profound effect on the virulence of Leishmania toward mice. Once infection has been established, disease progression in animals infected with wild-type Leishmania or \(g l f^- \) mutants is comparable, suggesting that Gal is not essential for the amastigote-macrophage interaction or the survival of amastigotes into these phagocytes. All effect observed might thus be due to the sole loss of LPG. Gal contributes significantly nevertheless to Leishmania major pathogenesis.

Acknowledgments—We thank Stephen M. Beverley for the gift of various vectors, W. Robert McMaster for providing gp63 antibody, and Anita Stolz and Andreas Pich for assistance with the mass spectrometry analyses. Helpful discussions with Werner Solbach and Tamás Laskay were greatly appreciated.

REFERENCES

1. Croft, S. L., Seifert, K., and Yardley, V. (2006) Indian J. Med. Res. 123, 399–410
2. McConville, M. J., and Ferguson, M. A. (1993) Biochem. J. 294, 305–324
3. van Zandbergen, G., Klinger, M., Mueller, A., Dannenberg, S., Gebert, A., Solbach, W., and Laskay, T. (2004) I. Immunol. 173, 6521–6525
4. McConville, M. J., and Blackwell, J. M. (1991) I. Biol. Chem. 266, 15170–15179
5. Winter, G., Fuchs, M., McConville, M. J., Sterhov, Y. D., and Overath, P. (1994) J. Cell. Sci. 107, 2471–2482
6. Sacks, D. L., Modi, G., Rowton, E., Spath, G., Epstein, L., Turco, S. J., and Beverley, S. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 406–411
7. Spath, G. F., Garraway, L. A., Turco, S. J., and Beverley, S. M. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 9536–9541
8. Spath, G. F., Epstein, L., Leader, B., Singer, S. M., Avila, H. A., Turco, S. J., and Beverley, S. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9258–9263
9. Garami, A., Mehlert, A., and Ilg, T. (2001) Mol. Cell. Biol. 21, 8168–8183
10. Hilley, J., Zawadzki, J. L., McConville M. J., Coombs, G. H., and Mottram, J. C. (2000)
11. Ilg, T. (2000) EMBO J. 19, 1953–1962
12. Ilg, T., Demar, M., and Harbecke, D. (2001) J. Biol. Chem. 276, 4988–4997
13. Turco, S. J., Spath, G. F., and Beverley, S. M. (2001) Trends Parasitol. 17, 223–226
14. Ilgotz, S. C., Zawadzki, J. L., Ralton, J. E., and McConville, M. J. (1999) EMBO J. 18, 2746–2755
15. McNeely, T. B., Rosen, G., Londen, M. V., and Turco, S. J. (1989) Biochem. J. 259, 601–604
16. Mensa-Wilmot, K., Garg, N., McGwire, B. S., Lu, H. G., Zhong, L., Armath, D. A., LeBowitz, J. H., and Chang, K. P. (1999) Mol. Biochem. Parasitol. 99, 103–116
17. Proudfoot, L., O’Donnell, C. A., and Liew, F. Y. (1995) Eur. J. Immunol. 25, 745–750
18. Tachado, S. D., Gerold, P., Schwartz, R., Novakovic, S., McConville, M., and Schofield, L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4022–4027
19. Zufferey, R., Allen, S., Barron, T., Sullivan, D. R., Denny, P. W., Almeida, I. C., Smith, D. F., Turco, S. J., Ferguson, M. A., and Beverley, S. M. (2003) J. Biol. Chem. 278, 44708–44718
20. McConville, M. J., Homans, S. W., Thomas-Oates, J. E., Dell, A., and Bacic, A. (1990) J. Biol. Chem. 265, 7385–7394
21. Pedersen, L. L., and Turco, S. J. (2003) Cell Mol. Life Sci. 60, 259–266
22. Bakker, H., Kleczka, B., Gerardy-Schahn, R., and Routier, F. H. (2005) Biol. Chem. 386, 657–661
