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GLYCOPINION

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The trypanosomatids are unicellular eukaryotic organisms, many of which are the cause of serious human disease. They undergo complex life-cycles, alternating between the tissues or blood of the mammalian host and the gut of an arthropod vector. Each stage is marked by biochemical and morphological changes, including changes in the expression of surface glycoconjugates. Members of the family include *Trypanosoma cruzi*, the cause of South American Chagas disease, *Trypanosoma brucei*, the cause of African sleeping sickness, and species of *Leishmania* which are responsible for a number of diseases throughout the tropical and subtropical world.

It is recognized that parasite surface glycoconjugates play a crucial role in various functions such as immune evasion, complement resistance, host-cell binding and interiorization, and parasite differentiation. As such, they represent key virulence determinants. They also contribute the major parasite surface antigens. In this review Susanne Zamze discusses the structure and function of parasite surface glycoconjugates, knowledge of which is essential in understanding host–parasite interactions at the molecular level.

The questions raised include:

- What are the similarities and differences between glycosylation in the protozoa and higher eukaryotes?
- How could a knowledge of parasite oligosaccharide structure lead to possible drug therapies?
- How do parasite surface glycoconjugates contribute to the survival of the parasite within the host? What is the significance of the glycan moieties?
- The trypanosomatids are thought to have separated from other eukaryotes about one billion years ago. What can be learnt about their evolution from their glycosylation?
- What can be learnt about alternative biosynthetic pathways from a study of parasites?

Glycosylation in parasitic protozoa of the Trypanosomatidae family

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Over the last few years enormous interest has been shown in the structures of the glycan moieties of various parasite surface glycoconjugates. Structures have been determined for the glyco-components of glycosylphosphatidylinositol (GPI) protein membrane anchors, for asparagine-linked oligosaccharides, and for the glycans of complex glycolipids. The following attempts to illustrate a few of the most salient observations with regard to the structures and possible functions of parasite surface glycans.

Leishmania glycolipids

The surfaces of *Leishmania* species are particularly rich in carbohydrate in the form of the glycan moieties of complex glycolipids. The parasite multiplies intracellularly in the phagolysosomes of mammalian host macrophages and must therefore recognize, bind to and penetrate its target cells. It must also survive in a harsh hydrolytic environment, both in the gut of the insect vector and with phagolysosomes, and evade complement lysis prior to host-cell penetration. There is now good evidence that many of these functions are conferred by its surface glycolipids and that the glycan portion is probably of key significance.

Two main classes of glycolipid have been identified on the surface of promastigotes (insect stage). These are the GIPLs (glycosylphosphatidylinositols), also known as GPIs (glycoinositolphospholipids), and the LPGs (lipophosphoglycans). The GIPL/GPIs are a heterogeneous group of low molecular weight glycolipids consisting of three to six saccharide residues linked to an alkylacylphosphatidylinositol [1, 2, 3, 4]. The LPGs are a similarly heterogeneous group of molecules. Their basic structural motif is of a large phosphoglycan sidechain attached, via a phosphorylated hexasaccharide core, to a lysoalkylphosphatidylinositol lipid moiety. The structure and function of LPGs are reviewed in Turco [5].

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The glycans of both GIPLs and LPGs show structural variation according to both species and life-cycle or growth stages. Structural variation in LPGs is limited almost entirely to the sidechain region. The sidechains from all three main species of *Leishmania* (*major*, *mexicana* and *donovani*) are based on the same linear backbone sequence, composed of repeating phosphodiester linked Gal$p\beta$1-4Man disaccharide units. Species variation arises due to differences in the degree of polymerization of the sidechain and by substitution of the backbone with other sugar residues. In the simplest case, that of *L. donovani* [6], there is negligible substitution; in the most complex, *L. major* [7], the backbone is substituted with sidebranches of variable composition containing between one and four saccharide units. In addition, some or all of the *L. major* sidechains appear to be capped at the non-reducing terminus with a mannose disaccharide. The possible significance of this modification to host–parasite interactions is not known.

