The human pathogen *Leishmania* synthesizes phosphoglycans (PGs) formed by variably modified phosphodisaccharide \([\text{6-Gal}[\beta-1\rightarrow4\text{Man}1-PO_4]\) repeats and mannoooligosaccharide phosphate \([\text{(Man}1-2\alpha,\beta\text{Man}1-PO_4]\) caps that occur lipid-bound on lipophosphoglycan, protein-bound on proteophosphoglycans, and as an unlinked form. PG repeat synthesis has been described as essential for survival and development of *Leishmania* throughout their life cycle, including for virulence to the mammalian host. In this study, this proposal was investigated in *Leishmania mexicana* using a spontaneous mutant that was fortuitously isolated from an infected mouse, and by generating a *lmexlpg2* gene deletion mutant (\(\Delta\text{lmexlpg2}\)), that lacks a Golgi GDP-Man transporter. The spontaneous mutant lacks PG repeats but synthesizes normal levels of mannoooligosaccharide phosphate caps, whereas the \(\Delta\text{lmexlpg2}\) mutant is deficient in PG repeat synthesis and down-regulates cap expression. In contrast to expectations, both *L. mexicana* mutants not only retain their ability to bind to macrophages, but are also indistinguishable from wild type parasites with respect to colonization of and multiplication within host cells. Moreover, in mouse infection studies, the spontaneous *L. mexicana* repeat-deficient mutant and the \(\Delta\text{lmexlpg2}\) mutant showed no significant difference to a wild type strain with respect to the severity of disease caused by these parasites. Therefore, at least in *Leishmania mexicana*, PG repeat synthesis is not an absolute requirement for virulence.

*Leishmania* are protozoan human pathogens that cycle between an extracellular promastigote stage residing in the digestive tract of vector sandflies, and an intracellular amastigote stage colonizing the phagolysosomal compartment of mammalian macrophages (1). A family of unique *Leishmania* glycoconjugates, the phosphoglycans (PGs), have been implicated as virulence factors of these parasites in the mammalian host and as essential molecules for survival and development in their sandfly vector (2). The phosphoglycan family of parasite molecules is characterized by linear or branched arrays of disaccharide phosphate repeats \((6\text{-Gal}[\beta-1\rightarrow4\text{Man}1-PO_4]\) that may carry glycan side chains and often terminate at their nonreducing ends with mannose-rich oligosaccharide caps (Fig. 1A). *Leishmania* PG repeats and caps exist 1) as free secreted phosphoglycan chains (3), 2) linked to a glycolipid core on lipophosphoglycan (LPG) (4, 5), and 3) attached to a large range of secreted and cell surface-associated parasite proteins collectively called proteophosphoglycans (PPGs) (6, 7).

A series of earlier investigations have demonstrated that developmental modifications of LPG in the *Leishmania* insect (promastigote) stages are instrumental for the phenomenon of vector competence, and that they may play an important role during transmission to the mammalian host (8–12). Furthermore, a recent study has shown unequivocally an essential role for both LPG and PPGs for successful colonization of *Phlebotomus papatasi* by *Leishmania major* (13).

The notion that phosphoglycan assembly in *Leishmania* is a specialized virulence pathway (14, 15) required for infection of a mammalian host was based on a number of investigations on LPG-deficient mutants, which were shown to be unable to colonize macrophages or mammals (16–18). This lack of virulence was explained by an apparently essential role of LPG in parasite uptake by macrophages, for resistance of the parasites to toxic and lytic host influences inside and outside the macrophage, and for modulation of the host immune response (19). By contrast to these studies, recent results in our laboratory have shown that LPG is not required for successful experimental infections of macrophages or mice by *Leishmania mexicana* (7), arguing against a crucial role for this molecule in virulence to mammals. These results did not, however, preclude a role for PG assembly as a virulence pathway, as the virulent LPG-deficient *L. mexicana* strains generated in this study still contained abundant PGs linked to various PPGs (7). Furthermore, one of the avirulent LPG-deficient *L. donovani* mutants (C3PO) generated by chemical mutagenesis in earlier studies (17) proved to be not only deficient for LPG, but appeared to lack also protein-bound PG repeats on the secreted acid phosphatase (SAP) (14). A defect in the gene *lgp2* encoding a Golgi GDP-Man transporter was identified as the cause for the down-regulation of PG repeat synthesis in this mutant (14, 20). These results taken together with the identification of abundantly expressed amastigote-specific PPGs in *Leishmania*-infected tissue (21, 22) raised the possibility that, instead of LPG, PG repeat-modified PPGs may be crucial for *Leishmania* virulence in the mammalian host (6, 19).

