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Galactofuranose metabolism: a potential target for antimicrobial chemotherapy

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Abstract. Galactofuranose-containing glycoconjugates are present in numerous microbes, many of which are pathogenic for humans. Metabolic aspects of the monosaccharide have proven difficult to elucidate, because galactofuranose metabolites and glycoconjugates are relatively unstable during analyses. Recent advances in biochemical and genetic approaches, however, have facilitated a better understanding of galactofuranose metabolism. This review summarizes our current information on its metabolism and a few selected glycoconjugates containing this furanose.

Key words. Galactofuranose; pathogenic microbes; microorganisms; arabinogalactan; lipophosphoglycan.

Introduction

Pathogenic microorganisms have been targeted by effective antimicrobial therapies for decades, yet despite the presence of an impressive array of antibiotics [1], in recent years the search for new antimicrobial therapies has become increasingly important. Of great concern is the surge in antibiotic resistance among many important human pathogens [1, 2]. One notable example is that of *Mycobacterium tuberculosis*, which despite several decades of successful chemotherapeutic treatment has reemerged through multidrug resistance to once again become one of the leading causes of death, with an annual mortality rate of approximately 3 million [3]. In addition, certain other devastating diseases still lack effective treatments or vaccines. Leishmaniasis, caused by the sand fly-mediated transmission of various species of the protozoan parasite *Leishmania*, is a major health threat worldwide, with an estimated 12 million cases, and approximately 350 million people at risk in 88 countries [4]. Traditionally, the standard leishmaniasis treatment has consisted of potentially dangerous, difficult-to-administer antimony-based compounds, with alternative treatments posing similar difficulties of toxicity and effectiveness [5]. In addition, unresponsiveness to pentavalent antimonial compounds is a rising concern in endemic areas [6]. These issues collectively reinforce the critical need for new rational approaches to antimicrobial drug design.

The search for molecules unique to pathogenic microorganisms has led to one potential chemotherapeutic target, the sugar galactofuranose (Gal*f*). In the pyranoic configuration, galactose is abundant in mammalian glycoconjugates. A conformational alteration in galactopyranose (Gal*p*) yields Gal*f* (fig. 1), which is much less abundant, having only been reported in microorganisms, such as bacteria, protozoa and fungi [7]. Importantly, Gal*f* has been shown to be present in numerous structures considered to be essential for virulence in many pathogenic organisms. These include the lipopolysaccharide (LPS) O-antigen of an increasing number of Gram-negative bacteria, [8–13]; the T1-antigen polysaccharide of *Salmonella* spp. [14] and extracellular or capsular polysaccharides of a variety of both Gram-positive and Gram-negative bacteria [15–19]. In addition, Gal*f* is a critical and abundant component of the arabinogalactan of *Mycobacterium* spp. [20]. Furthermore, Gal*f* has been shown to be present in surface glycoconjugate structures that are believed to play important roles in the pathogenesis of some protozoan parasites, such as the lipopeptidophosphoglycan (LPPG), glycoinositolphospholipids (GIPLs), and certain mucins of *Trypanosoma cruzi* [7, 21, 22], and the lipophosphoglycan (LPG) of *Leishmania* spp. [23–25]. Gal*f* is also a component of a variety of fungal cell surface glycoconjugates and glycans [26–31]. Importantly, Gal*f*

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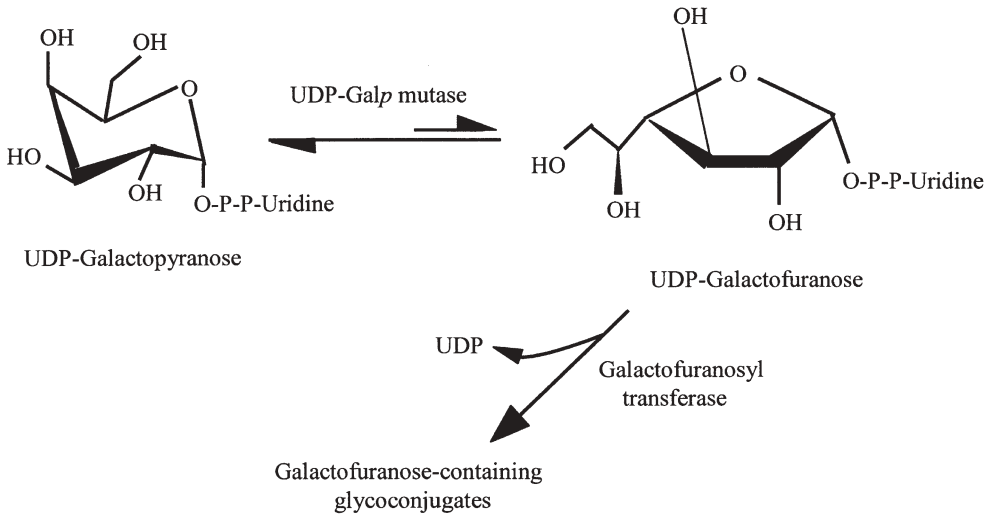

Figure 1. The conversion of UDP-Gal*p* to UDP-Gal*f* catalyzed by UDP-Gal*p* mutase [37], followed by transfer of Gal*f* from the nucleotide sugar to the glycoconjugate structure by Gal*f* transferase.

has not been reported to exist in mammals; rather, Gal*f*-containing epitopes have been shown to be highly antigenic [21, 31, 32]. Thus, the metabolism of Gal*f* has become a very attractive candidate as a target for new antimicrobial drugs.

