Deficiency in β1,3-Galactosyltransferase of a Leishmania major Lipophosphoglycan Mutant Adversely Influences the Leishmania-Sand Fly Interaction*

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To study the function of side chain oligosaccharides of the cell-surface lipophosphoglycan (LPG), mutagenized Leishmania major defective in side chain biosynthesis were negatively selected by agglutination with the monoclonal antibody WIC79.3, which recognizes the galactose-containing side chains of L. major LPG. One such mutant, called Spock, lacked the ability to bind significantly to midguts of the natural L. major vector, Phlebotomus papatasi, and to maintain infection in the sand fly after excretion of the digested bloodmeal. Biochemical characterization of Spock LPG revealed its structural similarity to the LPG of Leishmania donovani, a species whose inability to bind to and maintain infections in P. papatasi midguts has been strongly correlated with the expression of a surface LPG lacking galactose-terminated oligosaccharide side chains. An in vitro galactosyltransferase assay using wild-type or Spock membranes was used to determine that the defect in Spock LPG biosynthesis is a result of defective β1,3-galactosyltransferase activity as opposed to a modification of LPG, which would prevent it from serving as a competent substrate for galactose addition. The results of these experiments show that Spock lacks the β1,3-galactosyltransferase for side chain addition and that the LPG side chains are required for L. major to bind to and to produce transmissible infection in P. papatasi.

Protozoan parasites of the genus Leishmania spend the extracellular phase of their life cycle as flagellated promastigotes within the gut of their sand fly vectors. The lipophosphoglycan (LPG)1 is the most abundant molecule on the surface of L. major promastigotes. The molecule consists of a neutral oligosaccharide cap followed by a backbone structure made up of repeating PO₄-6-Gal(1→4)Man₁₁ units, which are linked by a phosphosidic lipid anchor. Species-specific polymorphisms in LPG structure may occur in the structure of the cap and in the composition or number of oligosaccharide side chains that branch from the repeat units (1).

Three types of LPG have been described, based upon the nature of sidechain substitutions (2). Type-1 LPG bears no side chains and is represented by the LPGs of the East African Leishmania donovani strains (Fig. 1) (3). Type-2 LPGs have glycan chains linked to the C-3 of galactose in the repeat units. Included in this group is Leishmania mexicana LPG, which bears glucose-containing side chains on approximately 30% of its repeats; Leishmania tropica LPG, which is highly substituted with over 19 different oligosaccharides (3, 4); and Leishmania major LPG, in which virtually every repeat is substituted with β1,3-galactose-terminated side chains (Fig. 1). Type-3 LPG is exemplified by Leishmania aethiopica. In this case, the C-2 position of mannose is substituted with a single α-mannose residue on approximately 35% of the repeat units.

LPG has been implicated as an adhesion molecule that mediates the interaction of procyclic promastigotes with the midgut epithelium of the sand fly vector (5). In order for Leishmania promastigotes to maintain infection in their sand fly vector, they must be anchored to the midgut epithelium during the passage of the digested bloodmeal. We previously demonstrated the significance of LPG polymorphisms for the establishment and maintenance of infection in Leishmania-vector pairs (6). Specifically, we showed that the β1,3-galactose-terminated side chains of L. major are critical for both in vitro binding to and in vivo infection of Phlebotomus papatasi midguts. The finding that only L. major, and not species which lack terminal β-galactose-containing side chains, is capable of this interaction implies the species-specific co-evolution of receptor:ligand pairs.

One approach to understanding how the structure of LPG dictates its function is to generate parasites that are deficient in distinct steps of LPG synthesis (6–10). The structural mutations of LPG can then be correlated with phenotypic characteristics such as infectivity for the vector (5, 6) and host (7). Using this strategy, the present report describes the biochemical and biological characterization of the L. major mutant, Spock. This represents the first description of a defect in LPG side chain biosynthesis resulting from a deficiency in β1,3-galactosyltransferase activity. The impact of this lesion, an impaired Leishmania-sand fly interaction, provides genetic evidence for previously published data that specific LPG oligosaccharides mediate the binding of Leishmania promastigotes to the sand fly midgut and that this trait alone can affect the ability to produce transmissible infections.