In this study, we investigated this question more stringently in *L. mexicana* using a fortuitously isolated mutant with an unknown defect and defined mutants generated by targeted disruption of the Golgi GDP-Man transporter gene of this species. These mutants were devoid of PG repeats, but synthesized...
normal or reduced levels of manno-oligosaccharide caps, respectively. Surprisingly, despite the lack of PG repeat synthesis in promastigotes as well as amastigotes, both mutants were as efficient or more efficient than wild type *L. mexicana* with respect to binding to and uptake into macrophages, and did not show any impairment with respect to survival, transformation and multiplication within macrophages. After experimental infections of mice, lesions developed with both mutants at high and low challenge doses and disease progression was comparable to that observed in infections with wild type parasites. These results suggest that, in contrast to previous assumptions, phosphoglycan synthesis is not an absolute requirement for infectivity of *L. mexicana* to mammals.

**MATERIALS AND METHODS**

 Parasites and Experimental Infections of Mice and Cultured Peritoneal Macrophages—Promastigotes of the *Leishmania mexicana* wild type (WT) strain MNYC/BZ/62/M379 and derived gene deletion mutants were grown at 27 °C in semi-defined medium 79 (SDM) supplemented with 4% heat-inactivated fetal calf serum as described previously (23). Infection of mice with either 10^7 or 10^5 stationary phase promastigotes, binding studies on, and infection of peritoneal cells were performed as outlined previously (7).

Immunofluorescence Microscopy (IFM) and Fluorescence-activated Cell Sorting (FACS) of Leishmania Promastigotes and Infected Macrophages—IFM and FACS studies on *Leishmania* promastigotes and infected macrophages were performed as described previously using the monoclonal antibodies (mAbs) (23) LT6 (directed against [-6Galβ1-3Galα1PO4-]n, n = unknown), L7.25 and AP3 (directed against...
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[Manα1–2]hexManα1–PO₄, LT17 (most likely directed against 6αGalβ1–3Galβ1–4Manα1–PO₄, x = unknown) and mAb L3.8 directed against a polypeptide epitope of L. mexicana leishmanolysin/gp63, as well as the biotinylated lectin concanavalin A (Sigma). The mAbs were diluted 1:2–1:10 (hybridoma supernatant) or 1:500–1:2000 (ascites fluid), and the lectin was used at 10 μg/ml. Bound mAbs and the biotinylated lectin were detected by incubation with Cy3-labeled goat anti mouse IgG/IgM (Dianova, 1:500) and fluorescein isothiocyanate-biotinylated lectin were detected by incubation with digoxigenin-labeled streptavidin (Sigma, 1:250), respectively.