Gal_f metabolism

The pathway of Gal*f* metabolism has long been a difficult area to unravel due to the instability of Gal*f* itself and a lack of effective and appropriate molecular tools. The uniqueness of Gal*f* as a component of important surface glycoconjugates of human pathogens, however, has in recent years led to a renewed interest in its metabolism. UDP-Gal*f* derived from UDP-Gal*p* was shown some time ago to be the source of Gal*f* within bacterial LPS [14, 33, 34], and more recently within *Leishmania donovani* LPG [35] and *Mycobacterium smegmatis* arabinogalactan [36]. UDP-Gal*p* is converted to UDP-Gal*f* by UDP-Gal*p* mutase, an enzyme which in recent years has been isolated and its activity directly demonstrated in several microorganisms that synthesize important Gal*f*-containing surface glycoconjugates, including *Escherichia coli* [37], *Mycobacteria* [36] and *Klebsiella* [38]. Gal*f* is then transferred from UDP-Gal*f* [14, 33] to the respective glycoconjugate molecules by specific galactofuranosyl transferases, as shown in figure 1 [39–41].

Target potential of Gal_f metabolism

For any microorganism, the likelihood that Gal*f* metabolism will be effectively targeted can be partially assessed based on whether the Gal*f*-containing surface glycoconjugates of that organism are essential for the viability or pathogenicity of the organism. LPS, which contains Gal*f* (fig. 2A), is well known as an important virulence factor for many Gram-negative bacterial species, playing an important role in protection from destruction by the mammalian immune system [42], and is a key mediator of septic shock [43]. The cell wall of mycobacteria is essential for viability [40, 44]. Mycobacterial arabinogalactan, a polysaccharide largely comprised of Gal*f*, covalently links the highly impermeable mycolic acid outer layer of the mycobacterial cell wall with the inner layer of peptidoglycan [20, 45–48] (fig. 2D). The metabolism of arabinan, which is directly linked to the Gal*f* component of arabinogalactan, is the target of the proven antituberculosis drug ethambutanol [44, 49], illustrating the effectiveness of targeting mycobacterial cell wall metabolism. In *Leishmania*, LPG (fig. 2C) is the most abundant surface glycoconjugate on the promastigote stage of the parasite, and in vitro data have suggested that LPG may play important roles within the mammalian host and the sand fly vector [50–57]. The fact that *Leishmania* expresses numerous other glycoconjugates, such as GIPLs, proteophosphoglycans (PPGs) and secreted acid phosphatase (sAP) [58], all of which share molecular domains with LPG, however, has made the conclusive demonstration of the roles of LPG in the mammalian infection process difficult.

Genetic, molecular and functional analysis

In recent years, several genetic loci involved in Gal*f* metabolism have been isolated and characterized in a number of pathogenic organisms, allowing investigation of the requirements for these enzymes in the pathogenesis of

