Parasitology

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Glycans in the roles of parasitological diagnosis and host–parasite interplay

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Review

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Abstract

The investigation of the glycan repertoire of several organisms has revealed a wide variation in terms of structures and abundance of glycan moieties. Among the parasites, it is possible to observe different sets of glycoconjugates across taxa and developmental stages within a species. The presence of distinct glycoconjugates throughout the life cycle of a parasite could relate to the ability of that organism to adapt and survive in different hosts and environments. Carbohydrates on the surface, and in excretory-secretory products of parasites, play essential roles in host–parasite interactions. Carbohydrate portions of complex molecules of parasites stimulate and modulate host immune responses, mainly through interactions with specific receptors on the surface of dendritic cells, leading to the generation of a pattern of response that may benefit parasite survival. Available data reviewed here also show the frequent aspect of parasite immunomodulation of mammalian responses through specific glycan interactions, which ultimately makes these molecules promising in the fields of diagnostics and vaccinology.

Introduction

As in many fields of biology, the primary goals of molecular and biochemical investigations of parasitic helminths have been directed towards characterizing the functional biology of their proteins and peptides. Studies on the contributions of helminth glycans, or carbohydrates, to protein structure and function, to parasite biology and to host–parasite interplay has lagged somewhat, although some pioneering work has called attention to these molecules. Norden and Strand (1985), for example, observed the relevance of a glycan present in *Schistosoma mansoni* extracts, strongly recognized by lectin concanavalin A, to the diagnosis of schistosomiasis. Methodological improvements in studies of glycans since the 1980s have allowed researchers to gain key insights into these molecules among various helminths and protozoans. Notably, the roles that glycans play in several aspects of parasitism, including contributions to antigenicity, pathogenicity, signalling, recognition, attachment and evasion of host defences, are being progressively taken into consideration. In this review we have compiled information about glycans of a wide range of parasites, including protozoans and helminths, discussing their involvement in pathogenicity, host–parasite interactions and their potential application for the development of diagnostic methods.

Glycans: an overview

Carbohydrates are organic molecules consisting of carbon, hydrogen and oxygen. These molecules are commonly called saccharides, glycans or sugars. Besides their central role in metabolism, carbohydrates are essential components of a broad range of biological processes that occur in eukaryotes and prokaryotes, including cell recognition, signalling and interaction, fertilization, virus invasion and replication and host–pathogen interactions (Handel *et al.*, 2005; van Liempt *et al.*, 2007; Fincher, 2009).

Glycans are often bound to proteins and lipids and the modified molecules are identified collectively as glycoconjugates. Proteins are glycosylated through complex biochemical pathways reliant on sequential action of a variety of enzymes, mainly glycosidases and glycosyltransferases. The attachment of oligo- or polysaccharides to the polypeptide structure is the most frequent post-translational modification observed in all living organisms (Spiro, 2002). By modifying the form of glycan linkage, and the structure of the glycan itself can directly influence the properties of a glycoprotein (Cipollo *et al.*, 2005; Varki, 2017).

In eukaryotic cells, the several reactions necessary for protein glycosylation are compartmentalized in the endoplasmic reticulum and Golgi apparatus. In these organelles, key glycosylation enzymes may generate a wide diversity of structures. *N*- and *O*-glycosylation are the two major forms of protein glycosylation. During these processes, carbohydrates are attached to specific glycosylation sites in the protein backbone. For *N*-glycosylation, the first residue of *N*-acetylglucosamine (GlcNAc) of a chitobiose core ($Man_3GlcNAc_2$) is attached to an