Cloning of the L. mexicana lpg2 Gene and Generation of Gene Knock-out Mutants—DNA techniques were performed as described previously (24). The L. donovani lpg2 gene was obtained from L. donovani 1S-2D genomic DNA by polymerase chain reaction (PCR) using the primers TCCGGATCCATGAACCATGTCAGCTGCTAG and GATCCTAGAAGCTTCTACGGATCTGCTAGTAC (14). Boldface bases indicate the restriction sites introduced by the PCR primers. The PCR product was subcloned into BamHI/HindIII-cut pQE30 (Qiagen). The digoxigenin-labeled PCR product was used to screen a λ-Dash-II library (25) and a dedicated pBSK+ (Stratagene) plasmid library of 1.5–2.5-kilobase pair as described previously (24) and the open reading frame corresponding to lmexlpg2 was identified by homology to L. donovani lpg2 DNA. Positive clones were subcloned into pBSK+ or pGEM-5z (Promega) and sequenced on an automated sequencer (Amersham Pharmacia Biotech) as described previously (24) and the open reading frame corresponding to lmexlpg2 was identified by homology to L. donovani lpg2 DNA. Double targeted replacement was performed by PCR amplification of the 5′-untranslated region (5′-UTR) of lmexlpg2 using the primers K01 (AGATCTAGAAGGTTGCGCGTGC) and K02 (AGTACTAGTAGTACGCTCAGCTCAGT) and by amplification of the 3′-UTR of lmexlpg2 using the primes KO3 (TCCGGATCCACATTGCTAGAGAAGCTG) and KO4 (GATATCCTGAGATTTGCAATGCGGTGGTGC) and by amplification of the 3′-UTR of lmexlpg2 using the primers KO5 (AGATCTAGAAGGTTGCGCGTGC) and KO6 (AGTACTAGTAGTACGCTCAGCTCAGT). The XhoI/Spel cut lmexlpg2 5′-UTR PCR DNA fragment, the BamHI/ClaI cut lmexlpg2 3′-UTR PCR DNA fragment, and a Spel/BamHI DNA fragment containing a hygromycin phosphotransferase gene (hyg) (26) were ligated consecutively into pBSK+. For the second lmexlpg2 gene replacement cassette, a Spel/BamHI fragment containing the plhemoycin-binding protein gene (phleo) was used (7). The hyg- and phleo-containing gene replacement cassettes were excised from the plasmids by XbaI/Bgl digestion and transfected into L. mexicana promastigotes as described previously (24). Selection on 96-well microtiter plates and an analysis of positive clones was performed as outlined previously (7). For gene addbac studies, the open reading frame of lmexlpg2 was amplified from a gene-containing plasmid using the primers TCCGGATCCAGAAGGUCCGCGTGC and CTCCGGATCCATGAACCATGTCAGCTGCTAGTAC. The BamHI/NcoI-cut PCR fragment was then cloned into pX (27). L. mexicana Δlpg2 promastigotes were transfected with this construct as described previously (24), and transfectants were selected by growth in SDM plus 5% heat-inactivated fetal calf serum containing 10–50 μg/ml G418 (Roche). The sequence data for the lmexlpg2-containing DNA fragment have been submitted to the EMBL data base under accession no. AJ278970.

Analytical Procedures—Production of SDS cell lysates; discontinuous SDS-polyacrylamide electrophoresis (SDS-PAGE); immunoblotting using the mAbs LT6, LT7, LT8,2 (23), and affinity-purified rabbit anti-SAP antibodies (28), as well as acid phosphatase enzyme assays (29) were performed as described previously. Total lipids from washed L. mexicana promastigotes were obtained by two extractions with chloroform/methanol/water (4:8:3). High performance thin layer chromatography (HPTLC; Silica 60, Merck, Darmstadt, Germany) of total lipids containing L. mexicana lmexlpg2 modified with phosphatidylglycerol (PG) (27) was performed as described earlier (30) using the solvent chloroform/methanol/1 M NH₄OH (60:30:3). Glycolipids on HPTLC plates were detected by visualization in 3% H₂SO₄ in acetic acid/methanol/1M NH₄OH (3:10:2).
selectively stained by orcinol/H$_2$SO$_4$. *Leishmania* promastigotes were metabolically labeled by incubating $5 \times 10^7$ cells/ml overnight at 27 °C with either 10 μCi/ml myo-[³H]inositol or 50 μCi/ml 2-[³H]o-mannose (Hartmann Analytics) in myo-inositol- or glucose/mannose-free SDM, respectively. HPTLC-separated radioactively labeled lipids were detected by spraying with En3Hance (DuPont) and fluorography. myo-[³H]inositol or 2-[³H]o-mannose-labeled delipidated cells were incubated with benzonuclease to cleave nucleic acids (7) and then separated by SDS-PAGE. Labeled compounds were detected by immersion of the polyacrylamide gel in Amplify$^{TM}$ (Amersham Pharmacia Biotech), followed by drying and fluorography.