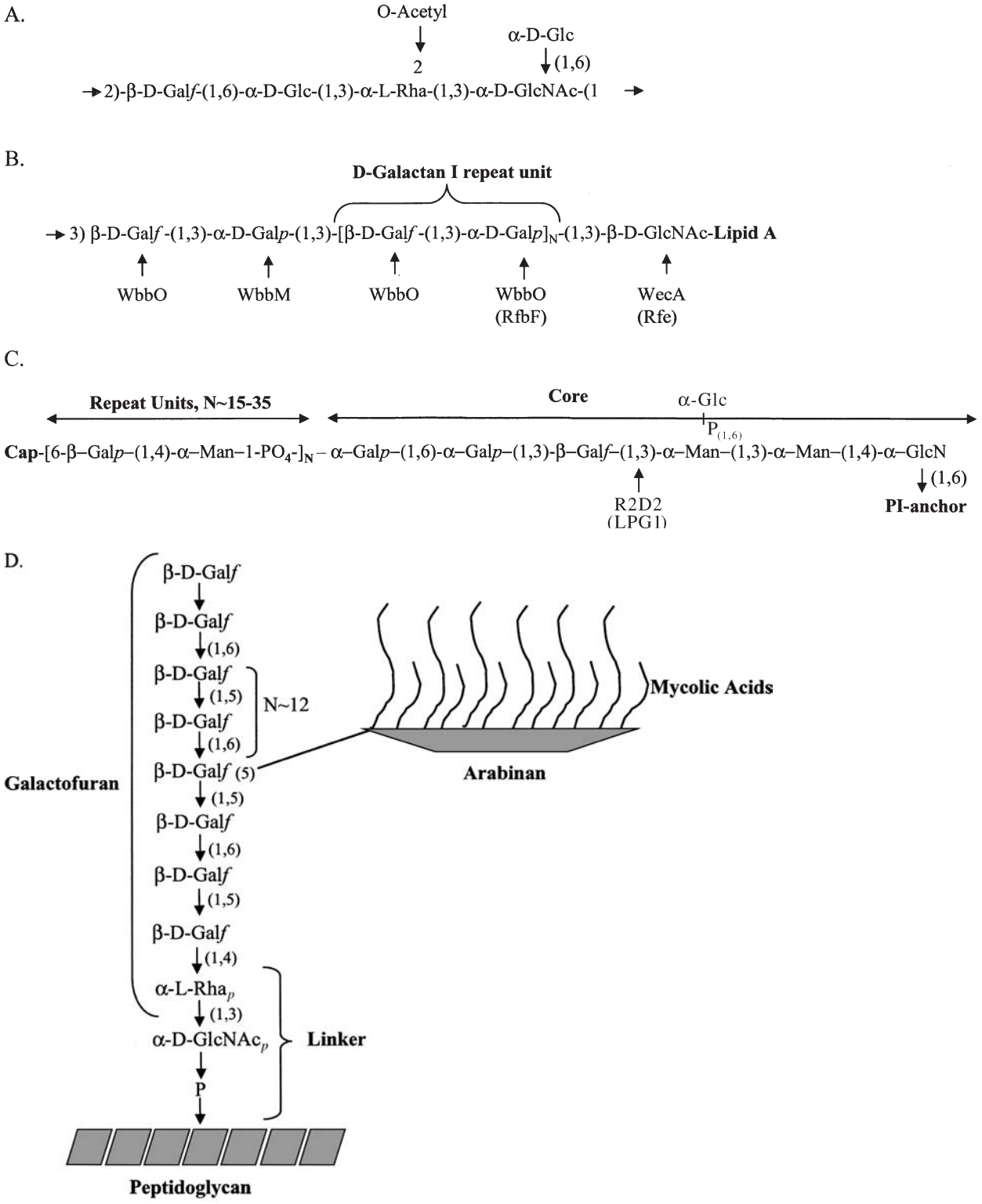

Figure 2. Gal*f*-containing glycoconjugate structures. (*A*) *Escherichia coli* K-12 LPS O-antigen repeating unit [42]; (*B*) *Klebsiella pneumoniae* O-antigen repeat unit galactan-I [39, 65, 67]; (*C*) *Leishmania donovani* LPG [56]; (*D*) Mycobacterial cell wall [40].

these organisms. Furthermore, the examination of the structure and function of these potential drug targets on the molecular level is an essential step in rational drug design. The remainder of this manuscript highlights recent data that have emerged in these areas.

Gram-negative bacteria

E. coli and *Klebsiella pneumoniae:* UDP-Gal$_p$ mutase and Gal$_f$ transferase

The gene encoding UDP-Gal*p* mutase, Glf (EC 5.4.99.9), was successfully identified and cloned from the *E. coli* K-12 *rfb* region, which is involved in the biosynthesis of *E. coli* LPS O-antigen (fig. 2A), and the purified protein was shown to demonstrate UDP-Gal*p* mutase activity by the interconversion of UDP-Gal*p* and UDP-Gal*f* in vitro (fig. 1) [37]. Biochemical characterization demonstrated that Glf is a flavoprotein to which FAD is noncovalently bound [37]. Subsequently, it was found that Glf was active independent of the redox state of bound FAD; however, a change in the redox state of enzyme-bound FAD to the reduced form enhances the catalytic efficiency of the enzyme [59, 60]. This same study also reported that neither $NADP^+$ nor NADPH is required for the reaction catalyzed by Glf [59]. Concurrently, the crystal structure of the *E. coli* mutase was resolved [61–63], and site-directed mutagenesis showed that enzyme-bound FAD is located within a cleft containing the active site of the mutase [63]. In contrast to the previous report, however, Sanders et al. [63], reported that the reduction of FAD is absolutely required for the enzymatic activity of Glf, resulting in the proposal that the substrate is transiently reduced during the reaction catalyzed by Glf. Further studies investigating the mechanism of catalysis by Glf showed that the reaction is not initiated by the oxidation of either the C2- or C3-OH of the sugar substrate, as synthetic C2- and C3-fluorinated UDP-Gal*f* analogues can function as substrates for the reduced UDP-Gal*p* mutase [60]. Similar results were observed using C2- and C3-fluorinated UDP-Gal*p* analogues as substrates for the *K. pneumoniae* UDP-Gal*p* mutase [64].

In *K. pneumoniae,* an analogous *rfbA-F* gene cluster is involved in directing the synthesis of D-galactan I, which comprises the O-antigen backbone structure of *K. pneumoniae* LPS (fig. 2B). Nomenclature changes recently adopted are reflected in figure 2B, and will be used here to avoid confusion [65]. Within this gene cluster, glf_{KPO1} (formerly $rfbD_{KPO1}$) was recently cloned and its protein product purified. Investigation of its activity in vitro showed that, as for *E. coli glf*, glf_{KPO1} encodes *K. pneumoniae* UDP-Gal*p* mutase (Glf_{KPO1}), capable of interconverting UDP-Gal*p* and UDP-Gal*f* [38]. In contrast to *E. coli* Glf, however, Glf_{KPO1} was shown to absolutely require NADH or NADPH for activity, possibly reflecting slight mechanistic differences between the organisms [38]. Further characterization using positional isotope exchange (PIX) experimentation revealed that the mechanism by which Glf_{KPO1} catalyzes the interconversion of UDP-Gal*p* and UDP-Gal*f* involves the cleavage of the anomeric C–O bond of the substrate [66], a result that was similarly confirmed for *E. coli* Glf [60].

wbbO (formerly $rfbF_{KPO1}$), also within the *K. pneumoniae rfbA-F* gene cluster, was recently cloned, expressed in *E. coli* K-12 strains, and shown in vitro to encode a product exhibiting both galactopyranosyl and galactofuranosyl transferase activities [67]. $WbbO_{KPO1}$ was demonstrated to be responsible for initiation of D-galactan I synthesis by adding a single D-galactan I repeat unit, comprising the disaccharide β-D-Gal*f*-(1,3)-α-D-Gal*p*, to the LPS core following addition of the initial GlcNAc residue to the lipid A core by WecA (formerly Rfe) (fig. 2B) [67]. A subsequent study showed that the galactopyranosyl transferase activity of $WbbO_{KPO1}$ participates only in the initial step of D-galactan I synthesis, since it recognizes the acceptor molecule for the initial Gal*p* residue as a GlcNAc residue. The galactofuranosyl transferase activity of $WbbO_{KPO1}$, however, can participate in extension of the D-galactan I polymer, since it recognizes Gal*p* as the acceptor for β-D-Gal*f* residues, resulting in formation of the β-D-Gal*f*-(1,3)-α-D-Gal*p* linkage [39]. A separate galactopyranosyl transferase, WbbM, then participates with WbbO in extension of the D-galactan I polymer chain (fig. 2B) [39].