Growth of promastigotes in axenic culture is associated with a transformation from non-infective (log-phase) to infective (stationary-phase) forms and with a modification of LPG structure [5, 8, 9, 10]. LPG modification takes two forms: an approximate doubling in the degree of polymerization of the sidechain in *L. donovani* and *L. major*, and an apparent structural modification of the repeating units in all three species. A similar transformation of promastigotes to that seen in culture, including LPG modification, occurs during the development of the parasite in the insect vector. LPG may be involved in this process [11].

LPG is also believed to have a number of functions related to the infection of host macrophages by promastigotes. Infective promastigotes show greater resistance to complement mediated lysis. The modified LPG sidechains of the infective form are thought to be important in protecting the surface membrane from complement-mediated damage [12]. Mutants, defective in LPG glycosylation, have been shown to be of decreased virulence [13] reminiscent to the association of the loss of O-antigen polysaccharide with increased sensitivity to complement, and an avirulent phenotype in bacterial pathogens. LPGs have also been implicated in the binding and entry of promastigotes into macrophages [14]. To this effect, LPGs appear to be major acceptors for C3b by activation of the classical pathway of complement and, consequently, may facilitate the uptake of opsonized parasites via macrophage CR1 and/or CR3 receptors. LPG is further thought to promote the initial survival of promastigotes within the phagolysosomal compartment [5, 13, 15] by inhibiting the macrophage oxidative burst and protecting the parasite surface from hydrolytic enzymes or oxygen free radicals. Recently, however, it has been shown that LPG is absent or expressed only in very low copy number, in the amastigote (intracellular form) of *L. donovani* [2]. LPG does not appear, therefore, to be required for continued intracellular survival in this species. However, LPG-like molecules, which may be related to intracellular survival, have been identified on the amastigote forms of *L. major* [16, 17].

There are still many questions as to the exact mechanism of leishmanial-macrophage infection [14]. For example, the relative contribution of other parasite components, in particular the major surface glycoprotein (gp63), to uptake, the role of serum opsonins as opposed to direct binding of parasite components to macrophage receptors, and of the relative use of different macrophage receptors. There is also evidence that the mode of uptake, and possibly of intracellular survival, may differ between species.

Unlike LPG, no specific functions can as yet be attributed to the GIPL glycolipids. However, the GIPLs [18] as well as LPG [2, 19] are a major surface component, occupying a considerable percentage of the cell surface and are likely to be functionally important in the plasma membrane. The GIPLs from *L. major* [1, 3, 4] show a partial structural homology with the LPG core glycan region [7, 20]; those from *L. donovani*, in contrast, are exclusively of an oligomannose type, containing between one and four mannose residues [2]. The GIPLs from *L. donovani* are expressed abundantly in both the promastigote and amastigote forms, whereas the LPG from this species is present only at the promastigote stage. The structures of the GIPL oligomannose moieties are also different between the two life-stages in this species.

LPGs and GIPLs both have novel glycan structures that are unique to the parasite. The LPG sidechain phosphoglycan is a particularly unusual structure for a eukaryotic glycoconjugate. Indeed, the occurrence of phosphodiester linked sugar repeating units shows greater similarity with bacterial teichoic acid-like molecules than with eukaryotic glycans. Galactofuranose and arabinose, two highly unusual components for eukaryotic glycans, are present in the LPG core, *L. major* GIPLs, and the *L. major* sidechain, respectively. Given these unusual structures, it is not surprising that the leishmanial glycolipids are highly immunogenic. The structural heterogeneity of the glycolipids is reflected in serological studies and this may be a useful criterion to adopt for the development of a serotyping system. Furthermore, there is evidence that LPG may be involved in the induction of a host protective immune response [21].