## RESULTS

### Isolation of a PG-deficient *L. mexicana* Mutant and Targeted Gene Replacement of the *L. mexicana* lmexlpg2 Gene

— Immunoblot studies on total cell lysates using the anti-([6-Galβ1–4Manα1PO$_4$–]$_x$) repeat mAb LT6 (Fig. 1A) showed that, as in the lmexlpg1 mutant (7), LPG-like molecules were completely absent in the PE repeat-negative mutant and in the lmexlpg2 mutant. However, in contrast to the lmexlpg1 mutant, PGP-like molecules were also absent (Fig. 3A). These results were confirmed by IFM and FACS analysis, which revealed a prominent flagellar pocket and a weak cell surface signal on lmexlpg1 mutants (Figs. 4F and 5A), but no reaction on either the PE repeat-negative mutant or the lmexlpg2 mutant (Figs. 4G and H) and 5A). With the distinct antipeptide mAb LT17 (Fig. 1A), which binds preferentially to PPGs versus LPG (Fig. 3B), similar results were obtained, except for a very weak signal in immunoblots of the lmexlpg2 mutant, which was also apparent in FACS analysis (Fig. 5). The anti-mannooligosaccharide cap antibody L7.25 (Fig. 1A) recognizes LPG and a large number of phosphoglycosylated parasite PPGs (Fig. 3C, lane 1) (7, 29). In the lmexlpg1 mutant, the...
immunoblot signal of LPG is absent, as expected (7), whereas the signal of PPGs is increased (Fig. 3, B and C, lanes 2). In the PG repeat-negative mutant, L7.25 recognizes the full set of PPGs, whereas LPG is absent. Instead, in the low molecular weight region near the front of the gel, a broad band of L7.25-reactive material is visible that is absent in either wild type parasites or the \( \Delta l m e x l p g 1 \) mutant (Fig. 3C, lanes 1–3). This material is reminiscent of the LPG precursor GIPL-6 from \( L. \) major (31), which is also reactive with L7.25.² FACS analysis (Fig. 5C) and IFM (data not shown) suggests that surface L7.25 epitopes are not down-regulated in the LT6-negative mutant. Mannooligosaccharide cap synthesis is, however, down-regulated in the \( \Delta l m e x l p g 2 \) mutant, as judged by immunoblotting (Fig. 3C, lane 4), FACS analysis (Fig. 5C), and IFM (data not shown), but not to undetectable levels (Fig. 3C, lane 4). This result indicates that, in contrast to repeat synthesis, the assembly of mannoooligosaccharide caps is only partially affected by the loss of the Golgi GDP-mannose transporter. In immunoblot and FACS analysis of the LPG2-deficient \( L. \) donovani mutant C3PO (14, 17) using mAb L7.25, we obtained similar results (data not shown). Concanavalin A, a lectin directed preferentially against \( \alpha \)-mannosyl residues, bound more avidly to the surface of \( \Delta l m e x l p g 2 \) mutants than to the wild type (Fig. 5D), while its binding to PG repeat-negative or \( \Delta l m e x l p g 1 \) parasites was unchanged (data not shown). Surface expression of the glycosylphosphatidylinositol membrane-anchored cell surface metalloproteinase leishmanolysin (gp63) was not diminished in either \( \Delta l m e x l p g 1 \), the PG repeat-negative mutant or \( \Delta l m e x l p g 2 \) (Fig. 4, A–D). FACS analysis suggested an increase of surface fluorescence by a factor of 2–3 in these mutants (Fig. 5E), most likely due to better access of the mAb L3.8 to surface gp63 in the absence of LPG rather than an increase in expression of this metalloproteinase (7). Attempts to purify