Mycobacteria

The basic structure of the mycobacterial cell wall core is shown in figure 2D [40]. The core consists of a highly impermeable outer layer composed of mycolic acids (C_{70}– C_{90} lipids) and an inner peptidoglycan layer. These two layers are connected by arabinogalactan, consisting of galactofuran, a structural component comprised of alternating β1,5- and β1,6-linked Gal*f* residues (N ~ 15), which is attached to the peptidoglycan via a disaccharide linker; and arabinan, which is attached to the mycolic acids (fig. 2D) [40]. The requirement for the integrity of the mycobacterial cell wall arabinogalactan component for the survival of *M. tuberculosis* is well known, as components of the drug regiment widely used to treat tuberculosis, including ethambutanol, isoniazid and ethionamide, effectively target mycolic acid or arabinan synthesis [36, 49, 68–70]. Arabinogalactan is rich in Gal*f* residues [71], which are clearly necessary for the integrity of the mycobacterial cell wall. Therefore, Gal*f* metabolism holds particularly good promise as a potential target in the search for new antimycobacterial drugs.

Recently, Weston et al. [36], demonstrated that UDP-Gal*p* mutase activity is present in *Mycobacterium smeg-*

matis, and that UDP-Gal*f* derived from UDP-Gal*p* is the Gal*f* donor in mycobacteria. This led to the identification, cloning and expression of the *M. smegmatis glf* gene (*Rv3809c*), encoding the UDP-Gal*p* mutase (Glf), which was shown to catalyze the interconversion of UDP-Gal*p* and UDP-Gal*f* using in vitro assays [36]. The amino acid sequence of *M. smegmatis* Glf is quite similar to *E. coli* and *K. pneumoniae* Glf proteins, with 42.5 and 40.9% identity at the amino acid level, respectively [36]. A subsequent study described the synthesis of arabinogalactan via the addition of Gal*f* residues to a linker unit intermediate (polyprenyl-P-P-GlcNAc-Rha), using UDP-Gal*p* as the donor of UDP-Gal*f* in the presence of Glf, followed by transfer of Gal*f* residues from UDP-Gal*f* to the linker by a specific galactofuranosyl transferase, identified as the product of the *M. tuberculosis* gene *Rv3808c* [72]. Shortly thereafter, a new assay was developed that used synthetic disaccharide neoglycolipid acceptors to demonstrate that the product of the *Rv3808c* locus, designated *glf*T, was a novel UDP-galactofuranosyl transferase that exhibits dual functions by transferring Gal*f* to the acceptor substrates via both $1\rightarrow5$ and $1\rightarrow6$ linkages, both of which are present in the mycobacterial arabinogalactan complexes [41]. Simultaneously, Pan et al. [40], generated a *glf* knockout mutant which could only be rescued by the presence of plasmids expressing both *glf* and the immediately downstream *rv3808c*, showing that both Glf (Rv3809c) and GlfT (Rv3808c) are essential for the growth of *M. smegmatis*. These studies demonstrate the requirement of galactofuran biosynthesis for mycobacterial growth, providing encouraging results for the target potential of Gal*f* metabolism in antimycobacterial therapy.

Eukaryotic pathogens

Important eukaryotic pathogens include members of the genus *Trypanosoma*, which are flagellate obligate parasites of a variety of hosts, ranging from insects to humans. These organisms possess important surface glycoconjugate structures, many of which are modified by Gal*f*, including the LPPG and GIPLs of *Trypanosoma cruzi* and the LPG of *Leishmania* spp. [7, 58, 73]. Other eukaryotic pathogens expressing Gal*f*-containing surface molecules include certain mycopathogens, such as *Paracoccidioides braziliensis* [31]. Only recent advances in *Leishmania* research regarding the Gal*f*-containing LPG will be highlighted here.

Leishmania spp.

The protozoan parasite *Leishmania* differs from the aforementioned microorganisms in that its major Gal*f*-containing glycoconjugate LPG is expressed in a stage-specific manner. The *Leishmania* life cycle and the properties of LPG have been reviewed elsewhere [50, 51, 56, 73–75]. LPG is expressed over the entire surface of the sand fly-borne procyclic parasite, and has been implicated as an important factor for promastigotes in the binding to and release from sand fly midguts [76]. LPG is abundant on the surface of the promastigote at the time of transmission to the mammalian host during sand fly feeding; however, shortly after transmission to the mammalian host, the parasite enters macrophages and dramatically downregulates LPG expression.

Earlier studies were performed in which a bank of *Leishmania* mutants generated by chemical mutagenesis was screened for the expression of defective LPG molecules [35, 77–81]. One such *L. donovani* mutant, R2D2, was found to synthesize LPG that was truncated at the Gal*f* residue within the core region (fig. 2C) [35]. Complementation by a single gene designated *LPG1* restored full-length LPG synthesis [81], leading to the proposal that *LPG1* encoded the Gal*f* transferase [35, 56, 81]. While this mutant was generated by chemical mutagenesis, however, subsequent studies led to the generation and investigation of targeted *LPG1* knockout mutants in several other species of *Leishmania,* specifically *L. mexicana* [82] and *L. major* [83, 84]. Importantly, each of these targeted mutants was defective only in LPG synthesis, yet retained normal levels of all the other important phosphoglycan-containing glycoconjugates.