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asparagine (Asn) residue of a consensus sequence composed of Asn-*X*-serine/threonine (Ser/Thr) (where *X* can be any amino acid except proline). For *O*-glycosylation, in most instances, a residue of *N*-acetylgalactosamine (GalNAc) is attached firstly to Ser/Thr residues of mucin and mucin-like proteins (Haslam et al., 2001). However *O*-glycans with mannose, GlcNAc or fucose residues occupying the first position are also observed in glycoproteins (Thaysen-Andersen and Packer, 2014). Following this step, a residue of Gal(β1-3) or GlcNAc(β1-6) is added into the first GalNAc giving rise to the core type 1 [Gal(β1-3)GalNAc] or type 2 [GlcNAc(β1-6)GalNAc] glycans, respectively. Subsequent additions of different monosaccharides or other glycan modifications may happen to the non-reducing termini, in specific configurations (Spiro, 2002; Varki, 2017). The availability of enzymes, carbohydrates and the characteristics of the protein undergoing glycosylation will define the repertoire of glycans that a specific protein, cell or organism will express. An individual protein can contain either *N*- or *O*-glycans, or a combination of both (Haslam et al., 2001; Thaysen-Andersen and Packer, 2014).

Parasite glycans and the host–parasite relationship

The mechanisms of protein glycosylation are highly conserved among eukaryotes, despite the genetic diversity of member taxa. Nonetheless, structural variation within the glycan cores and termini of glycoproteins and glycolipids of parasites have been observed (Fig. 1) (Haslam *et al.*, 2001; Hokke *et al.*, 2007). Therefore, is not surprising that diverse eukaryotic parasites were observed to express a wide diversity of glycans or glycoconjugates containing distinctive and unusual monosaccharides residues or terminal modifications (caps), or even oligosaccharides linked in rare configurations (Table 1). The generation of such specific molecules will depend on the cellular glycosylation machinery, which comprises enzymes such as nucleotide sugar synthases, glycosidases and glycosyltransferases, which perform the necessary glycosylation reactions (Haslam *et al.*, 2001; Nyame *et al.*, 2004). On the other hand, *protozoan parasites often display* oligosaccharides attached to phosphatidylinositol, forming what is known as glycosylphosphatidylinositol (GPI) anchors at their surface. These anchors are the most important and common post-translational modifications in proteins across the vast diversity of protozoan parasites (Mendonça-Previato *et al.*, 2003).

The diverse glycan repertoire of parasites often include moieties that also form the so-called 'host-like' glycans (van Die and Cummings, 2010). Motifs such as Lewis X [Gal1,4(Fucα1-3)GlcNAc; Le^X], LacdiNAc (GalNAcβ1,4GlcNAc; LDN), fucosylated LDN [GalNAcβ1,4(Fucα1-3)GlcNAc; LDNF], truncated *O*-glycans, known as *T* antigen (Galβ1-3GalNAcα1-*O*-Thr/Ser), as well as *Tn* antigen (GalNAc-*O*-Ser/Thr), and GPI anchors modified by different glycans are examples of structures found both in parasites and humans. Nevertheless, helminth parasites in general present *Tn* antigen in greater abundance than do human cells, whereas no sialic acid termini, other than the trans-sialidase-mediated sialylation in trypanosomatids (Schauer *et al.*, 1983), are observed in glycans isolated from parasites (Cummings, 2009; Hokke and van Diepen, 2017).

The glycan repertoire that a parasite presents throughout its life cycle is thought to be of fundamental importance to avoid detection and clearing from the host, ultimately enabling the establishment of a chronic infection. Many helminth-glycans can subvert host immune responses by suppressing it or by inducing T-helper 2 (Th2) type response, activate T regulatory cells (Tregs) and alternatively activate macrophages (Sher *et al.*, 2003; Maizels *et al.*, 2004; Tundup *et al.*, 2012). To mimic host glycan is, therefore, a parasite strategy for survival. The mimicked antigens modulate immune responses through direct interaction with receptors on the surface of antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages, presenting themselves as 'self-like' molecules and not as foreign antigens, consequently preventing the host from attacking the parasite (van Die and Cummings, 2010).