² T. Ilg, unpublished results.
LPG-like molecules from promastigotes of *L. mexicana* PG repeat-negative and Δlmexlp2 mutants by a standard method (32) were unsuccessful, as described previously for *L. mexicana Δlmexlp1* (data not shown; Ref. 7). Furthermore, absence of LPG in the LT6-negative and Δlmexlp2 mutants was confirmed by [3H]inositol labelings, which also suggested that there is no increase in gp63 expression in either mutant (Fig. 6).

The modification of *L. mexicana* SAP by phosphoglycans was assessed by immunoblots of culture supernatant. Wild type parasites secrete SAP composed of the two subunits SAP1 and SAP2 (Fig. 7A, lane 1), which are modified by mannoisooligosaccharide caps (Fig. 7B, lane 1) and LT17 binding sites (Fig. 7C, lane 1). Expression of these epitopes is up-regulated in Δlmexlp1 mutants, as indicated by a stronger signal and an increase in apparent molecular mass on immunoblots (Fig. 7A, B and C, lanes 2). The PG repeat-negative mutant showed a normal level of L7.25 epitopes, but LT17 epitopes were absent (Fig. 7, A–C, lanes 4), whereas SAP of the Δlmexlp2 mutant was devoid of either binding sites. This lack of phosphoglycan caps and repeats led to a considerable decrease in apparent molecular mass (Fig. 7A, lane 2). As indicated by immunoblots of total cell lysates (Fig. 3, A–C, lanes 4) and the FACS analyses (Fig. 5, A–D), the PG-deficient phenotype of Δlmexlp2 could be reversed by episomal expression of the *lmexlp2* gene from plasmid pX.

In summary, it appears that the PG repeat-negative mutant is completely defective in phosphoglycan repeat synthesis but synthesizes normal levels of mannoisooligosaccharide caps, while Δlmexlp2 is almost completely deficient for phosphoglycan repeats and severely down-regulates mannoisooligosaccharide cap synthesis.

Recent reports suggest that the *L. mexicana* phosphoglycan synthesis pathway is distinct from glycoinositolphospholipid (GIPL) synthesis pathway (33). The GIPL profiles of *L. mexicana Δlmexlp1*, the PG repeat-negative, and the Δlmexlp2 mutant are in agreement with this view, as no gross changes in abundance and composition of GIPLs could be detected in comparison with the wild type by either isolation and chemical staining (Fig. 8A), by [3H]mannose (Fig. 8B), or by myo-[3H]inositol labeling (data not shown). Some extra glycolipid bands observed in [3H]mannose labelings of Δlmexlp1 and Δlmexlp2 may be due to accumulating LPG core intermediates.