These *LPG1* mutants were examined to determine the role of LPG on *Leishmania* promastigotes within the sand fly vector. Both the *L. donovani LPG1* – mutant R2D2 and the *L. major LPG1* – knockout were only slightly defective in their survival and replication within the sand fly midgut, indicating that LPG does not play a major role in protection from digestive enzymes within the sand fly midgut. However, in agreement with their failure to bind sand fly midgut epithelial cells in vitro, the ability of these LPG-defective mutants to persist in the midgut of the sand fly when the initial bloodmeal was excreted was completely lost [84]. These data confirm that LPG plays an essential role in binding of the parasites to the midgut of the sand fly, a critical factor for transmission of *Leishmania* to the mammalian host during the natural disease process.

Until recently, the proposed roles of LPG during the mammalian infection had only been shown through in vitro assays [50–57]. Recently, however, two independent labs investigated the role of LPG during the mammalian infection using targeted *LPG1* mutants. In one study, an *L. mexicana LPG1* knockout was examined for its ability to infect macrophages and mice. In this study, Ilg et al. [82], showed that there were minimal differences between the LPG-deficient mutant and the wild-type parasites in their attachment to, uptake by and replication within macrophages. Furthermore, the LPG-deficient

parasites were as virulent as the wild-type *L. mexicana*, resulting in disseminated disease and death in both BALB/c (susceptible) and C57/BL6 (resistant) mice [82]. Concurrently, Spath et al. [83], reported that in contrast to the previous study, an *L. major* targeted *LPG1* – knockout was highly attenuated in BALB/c mice, with the restoration of virulence upon reintroduction of *LPG1*. Furthermore, the promastigote form of the *LPG1* – knockout was shown to be significantly impaired in its ability to survive within macrophages; however, disruption of the *LPG1* locus had no effect on the ability of lesion-derived amastigotes to survive within macrophages in vitro, which is an expected result, as amastigotes are known to significantly downregulate surface LPG expression [83].

The differences in the reported results of these two studies may reflect species-specific variations, or differences in techniques or protocols. While further studies regarding the roles of LPG and Gal*f* metabolism during mammalian infection are certainly warranted, the critical role of LPG, and therefore Gal*f* metabolism, in attachment of *Leishmania* promastigotes to the sand fly midgut is without dispute, confirming their importance in the pathogenic life cycle of *Leishmania* [73, 84].

Conclusions

Although homologous Gal*f* metabolism genes have been identified and examined in several species of pathogenic organisms, whether functions assigned to one enzyme can be translated as common to all other pathogenic microorganisms known to possess Gal*f* in their surface glycoconjugates remains an open question. The important roles of the surface glycoconjugates containing Gal*f* warrant continued exploration of the mechanisms of Gal*f* metabolism and their requirements for virulence. As investigation of Gal*f* metabolism continues to unfold on molecular and genetic levels, we may begin to see common elements that may be effectively exploited as targets within a wide range of pathogenic microorganisms.

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1 Neu H. C. (1992) The crisis in antibiotic resistance. Science **257:** 1064–1073
2 Cohen M. L. (1992) Epidemiology of drug resistance: implications for a post-antimicrobial era. Science **257:** 1050–1055
3 Bloom B. R. and Murray C. J. (1992) Tuberculosis: commentary on a reemergent killer. Science **257:** 1055–1064
4 Desjeux D. P. (2002) World Health Organization: programme for the surveillance and control of Leishmaniasis. Accessed 4/11/02 at http://www.who.int/emc/diseases/leish/index.html
5 Herwaldt B. L. (1999) Leishmaniasis. Lancet **354:** 1191–1199
6 Sereno D., Guilvard E., Maquaire S., Cavaleyra M., Holzmuller P., Ouaissi A. et al. (2001) Experimental studies on the evolution of antimony-resistant phenotype during the in vitro life cycle of *Leishmania infantum*: implications for the spread of chemoresistance in endemic areas. Acta Trop. **80:** 195–205
7 De Lederkremer R. M. and Colli W. (1995) Galactofuranose-containing glycoconjugates in trypanosomatids. Glycobiology **5:** 547–552
8 Jann B., Shashkov A. S., Kochanowski H. and Jann K. (1994) Structure of the O16 polysaccharide from *Escherichia coli* O16:K1: an NMR investigation. Carbohydr. Res. **264:** 305–311
9 Kol O., Wieruszeski J. M., Strecker G., Montreuil J., Fournet B., Zalisz R. et al. (1991) Structure of the O-specific polysaccharide chain from *Klebsiella pneumoniae* O1K2 (NCTC 5055) lipopolysaccharide. Carbohydr. Res. **217:** 117–125
10 Dmitriev B. A., Lvov V. L. and Kochetkov N. K. (1977) Complete structure of the repeating unit of the O-specific polysaccharide chain of *Shigella dysenteriae* type 3 lipopolysaccharide. Carbohydr. Res. **56:** 207–209
11 Altman E., Brisson J. R. and Perry M. B. (1988) Structure of the O-antigen polysaccharide of *Haemophilus pleuropneumoniae* serotype 3 (ATCC 27090) lipopolysaccharide. Carbohydr. Res. 1988. **179:** 245–258
12 Perry M. B. (1990) Structural analysis of the lipopolysaccharide of *Actinobacillus (Haemophilus) pleuropneumoniae* serotype 10. Biochem Cell Biol. **68:** 808–810
13 Stevenson G., Neal B., Liu D., Hobbs M., Packer N. H., Batley M. et al. (1994) Structure of the O antigen of *Escherichia coli* K-12 and the sequence of its rfb gene cluster. J. Bacteriol. **176:** 4144–4156
14 Sarvas M. and Nikaido H. (1971) Biosynthesis of T1 antigen in *Salmonella*: origin of D-galactofuranose and D-ribofuranose residues. J. Bacteriol. **105:** 1063–1072
15 Abeygunawardana C., Bush C. A., Tjoa S. S., Fennessey P. V. and McNeil M. R. (1989) The complete structure of the capsular polysaccharide from *Streptococcus sanguis* 34. Carbohydr. Res. **191:** 279–293
16 Abeygunawardana C., Bush C. A. and Cisar J. O. (1990) Complete structure of the polysaccharide from *Streptococcus sanguis* J22. Biochemistry **29:** 234-248
17 Abeygunawardana C., Bush C. A. and Cisar J. O. (1991) Complete structure of the cell surface polysaccharide of *Streptococcus oralis* C104: a 600-MHz NMR study. Biochemistry **30:** 8568–8577
18 Abeygunawardana C., Bush C. A. and Cisar J. O. (1991) Complete structure of the cell surface polysaccharide of *Streptococcus oralis* ATCC 10557: a receptor for lectin-mediated interbacterial adherence. Biochemistry **30:** 6528–6540
19 Bax A., Summers M. F., Egan W., Guirgis N., Schneerson R., Robbins J. B. et al. (1988) Structural studies of the *Escherichia coli* K93 and K53 capsular polysaccharides. Carbohydr. Res. **173:** 53–64
20 Besra G. S., Khoo K. H., McNeil M. R., Dell A., Morris H. R., and Brennan P. J. (1995) A new interpretation of the structure of the mycolyl-arabinogalactan complex of *Mycobacterium tuberculosis* as revealed through characterization of oligoglycosylalditol fragments by fast-atom bombardment mass spectrometry and 1 H nuclear magnetic resonance spectroscopy. Biochemistry **34:** 4257–4266
21 De Arruda M.V., Colli W. and Zingales B. (1989) Terminal β-D-galactofuranosyl epitopes recognized by antibodies that inhibit *Trypanosoma cruzi* internalization into mammalian cells. Eur. J. Biochem. **182:** 413–421
22 Previato J. O., Gorin P. A., Mazurek M., Xavier M. T., Fournet B., Wieruszesk J. M. et al. (1990) Primary structure of the oligosaccharide chain of lipopeptidophosphoglycan of epimastigote forms of *Trypanosoma cruzi*. J. Biol. Chem. **265:** 2518–2526
23 Turco S. J., Orlandi P. A. Jr, Homans S. W., Ferguson M. A., Dwek R.A. and Rademacher T. W. (1989) Structure of the phosphosaccharide-inositol core of the *Leishmania donovani* lipophosphoglycan. J. Biol. Chem. **264:** 6711–6715