Indeed, during the development and differentiation of the parasites within their mammal hosts, DCs are exposed to various parasite-derived antigens that modulate their functions and maturation (Linehan *et al.*, 2003; Van Liempt *et al.*, 2007; Terrazas *et al.*, 2010). Such modulation occurs through DC receptors, including the classes of Toll-like (TLR), the C-type Lectin receptor (CLRs) and other lectin receptors, that recognize specific glycan motifs (Akira *et al.*, 2006; Diebold, 2009; Terrazas *et al.*, 2010). It has been proposed that CLRs induce an intracellular signalling pathway upon the recognition of carbohydrates that might lead to impairment of TLR-mediated signalling (Geijtenbeek and Gringhuis, 2009).

Each different class of lectin receptors, including DC-SIGN (dendritic cell-specific ICAM3-grabbing non-integrin), MGL (macrophages galactose-type lectin) and MBL (mannose-binding lectin) have been demonstrated to recognize specific host-like or parasite-specific glycan moieties. DC-SIGN receptors, for example, interact with Lewis-X (Le^x), LDNF and high mannose *N*-glycans that are commonly found in extracts of various parasites (Table 1), but which are also expressed by human cells (Okano *et al.*, 2001; Geijtenbeek *et al.*, 2003; Gomez-Garcia *et al.*, 2006). Therefore, upon their recognition by DC receptors, TLR-mediated signalling and immune response against the parasite presenting these motifs will be dampened. Even though the mechanism of immune modulation through glycan receptors is not fully understood, it is known that DCs bind and internalize glycans derived from *S. mansoni* eggs through MGL, mannose receptor and DC-SIGN receptors, ultimately driving a T-helper 2 (Th2) response fundamental for the establishment of infection (Van Liempt *et al.*, 2007).

Alternatively, at the parasite surface, carbohydrates may present as a rigid and resilient layer that aid the parasite in evading cellular immune responses (Taratuto and Venturello, 1997; Kusel *et al.*, 2007). A classic example of this mechanism is observed during infection with the *Trypanosoma brucei* species complex in Africa. *Trypanosoma* encodes many genes belonging to the variant surface glycoprotein (VSG) family of proteins (discussed further below), each of them giving origin to a distinct glycoprotein that will present a unique immune signature to the host (Ferguson, 1999). Thereby, this parasite can constantly stall immune mediated killing of newly members of the blood-resident population. In the same context, changes of the glycan surface of schistosome cercariae, which completely replace their surface membrane of the tegumentary syncytium after host invasion, is thought to allow *Schistosoma* to survive and complete its life cycle within its definitive host after skin penetration (Jones *et al.*, 2004).

Glycoconjugates of protozoan parasites

Trypanosoma

Trypanosoma (Euglenozoa: Kinetoplastida: Trypanosomatidae) is a widespread genus of flagellated parasites of vertebrates and invertebrates. Two species of *Trypanosoma* primarily cause disease in humans, *T. cruzi* in the Americas causing Chagas disease and members of the *T. brucei* species complex in Africa. Two subspecies of the latter, *T. brucei gambiense* and *T. brucei rhodesiense,* cause blood infection that eventually reaches the central nervous system causing sleeping sickness in humans and nagana in cattle. *Trypanosoma brucei* survives freely in the bloodstream of its hosts