Each species of *Leishmania* displays a distinctive tissue tropism. The pathological consequence of infection differs accordingly. Whether the species-specific differences in GIPL and LPG play a role in mediating the different host–parasite interactions is an intriguing but unanswered question. Again, how the structural variations of GIPL and LPG with life-cycle stage may be related to the different host–parasite interactions is not known.

N-glycosylation

The N-linked glycans from the trypanosomatids show a far greater similarity with their mammalian counterparts than

the leishmanial glycolipids. However, unique aspects of N-glycosylation have been observed. The trypanosomatids, *T. cruzi*, *Leishmania* and various insect parasites, are unable to synthesize dol-P-Glc and are unique among wild type eukaryotic cells in transferring non-glucosylated oligosaccharides from dolichol pyrophosphate to asparagine residues in the polypeptide backbone. N-glycosylation in these trypanosomatids is reviewed in Bosch *et al.* [22]. The oligosaccharides transferred contain between nine and six mannose residues, depending on the species and life-cycle stage. For example, *L. mexicana* promastigotes transfer $Man_6GlcNAc_2$, *T. cruzi* epimastigotes (insect stage) $Man_9GlcNAc_2$ and *T. cruzi* trypomastigotes (infective bloodstream form) mainly $Man_7GlcNAc_2$. The $Man_9GlcNAc_2$ glycan has a classical branched structure and is processed by the removal of mannose residues to give rise to the typical $Man_{(9-5)}GlcNAc_2$ series. The $Man_7GlcNAc_2$ and $Man_6GlcNAc_2$ glycans have the same biantennary structures as the lipid-linked intermediates in higher eukaryotes. Processing of these structures consequently gives rise to a highly unusual series of biantennary oligomannoses on the mature glycoprotein. In accordance with these observations the dolichol pyrophosphate protein oligosaccharyltransferase enzyme from trypanosomatids shows a lack of specificity in a cell free assay with regard to the glucosylation and number of mannose residues in the lipid-linked glycan [23]. This is in marked contrast to higher eukaryotes where there is a marked preference for transfer of $Glc_3Man_9GlcNAc_2$.

Two unique modifications of protein bound oligomannoses have been described in the trypanosomatids. These are: possible substitution with terminal galactofuranose residues [24, 25], and the presence of a single terminal Glc residue on the $Man_6GlcNAc_2$ oligosaccharide of the major surface glycoprotein, gp63, from *L. mexicana* [26].

A transient glycosylation of protein bound N-linked oligomannose was first demonstrated in the trypanosomatids and has since been described for a variety of mammalian, plant and fungal cells. The process involves transfer followed by the rapid removal of a single α(1-3)-linked Glc residue to the Manα(1-3)-arm. Transfer occurs directly from UDP-Glc and is distinct from glucosylation of the biosynthetic intermediates [27]. In agreement with the observed patterns of glucosylation in the parasites, trypanosomatids contain glucosidase II activity, capable of removing α(1-3)-linked residues, but not glucosidase I activity responsible for removal of the terminal (1-2)-linked Glc of the $Glc_3Man_9GlcNAc_2$ intermediate [27]. Whether the Glc residue in gp63 is related to a biosynthetic intermediate or to the transient glucosylation of protein bound oligosaccharide is not known.

Details of the pathway of N-glycosylation in *T. brucei* are not known. The variant surface glycoprotein (VSG) from *T. brucei* contains typical oligomannose oligosaccharides but also non-typical oligomannoses containing four and three mannose residues [28, 29, 30]. The latter appear very rapidly during VSG biosynthesis and may possibly result from a separate biosynthetic pathway.

T. brucei lives extracellularly in the bloodstream of the mammalian host and, not surprisingly, has a very different strategy for survival from the intracellular parasites. The surface is covered by a single species of glycoprotein, the VSG. The parasites evade immune attack by sequential expression of immunologically distinct VSG coats. The VSG coat is also required to prevent lysis by complement in the absence of specific antibodies to the coat. All VSGs contain one or more N-linked oligosaccharide.