EXPERIMENTAL PROCEDURES

Materials—Materials were obtained as follows: medium 199, Dulbecco's modified Eagle's medium, glutamine, penicillin/streptomycin, and...
HEPES from Life Technologies, Inc.; phenyl-Sepharose CL-4B, octyl-Sepharose CL-4B, UDP-galactose, GDP-mannose, N-methyl-N-nitroso-N-nitroguanidine, adenine, and alkaline phosphatase (Escherichia coli) from Sigma–L. major lipases (17); N-acetyl galactosamine from Amersham Corp.; [3H] mannose from American Radiolabeled, Inc.; Sephadex G-150 from Pharmacia Biotech Inc.; AG-1 and AG-50 from Bio-Rad; peanut agglutinin and Ricinus communis agglutinin from Vector Laboratories (Burlingame, CA); and phycocerythrin-conjugated goat anti-mouse IgG from Molecular Probes, Inc. Phosphatidylinositol-specific phospholipase C was purified from Bacillus thuringiensis (8).

In a typical experiment, promastigotes (3 × 10^6) from the monoclonal antibody organization designation MHOM/L180/Friedlin, clone D1) were cultivated in medium 199 supplemented with 20% (v/v) fetal calf serum, 2 mM adenine, 2 mM glutamine, penicillin (1000 units/ml), and streptomycin (50 units/ml). Promastigotes were passaged daily to ensure exponential growth.

Generation of LPG Mutants—Procylic parasites were mutagenized according to the protocol of Iovannisci et al. (11). Approximately 10^6 parasites (10^6/ml) were incubated in DMEM containing 8 µg/ml N-methyl-N-nitroso-N-nitroguanidine (4, 10 min). The parasites were washed twice with fresh DMEM and cultured for 1 week in complete medium before selection. Parasites mutant in lipophosphoglycan synthesis were negatively selected by agglutination with the monoclonal antibody WIC79.3 (12). Promastigotes from log phase cultures were washed twice with cold DMEM and resuspended at 10^7/ml DMEM containing a 1:100 dilution of WIC79.3 ascites fluid. The suspension was incubated for 0.5 h at room temperature, and the agglutinated parasites were pelleted by low speed centrifugation (1000 rpm, 10 min). The supernatant was collected and centrifuged (3000 rpm, 10 min) to separate non-agglutinating parasites. i.e. WIC79.3 negative parasites that were then placed into complete medium and further subjected to 10 rounds of selection. The WIC79.3 negative parasites were cloned by limiting dilution and analyzed by FACs for WIC79.3 reactivity. FACs Analysis of LPG Expression—Log phase promastigotes were washed once with FACs buffer (phosphate-buffered saline, pH 7.2, 2,5% fetal calf serum). Washed parasites (10^7/ml) were incubated on ice for 0.5 h, on ice with 3 µg/ml WIC79.3 Fab fragments in round-bottom microtiter plates. The parasites were washed twice with FACs buffer and incubated (0.5 h, on ice) with fluorescein-conjugated goat anti-mouse IgG Fab (Jackson Immunoresearch Laboratories). After incubation with the second antibody, the parasites were washed twice and resuspended in FACs buffer containing 2% formaldehyde. Stained parasites were analyzed for fluorescence using a Becton Dickinson FACScan (San Jose, CA) and CellQuest software.

Agglutination Assays—Antibodies or lectins (50–150 µl) were serially diluted with DMEM containing 2% bovine serum albumin into flat-bottomed 1-ml microtiter plates. Washed parasites were added at a final density (2 × 10^6 cells/ml) and mixed. The percentage of free parasites compared to control wells (without lectin or antibody) was determined.

Metabolic Labeling and Extraction of LPG—Exponentially growing cells (1–3 × 10^5) were metabolically labeled with [3H]galactose (100 µCi/ml) in Dulbecco's modified Eagle's medium for 2 h at room temperature. LPG was extracted and solubilized in Solvent E (H_2O/ethanol/diethylether/pyridine/NH_4OH, 15:15:1:1:0.1) as described elsewhere (13). The LPG extract was evaporated to dryness under N_2, resuspended in 3 ml of 0.1 M NaCl, 0.1 M acetic acid, and applied to a 1-ml phenyl-Sepharose column equilibrated in the same buffer. The column was washed sequentially with 3 ml of equilibration buffer, 2 ml of 0.1 M acetic acid, and 1 ml of H_2O. LPG was eluted with Solvent E and evaporated to dryness (13). Gel Filtration of LPG—The lipid anchor of LPG was removed by resuspending lyophilized LPG in CHAPS buffer (50 mM HEPES, 5 mM EDTA, 0.2% CHAPS, pH 7.4) and incubating with phosphatidylinositol-specific phospholipase C (37°C, 24 h). The resultant phosphoglycan (PG) was dialyzed against water, lyophilized, and resuspended in 100 mM NH_4OH/1 mM EDTA. The PG was applied to a Sephadex G-150 column (1 × 85 cm) and eluted with 40 mM NH_4OH, 1 mM EDTA. Fractions were collected (0.6 ml) and counted by liquid scintillation. Void and retention volumes were determined with blue dextran and [3H]mannose, respectively.