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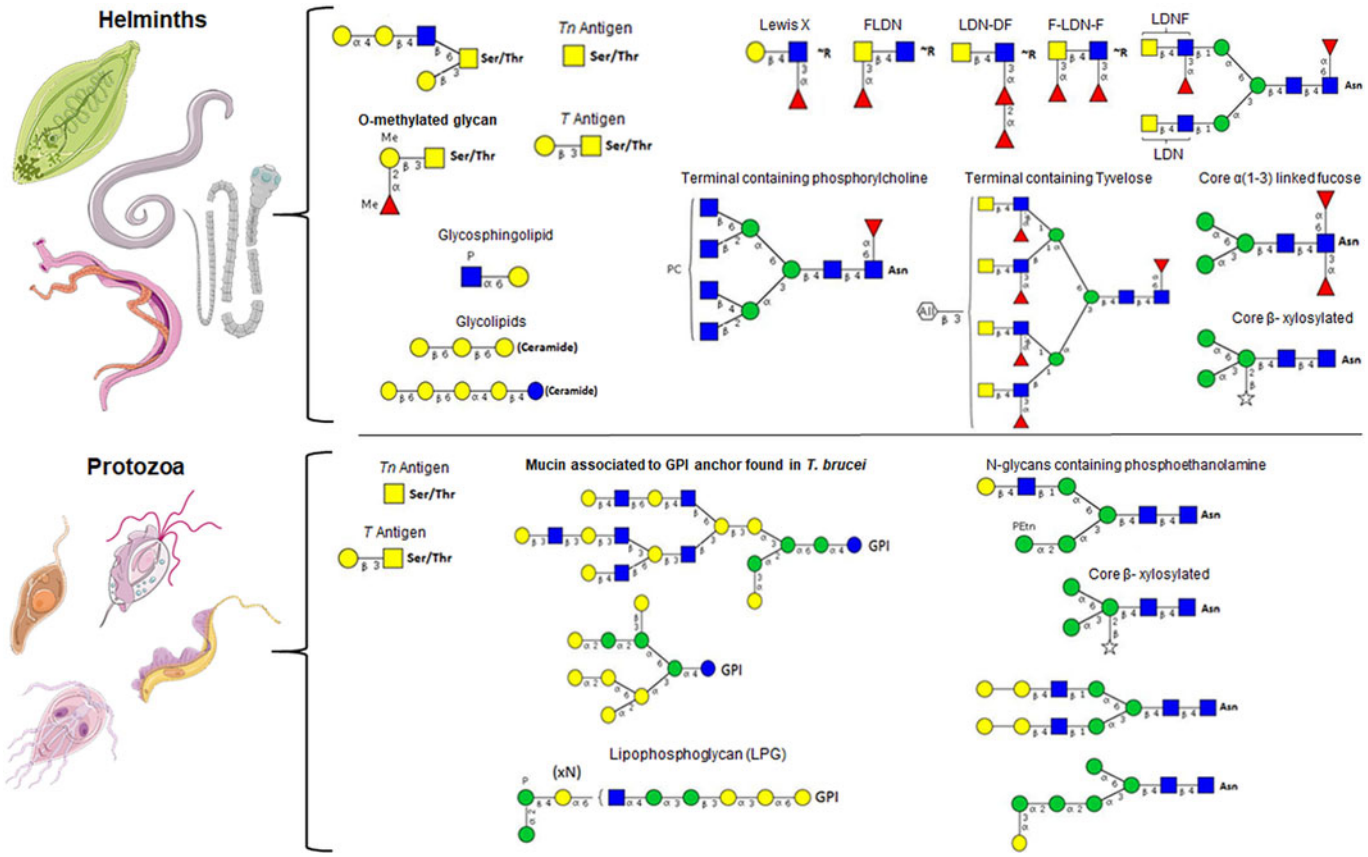

Fig. 1. Main glycan moieties found in helminth and protozoan parasites.

and, as previously mentioned, is covered by GPI-linked variant surface glycoprotein (VSG). This molecule contains a glycan portion that is immunogenic, and through an intriguing molecular switch, the VSG present in the parasite surface can be replaced randomly and repeatedly by another VSG that will contain a distinct glycan profile. The new VSG will also be expressed in the progeny of the original parasite, produced through asexual reproduction, and the new clonal population will be initially resistant to the host immune response. More than a thousand VSG variants have already been identified (Bangs, 2018).

Remarkably, *T. brucei* can also vary its glycan profile throughout the multiple morphological forms that constitute its life cycle. Complex *N*-glycans are absent or expressed at a very low level in procyclic forms found in the vector mid-gut, while trypomastigote forms present in the bloodstream of its mammal host express these glycans in substantial amount (Hwa and Khoo, 2000). Additionally, *T. brucei* has been demonstrated to express moieties containing oligomannose, paucimannose and complex *N*-linked glycans that contain large poly-*N*-acetyllactosamine structures.