Lectin binding studies have indicated that this carbohydrate is not exposed at the surface of the bloodstream form of *T. brucei* [31] but is exposed at the surface of the related African parasites *T. congolense* [32] and *T. equiperdum* [33]. Carbohydrate is also exposed at the surface of the procyclic (insect) forms of all the African trypanosomatids, including *T. brucei* [34], and may be involved in a lectin-like interaction of this life stage with the insect gut.

N-glycosylation does not appear to be required for the intracellular transport and deposition of VSG on the cell surface [30, 35, 36] and no specific function has so far been attributed to the VSG N-glycans from *T. brucei*. They do, however, appear to be necessary for the formation of typical VSG dimeric units and may be involved in maintaining the long term stability of the coat [36]. Some of the N-linked glycans, namely the poly-*N*-acetyllactosamines, on *T. brucei* VSG are extremely large and complex [29]. Since *T. brucei* is efficiently adapted to co-exist with its natural host species, it is difficult to understand why the parasite should expend energy to elaborate complex glycans if they are not of some biological relevance.

The basic functions of N-glycosylation in the protozoa, in many cases, are likely to be the same as those in other eukaryotic cells, such as correct protein folding, transport, secretion or surface expression and to confer protease resistance. Some of the trypanosomatid glycoproteins have enzymic activity or are membrane bound surface receptors and the N-links may be important to their activity [37]. N-glycosylation is known to be necessary for the stability of *Leishmania* gp63 [38], which itself has protease activity, and for the protease resistance of surface bound VSG in *T. congolense* [39].

Novel N-linked glycans are potentially antigenic. Whether they are involved in any way in mediating host–parasite interactions is not known. The sera of patients with trypanosomatid infections contain elevated levels of antibody against Galα(1-3)Gal moieties [40]. This correlates with the known expression of the Galα(1-3)Gal epitope on N-linked glycans in *T. cruzi* [41] and *T. brucei* [29] and on leishmanial glycolipids [4], the latter also containing the possibly cross reacting Galα(1-3)Gal*f* structure [1, 4].

GPI membrane anchors

The GPI moiety provides a stable means of anchoring a protein in the plasma membrane and is the main mechanism used for membrane anchorage in the trypanosomatids. For a recent review on GPI anchors refers to Thomas *et al.* [42]. The GPI glycans of the trypanosomatids contain the same conserved glycan core structure found in all eukaryotes investigated so far. The GPI from *T. brucei* VSG, in addition, contains a unique glycan sidebranch composed of a variable number of α-linked Gal residues. As with the VSG N-linked oligosaccharides no function can as yet be attributed to the Gal sidebranch. It is conceivable that both are necessary to maintain the structural integrity of the VSG coat. The α-Gal branch is absent from procyclin, the major surface glycoprotein of the procyclic insect form of *T. brucei* [43] and the GPI anchor from *L. major* gp63 similarly contains the conserved core glycan without any modification to the basic structure [44].

The surface glycoconjugates from the various trypanosomatids discussed are very different reflecting the different life-cycles and host specificities. It seems that few, if any, generalizations can be made about their glycosylation and that each glycoconjugate requires separate study to ascertain its function and the possible role of the glycan moiety. The process of binding and internalization of the intracellular parasites, particularly of *T. cruzi*, is not well understood. In the case of *T. cruzi*, preliminary evidence involving the use of inhibitors and enzymic modification of surface glycoconjugates suggests that glycans present on both the parasite and host cell are involved in the process [45, 46].

The trypanosomatid glycans, in many cases, have unique structures distinct from those of the mammalian host, and as such are potential targets for drug therapy and possibly vaccine development. The leishmanial glycolipids, being structurally distinct, immunogenic and involved in host-cell infectivity would appear to offer particularly good prospects in this respect.

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