23. Beverley, S. M., Owens, K. L., Showalter, M., Griffith, C. L., Doering, T. L., Jones, V. C., and McNeil, M. R. (2005) Eukaryot. Cell 4, 1147–1154
24. Koplin, R., Brisson, J. R., and Whitfield, C. (1997) J. Biol. Chem. 272, 4121–4128
25. Nauj, A. M., Bollinger, A., Wenzel, W., Holz, M., and Navashin, M. N. (2004) J. Biol. Chem. 279, 765–774
26. Roper, J. R., Guther, M. L., Macrae, J. I., Prescott, A. R., Hallyburton, I., Clayton, C. A., and Beverley, S. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9376–9380
27. Scherman, M. S., Besra, G. S., Duncan, K., and McNeil, M. R. (1997) Biochem. J. 326, 377–383
28. de Ibarra, A. A., Howard, J. J., and Snary, D. (1982) Parasitology 85, 523–531
29. van Zandbergen, G., Bollinger, A., Wenzel, W., Kamhawi, S., Voll, R., Muller, A., Holscher, C., Herrmann, M., Sacks, D., Solbach, W., and Laskay, T. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 13837–13842
30. Griffith, C. L., Klutts, J. S., Zhang, L., Levery, S. B., and Doering, T. L. (2004) J. Biol. Chem. 279, 51669–51676
31. Rooper, J. R., Guther, M. L., Macrae, J. I., Prescott, A. R., Hallyburton, I., Acosta-Serrano, A., and Ferguson, M. A. (2005) J. Biol. Chem. 280, 19728–19736
32. Ivens, A. C., Peacock, C. S. W., Lamb, I. A., Murphy, L., Aggarwal, G., Berriman, M., Sisk, E., Rajandream, M. A., Adlem, E., Aert, R., Anupama, A., Apostolou, Z., Attipoe, P., Bason, N., Baier, S., Beck, A., Beverley, S. M., Bistau, C., Bottger, K., Brunchi, C. V., Collins, M. D., Cid, A., Clayton, C., Coulon, R. M., Cronin, A., Cruz, A. K., Davies, R. M., De Gaudenzi, J., Dobson, D. E., Duesterhoeft, A., Fazeltine, G., Fosker, N., Frasch, A. C., Fraser, A., Fuchs, M., Gabel, C., Goble, A., Goffaux, A., Harris, D., Hertz-Fowler, C., Hilbert, H., Horn, D., Huang, Y., Klages, S., Knights, A., Kube, M., Larke, N., Litvin, L., Lord, A., Louie, T., Marr, M., Masuy, D., Matthews, K., Michaeli, S., Mottram, J. C., Muller-Auer, S., Munden, H., Nelson, S., Norbertczak, H., Oliver, K., O’neil, S., Pentony, M., Pohl, T. M., Price, C., Purnelle, B., Quail, M. A., Rabinowitz, E., Reinhardt, R., Rieger, M., Rinta, J., Robben, J., Robertson, L., Ruiz, J. C., Rutter, S., Saunders, D., Schafer, M., Schein, J., Schwartz, D. C., Seeger, K., Seyler, A., Sharp, S., Shin, H., Sivam, D., Squares, R., Squares, S., Tosato, V., Vogt, C., Volckaert, G., Wambutt, R., Warren, T., Wedler, H., Woodward, J., Zhou, S., Zimmermann, W., Smith, D. F., Blackwell, J. M., Stuart, K. D.,
Barrell, B., and Myler, P. J. (2005) Science 309, 436–442
44. Schneider, P., Schnur, L. F., Jaffe, C. L., Ferguson, M. A., and McConville, M. J. (1994) Biochem. J. 304, 603–609
45. Ralton, J. E., and McConville, M. J. (1998) J. Biol. Chem. 273, 4245–4257
46. Opperdoes, F. R., and Szikora, J. P. (2006) Mol. Biochem. Parasitol. 147, 193–206
47. Proudfoot, L., Schneider, P., Ferguson, M. A., and McConville, M. J. (1995) Biochem. J. 308, 45–55
48. Schneider, P., Rosat, J. P., Ransijn, A., Ferguson, M. A., and McConville, M. J. (1993) Biochem. J. 295, 555–564
49. Weller, C. T., McConville, M., and Homans, S. W. (1994) Biopolymers 34, 1155–1163
50. Suzuki, E., Tanaka, A. K., Toledo, M. S., Takahashi, H. K., and Straus, A. H. (2002) Infect. Immun. 70, 6592–6596

Leishmania major UDP-galactopyranose Mutase