Poly-*N*-acetyllactosamine is produced by *N*-acetylglucosaminyltransferases. A search of the *T. brucei* genome did not identify any orthologues of conventional *N*-acetylglucosaminyltransferases, a perplexing result given the abundance of this glycan within the parasite. Damerow *et al.* (2014, 2016) resolved this conundrum when they characterized two highly divergent genes encoding members of the *β*3-glycosyltransferase family in *T. brucei,* namely *N*-acetylglucosaminyltransferases I and II (TbGnTI and II) (Damerow *et al.*, 2014; 2016). Surprisingly, TbGnTI and TbGnTII are not essential for parasite survival *in vitro* and trypanosomes adapted their *β*3-glycosyltransferases members to catalyse specific glycosidic linkages (Damerow *et al.*, 2014; 2016). These data show essential aspects of *Trypanosoma* protein glycosylation that may assist in identifying druggable targets. Other glycoconjugates of trypanosomatid parasites include various glycoinositolphospholipids (GIPLs), and an unusual GPI anchored mucin. *Trypanosoma cruzi O*-glycans contain a quite unique mix of GlcNAc, galactose, galactopyranose, galactofuranose and other linked carbohydrates residues (Todeschini *et al.*, 2001). In addition, *T. cruzi*, present an unusual glycan with phosphate attached to Galp terminal residues is found in the gp72 glycoprotein of the epimastigote form possibly having a role in inhibiting parasite to differentiate into metacyclic trypomastigote stage (Allen *et al.*, 2013).

Although trypanosomatids do not synthesize sialic acid, they do express trans-sialidase (TS) enzymes that catalyse the transfer of sialic acid from host glycoconjugates onto the parasite surface (Costa *et al.*, 1998), an unique ability that is thought to be critical for the success of the infection and virulence of the parasite (San Francisco *et al.*, 2017). Indeed, the activity of TS seems to guarantee the survival *of T. cruzi* by suppression of host responses, as the sialylated glycans on the parasite surface interact with DC receptors to suppress response, mainly *via* the lectin receptor sialic acid-binding protein (Siglec-E) (Erdmann *et al.*, 2009). This interaction leads to lower production of IL-12 and IFN-*γ*. Vaccines against *T. cruzi* based on recombinant TS have demonstrated 60% efficacy in experimental infections in A/As rats (Pereira-Chioccola *et al.*, 1999). Similarly, Hoft *et al.* (2007) used only the enzymatic domain of TS to formulate another vaccine that showed what the author considered a good protection against the acute *T. cruzi* infection, a response also maintained during the chronic phase. In addition, Hoft and colleagues demonstrated that intranasal delivery of a soluble recombinant TS induced both TS-specific CD4(+) and CD8(+) T cells associated with protective immunity, results further confirmed by Giddings *et al.* (2010). Even though these results are promising,

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Table 1. Main glycan structures described in helminths and protozoa