Separation and Detection of LPG Oligosaccharides—Purified LPG was depolymerized by mild acid hydrolysis (0.02 N HCl, 5 min, 100°C), and the aqueous-soluble fragments were treated with 0.25 units of E. coli alkaline phosphatase (37°C, 18 h). The dephosphorylated fragments were labeled with 37°C, 18 h) with 8-aminonaphthaline-1,3,6-trisulfate and subjected to electrophoresis using the O-linked oligosaccharide profiling gel according to the manufacturer's instructions (GLYKOFACE electrophoresis products, GLYKO, Inc. Novato, CA). The fluorophore-labeled fragments were visualized with a GLYKO UV imager.

Quantitation of Repeat Units in LPG—Purified LPG (0.5 mg) was deamidated by incubation (18 h, 37°C) with 300 µl of 0.5 M NaOH, and 300 µl of 0.25 M sodium acetate to remove the phosphatidylinositol anchor and generate the phosphogycan derivative containing 2,5-anhydromannose at the reducing end. The 2,5-anhydromannose residue was reduced to 2,5-anhydromannitol with the addition of 2 mg/ml NaBH_4 in 1 M NH_4OH, and then the PG was hydrolyzed with 2N trifluoroacetic acid to generate phosphorylated and neutral monosaccharides. Phosphorylated monosaccharides (primarily galactose 6-phosphate) were removed by anion exchange chromatography, and the neutral monosaccharides were subjected to alditol acetate derivatization in preparation for gas chromatography-mass spectrometry. Briefly, monosaccharides were reduced with 2 mg/ml NaBH_4 in 1 M NH_4OH (2 h, room temperature), and borate was removed by the repeated addition of methanol-acetic acid (91, v/v) and evaporation. Dried samples were O-acetylated by incubation (20 min, 121°C) with 0.1 ml of acetic anhydride and 0.1 ml of pyridine and dried under nitrogen. The derivatized monosaccharides were dissolved in 1 ml of dichloromethane and extracted three times with water. The lower phase was dried under nitrogen at room temperature and resuspended in methanol for gas chromatography using an SP2380 column.

In [LPG Biotransformation—LPG samples were harvested at a density of 3–5 × 10^7 cells/ml, and membranes were prepared as described previously (14). Standard incubation mixtures (400 µl) contained 0.4 mg of membrane protein, 50 mM HEPES (pH 7.4), 25 mM KCl, 5 mM MgCl_2, 0.1 mM N^3- tosyI -lsine chloromethyl ketone, 1 µg/ml leupeptin, 1 mM ATP, 0.5 mM dithiothreitol, and 4 µCi UD[3H]galactose (specific activity, 15.3 Ci/mmol). For experiments in which LPG was used as an exogenous acceptor for side chain addition, 200 µg of purified LPG from either L. donovani or Spock was added. To determine the ability of isolated membranes to synthesize complete LPG, 10 µg GDP-mannose was included in the assay. The mixture was incubated for 1 h at 26°C. The reaction was terminated with the addition of 3 volumes of distilled water, and the membranes were pelleted in a microfuge. LPG was released from the membranes by de-polymerized by hydrolysis with 40 mM trifluoroacetic acid (100°C, 8 min). The hydrolysate was lyophilized to remove trifluoroacetic acid, and samples were resuspended in 15 µl Tris (pH 9) and dephosphorylated with alkaline phosphatase. The samples were desalted over Bio-Rad AG1-X8 and AG50-X12 columns and resuspended in MeOH:H_2O (1:1) for application to a Whatman 3-mm chromatography paper and developed 36 h in a developing chamber. The paper chromatography protein pre-equilibrated with 1-butanol:pyridine:H_2O (6:4:3). The paper was cut into 1-cm segments and counted by liquid scintillation. Migration distances were compared to oligosaccharide standards, which were visualized using the periodic acid/silver nitrate method (15).