24 McConville M. J., Homans S. W., Thomas-Oates J. E., Dell A. and Bacic A. (1990) Structures of the glycoinositolphospholipids from *Leishmania* major. A family of novel galactofuranose-containing glycolipids. J. Biol. Chem. **265:** 7385–7394

25 Ilg T., Etges R., Overath P., McConville M. J., Thomas-Oates J., Thomas J. et al. (1992) Structure of *Leishmania mexicana* lipophosphoglycan. J. Biol. Chem. **267:** 6834–6840

26 Unkefer C. J. and Gander J. E. (1979) The 5-O-beta-D-galactofuranosyl-containing glycopeptide from *Penicillium charlesii*. Carbon 13 nuclear magnetic resonance studies. J. Biol. Chem. **254:** 12131–12135

27 Parra E., Jimenez-Barbero J., Bernabe M., Leal J. A., Prieto A. and Gomez-Miranda B. (1994) Structural investigation of two cell-wall polysaccharides of *Penicillium expansum* strains. Carbohydr. Res. **257:** 239–248

28 Takayanagi T., Kimura A., Chiba S. and Ajisaka K. (1994) Novel structures of N-linked high-mannose type oligosaccharides containing α-D-galactofuranosyl linkages in *Aspergillus niger* α-D-glucosidase. Carbohydr. Res. **256:** 149–158

29 Nakajima T., Yoshida M., Nakamura M., Hiura N. and Matsuda K. (1984) Structure of the cell wall proteogalactomannan from *Neurospora crassa*. II. Structural analysis of the polysaccharide part. J. Biochem. **96:** 1013–1020

30 Barr K., Laine R. A. and Lester R. L. (1984) Carbohydrate structures of three novel phosphoinositol-containing sphingolipids from the yeast *Histoplasma capsulatum*. Biochemistry **23:** 5589–5596

31 Levery S. B., Toledo M. S., Straus A. H. and Takahashi H. K. (1998) Structure elucidation of sphingolipids from the mycopathogen *Paracoccidioides brasiliensis*: an immunodominant beta-galactofuranose residue is carried by a novel glycosylinositol phosphorylceramide antigen. Biochemistry **37:** 8764– 8775

32 Golgher D. B., Colli W., Souto-Padron T. and Zingales B. (1993) Galactofuranose-containing glycoconjugates of epimastigote and trypomastigote forms of *Trypanosoma cruzi*. Mol Biochem Parasitol. **60:** 249–264

33 Trejo A. G., Chittenden G. J., Buchanan J. G. and Baddiley J. (1970) Uridine diphosphate alpha-D-galactofuranose, an intermediate in the biosynthesis of galactofuranosyl residues. Biochem J. **117:** 637–639

34 Nikaido H. and Sarvas M. (1971) Biosynthesis of TI antigen in salmonella: biosynthesis in a cell-free system. J. Bacteriol. **105(3):** 1073–1082

35 Huang C. and Turco S. J. (1993) Defective galactofuranose addition in lipophosphoglycan biosynthesis in a mutant of *Leishmania donovani*. J. Biol. Chem. **268:** 24060–24066

36 Weston A., Stern R. J., Lee R. E., Nassau P. M., Monsey D., Martin S. L. et al. (1998) Biosynthetic origin of mycobacterial cell wall galactofuranosyl residues. Tuber. Lung Dis. **78:** 123–131