**Fig. 8.** Silica gel 60 HPTLC analysis of the predominant *L. mexicana* promastigote glycolipids. A, total lipids from 2 × 10⁶ promastigotes were loaded onto a HPTLC plate. After chromatography, glycolipids were visualized by orcinol/H₂SO₄ spraying. B, 50,000 cpm of total lipids from [3H]mannose-labeled promastigotes were loaded onto a HPTLC plate. After chromatography, glycolipids were visualized by fluorography. The positions of the abundant *L. mexicana* GIPLs are indicated by the bars, the start and front of the TLCs are marked by S and F, respectively. Asterisks mark putative LPG core intermediates accumulating in Δlmexlp1 and Δlmexlp2 mutants. A and B, lanes 1, wild type; lanes 2, PG repeat-negative mutant; lanes 3, Δlmexlp1; lanes 4, Δlmexlp2.
phage binding experiments, Δmexlp2 mutants appeared to be more efficient in attachment than either wild type parasites or gene addback mutants (Fig. 9A). Transformation to amastigotes and intracellular survival in long term infection studies of macrophages was very similar for Δmexlp2, Δmexlp1, and PG repeat-negative L. mexicana mutants compared with wild type parasites or gene addback Δmexlp2 mutants (Fig. 9B). Intracellular multiplication of Δmexlp2, Δmexlp1, and PG repeat-negative L. mexicana mutant amastigotes appeared to be even more pronounced than that of wild type parasites or gene addback Δmexlp2 mutants (Fig. 9C). Immunofluorescence studies on infected macrophages demonstrated that, as expected, in L. mexicana Δmexlp2-parasitized macrophages PG repeats and mannooligosaccharide caps are largely absent (Fig. 10, A, B, E, F, I, and J) or wild type (C, D, G, H, K, and L) promastigotes per host cell. Infected macrophages were labeled after 3 days in culture with the mAbs LT6 (A and C), LT17 (E and G), and L7.25 (I and K). Counterstaining of the same specimen for DNA was performed with 4,6-diamidino-2-phenylindole (Dapi; B, D, F, H, J, and L). Leishmania-infected macrophages that can be recognized by the intracellular spotlike 4,6-diamidino-2-phenylindole signals of the parasite kinetoplasts are marked by arrows, whereas uninfected cells are marked by asterisks. The exposure times are identical for specimens stained with the same antibody.
The L. mexicana PG Repeat-negative and L. mexicana Δlmexlpg2 Mutants Remain Infective to Mice—PG repeat synthesis in general (15) and the Golgi GDP-mannose transporter LPG2 specifically (2, 14) have been implicated in a specialized virulence pathway of Leishmania that is thought to be required for their infectivity to mammals. To investigate these proposals more stringently, BALB/c mice were infected with wild type, Δlmexlpg2, PG repeat-negative, and, for comparison, with L. mexicana wild type PG-deficient mutant promastigotes. Mice were challenged with either 10^7 (A and B) or 10^6 (C and D) L. mexicana promastigotes in the right hind footpad. The swelling caused by L. mexicana wild type, Δlmexlpg2, and Δlmexlpg2 + pXlmexlpg2 are shown in A and C, whereas the courses of the disease elicited by Δlmexlpg1 and the PG-negative mutant compared with the same wild type curve as in A and C are shown in B and D. The infection experiments were performed in triplicate, and the standard error is indicated.
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Δlmexlpg1 L. mexicana promastigotes. Mice infected with 10^7 parasites immediately developed footpad lesions with all parasite strains, whereas a challenge dose of 10^5 parasites led to a 30–40-day delay in lesion formation (Fig. 11, A–D). Surprisingly, only minor differences in lesion development were observed between L. mexicana wild type parasites or L. mexicana Δlmexlpg2 + pΔlmexlpg2 addback mutants and PG repeat-negative or Δlmexlpg2 mutants (Fig. 11, A–D). As observed previously (7) disease progression caused by the L. mexicana Δlmexlpg1 mutant, which is selectively deficient in LPG synthesis, was faster than in all other strains and mutants investigated in this study. L. mexicana Δlmexlpg2 and PG repeat-negative parasites could be reisolated from lesion tissue, draining lymph nodes and the spleen of infected animals, suggesting that they retained the ability to metastasize in mice. Lack of PG repeat expression in reisolated promastigotes was confirmed by IFM analysis. These results indicate that, in contrast to expectation, PG repeat synthesis in L. mexicana is not required for efficient experimental infection of BALB/c mice.