Sand Fly Infection and Dissection—P. papatasi sand flies were reared and maintained in the Department of Entomology, Walter Reed Army Institute of Research. 3–5-day-old female sand flies were anesthetized with CO2, and their midguts were dissected. The number of midguts in infected flies was determined by placing individual midguts into a microcentrifuge tube containing 30 µl of phosphate-buffered saline, pH 7.4. Each gut was homogenized with a Teflon-coated microtissue grinder, and released promastigotes were counted in a hemocytometer.

In Vitro Assay for Promastigote Binding to Sand Fly Midguts—Binding of promastigotes to sand fly midguts was quantitated by an modification of an in vitro technique (6). 3–5-day-old nonfed female sand flies, maintained on 30% sucrose, were dissected in phosphate-buffered saline. Heads, crops, hindguts, and Malpighian tubules were removed, and the isolated midguts were opened along the length of the abdominal segment with a fine needle. Midguts (7–10 per group) were placed in a concave well of a microscope chamber slide. Leishmania promastigotes (2.5 × 10^7) in 40 µl were added to the guts and incubated for 45 min at room temperature. The guts were then individually washed by placing them in successive drops of phosphate-buffered saline. Guts were homogenized, and released promastigotes were counted as described above.
RESULTS

Generation of LPG-defective Mutants—L. major procyclic promastigotes were mutagenized with nitrosoguanidine and subjected to several rounds of negative selection with the monoclonal antibody WIC79.3. This antibody recognizes the mono- and di-β1,3-galactose side chains of wild-type LPG (Fig. 1)(12,17). Selection enrichment was monitored by FACS analysis, and WIC79.3 negative parasites were cloned by limiting dilution. Fig. 2 compares by FACS analysis the WIC79.3 reactivity of the wild-type L. major (Fig. 2A) to that of one mutant, Spock (Fig. 2B), which was chosen for further analysis. Spock has maintained its WIC79.3 negative phenotype through daily passage in laboratory culture. Species typing analyses by polymerase chain reaction amplification of the mini-exon repeats and by isoenzymes validated that Spock was derived from the parental L. major strain.

Lectin and Antibody Agglutination—Spock’s lack of WIC79.3 reactivity could be explained either by a complete absence of LPG or by the loss of the specific WIC79.3 epitope on LPG. To discriminate between these possibilities, an agglutination assay with the monoclonal antibody CA7AE was performed (Fig. 3A). CA7AE was raised against L. donovani LPG (Fig. 1) and recognized phosphorylated disaccharide repeats that were unmodified by side-chain oligosaccharides (18). As expected, L. donovani promastigotes were agglutinated at all CA7AE dilutions tested. Appreciable agglutination of wild-type L. major parasites did not occur because the abundant, β1,3-galactose-containing side chains of its LPG mask the CA7AE epitope. In contrast, Spock is significantly agglutinated by high dilutions (1:5000) of CA7AE ascites. This result indicates that Spock synthesizes LPG that is relatively unmodified by side-chain oligosaccharides, similar to that of L. donovani LPG.

Fig. 3B shows the results of an agglutination assay with peanut lectin (peanut agglutinin). Peanut agglutinin recognizes terminal galactose residues in a β1,3-linkage to the penultimate sugar (galactose, in the case of L. major LPG) (19). While wild-type L. major parasites were completely agglutinated at all lectin concentrations tested, Spock parasites remained unagglutinated at concentrations as high as 50 μg/ml, indicating the absence of terminally exposed β-galactose residues. This is in contrast to the sensitivity of L. donovani to peanut lectin, which agglutinates the parasite at concentrations above 6 μg/ml, reflecting the presence of a terminal β-galactose residue in the neutral cap.

Characterization of in Vivo Labeled LPG from Spock—The relative ability of Spock to synthesize LPG was examined by metabolic labeling of the cells with [3H]galactose and [3H]
mannose, extraction of LPG by organic solvents, and purification of the glycoconjugate on a column of phenyl-Sepharose. Incorporation of radioactive galactose and mannose precursors showed a slight decrease and increase, respectively, into Spock LPG compared to wild type (data not shown). To obtain information regarding the relative sizes of the radioactive LPGs, wild-type L. major, Spock, and L. donovani LPGs were delipitated by nitrous acid deamination and then were subjected to gel filtration on Sephadex G-150 (Fig. 4). Wild-type L. major LPG eluted first, followed by Spock and then L. donovani, indicating that the wild-type L. major synthesizes a slightly larger glycoconjugate than either the mutant or L. donovani. In addition, the length of each PG was assessed by determining the ratio of mannose:2,5-anhydromannose (27.4 (wild-type L. major)), 20.0 (Spock), and 16.4 (L. donovani)). These ratios reflect the relative number of repeats in LPG assuming a similar number of mannose residues in the glycan core and cap domains.