37 Nassau P. M., Martin S. L., Brown R. E., Weston A., Monsey D., McNeil M. R. et al. (1996) Galactofuranose biosynthesis in *Escherichia coli* K-12: Identification and cloning of UDP-galactopyranose mutase. J. Bacteriol. **178:** 1047–1052

38 Koplin R., Brisson J. R. and Whitfield C. (1997) UDP-galactofuranose precursor required for formation of the lipopolysaccharide O antigen of *Klebsiella pneumoniae* serotype O1 is synthesized by the product of the rfbDKPO1 gene. J. Biol. Chem. **272:** 4121–4128

39 Guan S., Clarke A. J. and Whitfield C. (2001) Functional analysis of the galactosyltransferases required for biosynthesis of D-galactan I, a component of the lipopolysaccharide O1 antigen of *Klebsiella pneumoniae*. J. Bacteriol. **183:** 3318–3327

40 Pan F., Jackson M., Ma Y. and McNeil M. (2001) Cell wall core galactofuran synthesis is essential for growth of mycobacteria. J. Bacteriol. **183:** 3991–3998

41 Kremer L., Dover L. G., Morehouse C., Hitchin P., Everett M., Morris H. R. et al. (2001) Galactan biosynthesis in *Mycobacterium tuberculosis*. Identification of a bifunctional UDP-galactofuranosyltransferase. J. Biol. Chem. **276:** 26430–26440

42 Whitfield C. (1995) Biosynthesis of lipopolysaccharide O antigens. Trends Microbiol. **3:** 178–185

43 Heine H., Rietschel E. T. and Ulmer A. J. (2001) The biology of endotoxin. Mol. Biotechnol. **19:** 279–296

44 Barry C.E. 3rd (1997) New horizons in the treatment of tuberculosis. Biochem. Pharmacol. **54:** 1165–1172

45 Daffe M., Brennan P. J. and McNeil M. (1990) Predominant structural features of the cell wall arabinogalactan of *Mycobacterium tuberculosis* as revealed through characterization of oligoglycosyl alditol fragments by gas chromatography/mass spectrometry and by 1H and 13C NMR analyses. J. Biol. Chem. **265:** 6734–6743

46 McNeil M., Daffe M. and Brennan P. J. (1991) Location of the mycolyl ester substituents in the cell walls of mycobacteria. J. Biol. Chem. **266:** 13217–13223

47 McNeil M., Daffe M. and Brennan P. J. (1990) Evidence for the nature of the link between the arabinogalactan and peptidoglycan of mycobacterial cell walls. J. Biol. Chem. **265:** 18200–18206

48 McNeil M., Besra G. S. and Brennan P. J. (1996) Chemistry of the mycobacterial cell wall. In: Tuberculosis, pp. 171–186, Rom W. N. and Garay S. M. (eds), Little, Brown, Boston

49 Takayama K. and Kilburn J. O. (1989) Inhibition of synthesis of arabinogalactan by ethambutol in *Mycobacterium smegmatis*. Antimicrob. Agents Chemother. **33:** 1493–1499

50 Turco S. J. and Descoteaux A. (1992) The lipophosphoglycan of *Leishmania* parasites. Annu. Rev. Microbiol. **46:** 65–94

51 Descoteaux A. and Turco S. J. (1999) Glycoconjugates in *Leishmania* infectivity. Biochim. Biophys. Acta Mol. Basis Dis. **1455:** 341–352

52 McConville M. J. and Homans S. W. (1992) Identification of the defect in lipophosphoglycan biosynthesis in a non-pathogenic strain of *Leishmania* major. J. Biol. Chem. **267:** 5855–5861

53 Sacks D. L. (1989) Metacyclogenesis in *Leishmania promastigotes* (1989) Exp. Parasitol. **69:** 100–103

54 Puentes S. M., Da Silva R. P., Sacks D. L., Hammer C. H. and Joiner K. A. (1990) Serum resistance of metacyclic stage *Leishmania major promastigotes* is due to release of C5b-9. J. Immunol. **145:** 4311–4316

55 Puentes S. M., Sacks D. L., da Silva R. P. and Joiner K. A. (1988) Complement binding by two developmental stages of *Leishmania major promastigotes* varying in expression of a surface lipophosphoglycan. J. Exp. Med. **167:** 887–902

56 Beverley S. M. and Turco S. J. (1998) Lipophosphoglycan (LPG) and the identification of virulence genes in the protozoan parasite *Leishmania*. Trends Microbiol. **6:** 35–40

57 Desjardins M. and Descoteaux A. (1997) Inhibition of phagolysosomal biogenesis by the *Leishmania lipophosphoglycan*. J. Exp. Med. **185:** 2061–2068

58 Guha-Niyogi A., Sullivan D. R. and Turco S. J. (2001) Glycoconjugate structures of parasitic protozoa. Glycobiology **11:** 45R–59R

59 Zhang Q. and Liu H. (2000) Studies of UDP-galactopyranose mutase from *Escherichia coli*: An unusual role of reduced FAD in its catalysis. J. Am. Chem. Soc. **122:** 9065–9070

60 Zhang Q. and Liu H. (2001) Mechanistic investigation of UDP-galactopyranose mutase from *Escherichia coli* using 2- and 3-fluorinated UDP-galactofuranose as probes. J. Am. Chem. Soc. **123:** 6756–6766

61 McMahon S. A., Leonard G. A., Buchanan L. V., Giraud M. F. and Naismith J. H. (1999) Initiating a crystallographic study of UDP-galactopyranose mutase from *Escherichia coli*. Erratum. Acta Crystallogr. D Biol. Crystallogr. **55:** 1108