DISCUSSION

Previous reports have proposed that PG repeat synthesis in Leishmania belongs to a specialized virulence pathway that is essential for survival and development of the parasites throughout their life cycle and for virulence in the mammalian host (14, 15, 19). PG repeat-modified molecules include free PG chains, lipid-containing LPG, and the protein-containing PPGs (6, 15). Although a crucial role for phosphoglycan synthesis has been unequivocally demonstrated for Leishmania promastigotes within the sandfly (13), and an important role for these molecules during transmission from the insect vector to the mammalian host appears likely (8, 34), the importance of PG-modified compounds during actual disease formation by Leishmania in the mammalian host has been questioned more recently (7). In experimental infections with L. mexicana, LPG-deficient lines are indistinguishable from wild type in their infectivity to macrophages and mice; furthermore, a recent study on L. major suggests that this is also the case for this Leishmania species, once transmission has been achieved (35). Although LPG is absent in these virulent LPG-deficient mutants, they still synthesize abundant PPGs, which raises the possibility that these molecules may be the crucial products of the proposed virulence pathway (6, 15, 19). Important arguments for this notion were 1) the discovery of an avirulent PG repeat-negative L. donovani mutant that was deficient in the Golgi GDP-Man transporter LP2 (14, 17, 20) (it should be noted, however, that recovery of virulence by reexpression of lpg2 gene in the L. donovani Δlpg2 mutant has not been tested, which raised the possibility that the loss of virulence may have been unrelated to the loss of PG synthesis); 2) the multitude of proposed biological and pharmacological activities for LPG and PG chains (19), which suggested an essential function; and 3), a range of functional studies on amastigote-expressed aPPG (6), which indicated an important role for this molecule for the colonization of mammalian tissue by Leishmania.

The data collected in this study show that, in contrast to the common expectation, at least in L. mexicana, PG repeat synthesis is not a requirement for virulence to the mammalian host. The spontaneous, undefined PG repeat-negative mutant and a defined Δlmexlpg2 mutant are as efficient in colonizing macrophages and BALB/c mice as their parental wild type strain. Both the PG repeat-negative mutant and the Δlmexlpg2 mutant lack wild type LPG and PG repeats on PPGs such as SAP, mPPG (this study), and fPPG (Ref. 6; data not shown). By contrast, mannooligosaccharide cap structures appear to be present at normal levels in the PG-negative mutant, whereas the Δlmexlpg2 mutant down-regulates the expression of such epitopes considerably on cellular proteins and to undetectable levels on secreted SAP and FPPG. This suggests that, like PG repeat synthesis, mannooligosaccharide cap synthesis occurs primarily in the Leishmania Golgi. However, the presence of some cap epitopes in Δlmexlpg2 promastigotes suggests that these are synthesized in a manner or at a location that does not require the Golgi GDP-Man transporter. By contrast, in immunofluorescence studies on Leishmania-infected macrophages, neither PG repeats nor mannooligosaccharide caps could be detected by monoclonal antibodies, which indicates that amastigotes do not even require this residual cap synthesis observed in promastigotes. The increase in concanavalin A binding on L. mexicana Δlmexlpg2 promastigotes could be due to the increased exposure of α-Man-terminating GIPLs (30) or N-glycans on the cell surface or the PG repeat-deficient parasites. This increase of potential mannose/fucose receptor binding sites may explain the more avid macrophage binding of mutant versus wild type parasites. The synthesis of the abundant cell surface GIPLs is not affected in any of the virulent PG repeat-deficient mutants, which is in agreement with a potentially essential role for these glycolipids (41). The results of this study provide also an explanation for our recent finding that deletion of the gene ppg2 (36) encoding the dominant secreted PPG of amastigotes, the aPPG (22), has only a minor impact on parasite virulence to macrophages or mice (37).2

It is possible that in other Leishmania species like L. donovani and L. major, the Golgi GDP-Man transporter LP2 or PG synthesis in general, are of greater importance to the invading promastigotes and/or the disease-causing amastigotes. Final conclusions on this topic will require studies on mutants of these organisms. In L. mexicana, amastigote-expressed aPPG has been implicated in parasite virulence to the mammalian host (42, 43). Our more recent results here argue against a central role of aPPG in the disease process caused by this Leishmania species. However, it should be kept in mind that experimental infections of mice mimic the natural situation only incompletely. It is possible that, in the natural life cycle, aPPG and other PPGs provide a subtle advantage to mammalian stage parasites that may, for instance, translate into higher transmission rates.

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