Characterization of the Repeating Units of Spock LPG—To confirm that Spock produces an LPG defective in the synthesis of side chain sugars recognized by WIC79.3 and peanut agglutinin, [3H]galactose-labeled LPGs from wild-type L. major, Spock, and L. donovani LPGs were depolymerized with mild acid, derivatized with 8-aminonaphthalene-1,3,6-trisulfate, and electrophoresed. Lane 4 contains oligoglucose standards. Arrows indicate the migration of oligosaccharide standards: a, Gal-Gal-Gal-Man; b, Ara-Gal-Gal-Man; c, Gal-Gal-Man; d, Gal-Man; G5 to G2 represent the migration distance of oligoglucose standards.

Glyko-FACE analysis of oligosaccharide fragments. LPG-isolated wild-type L. major (lane 1), Spock (lane 2), and L. donovani (lane 3) were depolymerized with mild acid and electrophoresed. Lane 4 contains oligoglucose standards. Arrows indicate the migration of oligosaccharide standards: a, Gal-Gal-Gal-Man; b, Ara-Gal-Gal-Man; c, Gal-Gal-Man; d, Gal-Man; G5 to G2 represent the migration distance of oligoglucose standards.
alkaline phosphatase. The fragments were derivatized at the reducing ends with a fluorophore and subjected to electrophoresis. Fluorescent bands were visualized by digital UV imaging (Fig. 6). In lane 1, the most prominent fragment is the trisaccharide from the wild-type *L. major* LPG, with lesser amounts of two tetrasaccharides and the Gal-Man disaccharide. The characteristic disaccharide repeating unit (Gal-Man) of the *L. donovani* LPG is shown in lane 3. The pattern of fragments from Spock LPG (lane 2) is virtually identical to that of *L. donovani*, indicating the lack of side chains. There was no change in the pattern of Spock LPG fragments as a function of the growth phase (data not shown).

Analysis of the Defect in a Cell-free System of LPG Biosynthesis—The results from experiments using intact Spock cells suggested that the LPG from these mutants did not have the characteristic side chain sugars initiated by a β1,3-galactose addition to the PO₄-Gal-Man backbone units. To investigate if the defect was due to the absence of β1,3-galactosyltransferase activity or a possible modification of the LPG that precludes side chain addition, a cell-free glycosylation assay was used (14). Membranes from wild-type *L. major* or Spock were incubated with exogenous Spock or *L. donovani* LPG and UDP-[^3H]galactose. The repeating units of the prelabeled LPG were analyzed for the presence of oligosaccharide side chains by subjecting the material to mild acid hydrolysis and dephosphorylation with alkaline phosphatase. The fragments generated were resolved by descending paper chromatography.

When wild-type or Spock membranes were incubated in the absence of exogenous LPG (Fig. 7, A and D, respectively), no incorporation of[^3H]galactose from UDP[^3H]galactose was observed. This experiment confirms the lack of de novo LPG biosynthesis in the absence of exogenously added GDP-mannose. Analysis of fragments from both exogenously supplied *L. donovani* and Spock LPG (Fig. 7, B and C, respectively) that had been incubated with wild-type *L. major* membranes revealed the presence of trisaccharide fragments, indicating that both LPGs are competent acceptors for β1,3-galactose addition. In contrast, analysis of LPG fragments generated from the incubation of Spock and *L. donovani* LPG with Spock membranes showed no trisaccharides, indicating that the Spock membranes lack the enzyme for side chain addition (Fig. 7, E and F, respectively). However, Spock membranes are enzymatically active and capable of in vitro assembly of the PO₄-Gal-Man repeat units when both UDP[^3H]galactose and GDP-mannose are included in the incubation medium. Incorporation of the radiolabel into wild-type and Spock LPG was similar (44,600 cpm versus 66,680 cpm, respectively). Analysis of the wild-type LPG fragments showed the presence of tri- and tetrasaccharides (Fig. 8A), while fragments generated with Spock membranes were predominantly disaccharides (Fig. 8B).