62 Sanders D. A., McMahon S. A., Leonard G. L. and Naismith J. H. (2001) Molecular placement of experimental electron density: a case study on UDP-galactopyranose mutase. Acta Crystallogr. D Biol Crystallogr. **57 (Pt 10):** 1415–1420

63 Sanders D. A., Staines A. G., McMahon S. A., McNeil M. R., Whitfield C. and Naismith J. H. (2001) UDP-galactopyranose mutase has a novel structure and mechanism. Nat. Struct. Biol. **8:** 858–863
64 Barlow J. N. and Blanchard J. S. (2000) Enzymatic synthesis of UDP-(3-deoxy-3-fluoro)-D-galactose and UDP-(2-deoxy-2-fluoro)-D-galactose and substrate activity with UDP-galactopyranose mutase. Carbohydr. Res. **328:** 473–480
65 Reeves R., Hobbs M., Valvano M. A., Skurnik M., Whitfield C., Coplin D. et al. (1996) Bacterial polysaccharide synthesis and gene nomenclature. Trends Microbiol. **4:** 495–503
66 Barlow J. N., Girvin M. E. and Blanchard J. S. (1999) Positional isotope exchange catalyzed by UDP-galactopyranose mutase. J. Am. Chem. Soc. **121:** 6968–6969
67 Clarke B. R., Bronner D., Keenleyside W. J., Severn W. B., Richards J. C. and Whitfield C. (1995) Role of Rfe and RfbF in the initiation of biosynthesis of D-galactan I, the lipopolysaccharide O antigen from *Klebsiella pneumoniae* serotype O1. J. Bacteriol. **177:** 5411–5418
68 Ramaswamy S. and Musser J. M. (1998) Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tuber. Lung Dis. **79:** 3–29
69 Quemard A., Lacave C. and Laneelle G. (1991) Isoniazid inhibition of mycolic acid synthesis by cell extracts of sensitive and resistant strains of *Mycobacterium aurum*. Antimicrob. Agents Chemother. **35:** 1035–1039
70 Baulard A. R., Betts J. C., Engohang-Ndong J., Quan S, McAdam R. A., Brennan J. et al. (2000) Activation of the prodrug ethionamide is regulated in mycobacteria. J. Biol. Chem. **275:** 28326–28331
71 McNeil M., Wallner S. J., Hunter S. W. and Brennan J. (1987) Demonstration that the galactosyl and arabinosyl residues in the cell-wall arabinogalactan of *Mycobacterium leprae* and *Mycobacterium tuberculosis* are furanoid. Carbohydr. Res. **166:** 299–308
72 Mikusova K., Yagi T., Stern R., McNeil M. R., Besra G. S., Crick D. C. et al. (2000) Biosynthesis of the galactan component of the mycobacterial cell wall. J. Biol. Chem. **275:** 33890–33897
73 Ilg T. (2001) Lipophosphoglycan of the protozoan parasite *Leishmania*: stage- and species-specific importance for colonization of the sandfly vector, transmission and virulence to mammals. Med. Microbiol. Immunol. **190:** 13–17
74 McConville M. J. and Ferguson M. A. J. (1993) The structure, biosynthesis and function of glycosylated phosphatidylinositols in the parasitic protozoa and higher eukaryotes. Biochem. J. **294:** 305–324
75 Sacks D. and Kamhawi S. (2001) Molecular aspects of parasite-vector and vector-host interactions in Leishmaniasis. Annu. Rev. Microbiol. **55:** 453–483
76 Sacks D. L., Saraiva E. M., Rowton E., Turco S. J. and Pimenta P. F. (1994) The role of the lipophosphoglycan of *Leishmania* in vector competence. Parasitology **108 Suppl.:** S55–S62
77 King D. L. and Turco S. J. (1988) A ricin agglutinin-resistant clone of *Leishmania donovani*. Mol. Biochem. Parasitol. **28:** 285–294
78 Elhay M., Kelleher M., Bacic A., McConville M. J., Tolson D. L., Pearson T. W. et al. (1990) Lipophosphoglycan expression and virulence in ricin-resistant variants of *Leishmania major*. Mol. Biochem. Parasitol. **40:** 255–268
79 McNeely T. B. and Turco S. J. (1990) Requirement of lipophosphoglycan for intracellular survival of *Leishmania donovani* with human monocytes. J. Immunol. **144:** 2745–2750
80 McNeely T. B., Tolson D. L., Pearson T. W. and Turco S. J. (1990) Characterization of *Leishmania donovani* variant clones using anti-lipophosphoglycan monoclonal antibodies. Glycobiology **1:** 63–69
81 Ryan K. A., Garraway L. A., Descoteaux A., Turco S. J. and Beverley S. M. (1993) Isolation of virulence genes directing surface glycosyl-phosphatidylinositol synthesis by functional complementation of *Leishmania*. Proc. Natl. Acad. Sci. USA **90:** 8609–8613
82 Ilg T. (2000) Lipophosphoglycan is not required for infection of macrophages or mice by *Leishmania mexicana*. EMBO J. **19:** 1953–1962
83 Spath G. F., Epstein L., Leader B., Singer S. M., Avila H. A., Turco S. J. et al. (2000) Lipophosphoglycan is a virulence factor distinct from related glycoconjugates in the protozoan parasite *Leishmania major*. Proc. Natl. Acad. Sci. USA **97:** 9258–9263
84 Sacks D. L., Modi G., Rowton E., Spath G., Epstein L., Turco S. J. et al. (2000) The role of phosphoglycans in *Leishmania*-sand fly interactions. Proc. Natl. Acad. Sci. USA **97:** 406–411