In Vitro Binding of Spock to Sand Fly Midguts—Isolated *P. papatasi* midguts were incubated with wild-type *L. major* or Spock promastigotes. After washing, the guts were homogenized, and released parasites were counted. The results
are shown in Fig. 9. The average number of wild-type promastigotes per midgut was approximately 7,500, while Spock exhibited significantly less binding (approximately 1,800 parasites/midgut).

Survival of Spock in *P. papatasi*—2 days after sand flies were membrane-fed on bloodmeals containing wild-type *L. major* or Spock, all flies in each group harbored similar numbers of viable promastigotes (data not shown). By day 5 postinfection, after passage of the digested bloodmeal, the parasite burden differed substantially in the three groups (wild type, 43,000 parasites/midgut; Spock, 800 parasites/midgut; *L. donovani*, 28 parasites/midgut), indicating that Spock, like *L. donovani*, is unable to maintain significant infection in *P. papatasi* vector (Fig. 10).

**DISCUSSION**

The ability of *Leishmania* parasites to bind to the sand fly midgut during excretion of the digested bloodmeal is essential for the development of transmissible infections. 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. The leishmanial receptor of *P. papatasi* is highly specific for the galactose-terminated oligosaccharide branches of *L. major* LPG (6). *L. major*, being the only Old World *Leishmania* to express abundant side chains terminating in galactose, appears to have yielded to a selective pressure that enables it to exploit a widespread phlebotomine species that is otherwise refractory to parasites expressing relatively unsubstituted LPGs.

The present report describes the interaction of a *L. major* LPG mutant, defective in β1,3-galactose addition, with the sand fly vector. This work furnishes genetic evidence for the previously published contention that the binding of *L. major* promastigotes to the midgut epithelium is mediated by the extensive side chain oligosaccharides (5, 6). The mutant, Spock, was generated by nitrosoguanidine treatment of wild-type *L. major* and negative selection with the monoclonal antibody WIC79.3. This monoclonal antibody is specific for the galactose-terminated oligosaccharide side chains of *L. major* LPG (7) and does not recognize this moiety on other glycoconjugates such as glycoproteins (23). Therefore, the use of WIC79.3 allowed us to specifically isolate parasites defective in LPG side-chain biosynthesis. The lack of these side chains diminished Spock's ability to bind to *P. papatasi* midguts in vitro binding assays and to maintain infections in sand flies after passage of the bloodmeal. Thus, the mutant *L. major* Spock displayed a biological phenotype similar to that of *L. donovani*. The data provide strong evidence that the inability of *L. donovani* to utilize *P. papatasi* as a natural vector can be explained solely on the basis of its LPG lacking appropriate side-chain additions, regardless of any other interspecies molecular differences that might exist.

Analysis of depolymerized, dephosphorylated LPG fragments from Spock indicated that the defect in Spock LPG biosynthesis lies in the β1,3 addition of galactose to the 3-hydroxyl of the galactose residue in the disaccharide repeats of the LPG backbone. Membranes from wild-type *L. major* but not from Spock were able to add galactose-initiated side chains to Spock LPG, demonstrating that the molecule is a competent substrate for oligosaccharide modification. It follows that the genetic defect of Spock involves the deficiency of a galactosylation enzyme (i.e., the absence of a β1,3-galactosyltransferase) rather than a covalent modification of the Gal-Man repeat unit, which would prevent side chain addition to the galactose residue. The lack of β1,3-galactosyltransferase activity in *L. donovani* has been demonstrated previously by Ng and co-workers (14).

A mammalian β1,3-galactosyltransferase has been de-
scribed, the deficiency of which leads to Tn-syndrome (24, 25). When the Tn antigen (O-linked α-N-galactosamine) or its sialylated derivative is not β1,3-galactosylated, hematopoietic cells bearing the antigen bind serum anti-a-GalNAc antibodies, causing the mild hemolytic anemia and thrombocytopenia observed in patients with this rare acquired disorder. Preliminary studies indicate that the transferase deficiency in Tn-syndrome results from the silencing of the galactosyltransferase gene or possibly its transcriptional control unit by DNA methylation. However, the enzyme has not been isolated nor has its gene been cloned, so molecular studies of the deficiency have not been performed.

Spock represents the first example of a Leishmania mutant defective in assembly of side chain sugars of LPG. Thus, Spock provides an important tool for the delineation of the biosynthetic pathway and functional significance of LPG. Experiments are currently underway to isolate the galactosyltransferase gene by functional complementation of Spock with wild-type L. major DNA using methods that enabled the isolation and characterization of the putative galactofuranosyltransferase gene of L. donovani (10).

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