Parasite	Glycan name	Reference
Helminths		
Trematodes		
Schistosoma sp	• Lewis X (Le^x) • Pseudo Le^y • LacdiNAc (LDN) • LDNF • FLDN • F-LDN-F • LDN-DF • DF-LDNF-DF • Truncated *O*-glycans T e Tn, • High mannose glycans ($Man_{5-9}GlcNAc$) • N-linked glycan containing β_{1-2} xylose • N-linked glycan containing α_3 fucose • Circulating Cathodic Antigen (CCA) • Circulating Anodic Antigen (CCA)	Wisnewski *et al.* (1993) Bergwerff *et al.* (1994) Haslam *et al.* (1996, 1998 van Die *et al.* (1999) Cummings and Nyame (1999) Hokke *et al.* (2007) Nyame *et al.* (2002) Wuhrer *et al.* (2006) van Die and Cummings (2006) Peterson *et al.* (2009)
Fasciola hepatica	• LDN • LDNF • Truncated *O*-glicídios T e Tn • Glycosphingolipid	Vervelde *et al.* (2003) Freire *et al.* (2003) Wuhrer *et al.* (2004)
Opisthorchis viverrini	• Mono-fucosylated N-linked glycans • Truncated, hybrid and complex glycans with 1–4 antennas • *O*-glycan mucin type, containing 1–5 antennas, ($Gal\beta_{1-3}GalNAc$)	Talabnin *et al.* (2013)
Nematodes		
Ascaris suum	• $Man_{5-9}GlcNAc$ • N-linked glycan containing phosphorylcholine terminals • *O*-glycans • Glycosphingolipid with phosphorylcholine terminals • Glycosphingolipid containing 3-sulfogalactosylcerebroside	Lochnit *et al.* (1998) Dell *et al.* (1999) Poltl *et al.* (2007)
Toxocara canis	• Truncated *O*-glycans T e Tn • *O*-Methylated glycans	Khoo *et al.* (1991) Casaravilla *et al.* (2003)
Dictyocaulus viviparous	• $Man_{5-9}GlcNAc$ • Lex	Haslam *et al.* (2000)
Trichinella spiralis	• LDN e LDNF (with or without phosphorylcholine terminals) • Tyvelose β_3-linked • $Man_{5-9}GlcNAc$ • N-linked glycans containing α_3-fucose	Wisnewski *et al.* (1993) Haslam *et al.* (1996, 1998) van Die *et al.* (1999) Morelle *et al.* (2000) van Die and Cummings (2006)
Haemonchus contortus	• LDN • LDNF • $Man_{5-9}GlcNAc$ • N-linked glycans containing α_3-fucose	Wisnewski *et al.* (1993) Haslam *et al.* (1996, 1998) van Die *et al.* (1999) Geldholf *et al.* (2005) van Die and Cummings (2006)
Dirofilaria immitis	• LDN • LDNF	Nyame *et al.* (1998)
Wuchereria bracofiti	• N-linked glycans containing phosphorylcholine terminals	Dell *et al.* (1999)
Onchocerca volvulus	• $Man_{5-9}GlcNAc$	Haslam *et al.* (1999) Wuhrer *et al.* (2000)

(*Continued*)

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Table 1. (*Continued.*)

Parasite	Glycan name	Reference
	• N-linked glycans containing phosphorylcholine terminals • Glycolipids containing phosphorylcholine terminals	
Onchocerca gibsoni	• $Man_{5-9}GlcNAc$ • N-linked glycans containing phosphorylcholine terminals	Haslam *et al.* (1999)
Acanthocheilonema viteae	• $Man_{5-9}GlcNAc$ • N-linked glycans containing phosphorylcholine terminals	Haslam *et al.* (1999)
Nippostrongylus brasiliensis	• Truncated *O*-glycans T e Tn	Casaravilla *et al.* (2003)
Cestodes		
Echinococcus multilocularis	• Truncated *O*-glycans T e Tn • Mucin	Ingold *et al.* (2000) Hülsmeier *et al.* (2002)
Echinococcus granulosus	• Truncated *O*-glycans T e Tn • $Man_{5-9}GlcNAc$ • Truncated glycans • N-linked glycans containing phosphorylcholine terminals • Glycosphingolipid	Khoo *et al.* (1997) Alvarez Errico *et al.* (2001) Paschinger *et al.* (2012) Wuhrer *et al.* (2004)
Mesocestoides vogae	• Truncated *O*-glycans T e Tn, sialyl-Tn[a]	Medeiros *et al.* (2008) Van Die and Cummings (2010)
Metacestoides corti	• Truncated *O*-glycans T e Tn	Freire *et al.* (2003)
Taenia hydatigena	• Truncated *O*-glycans T e Tn	Freire *et al.* (2003)
Taenia crassiceps	• N-linked glycans containing α_3-fucose • $Man_{5-9}GlcNAc$ • Glycolipids • Glycosphingolipid	Nyame *et al.* (2004) Lee *et al.* (2005)
Taenia solium	• $Man_{5-9}GlcNAc$ • Complex and truncated N-linked glycans, with or without fucose • Glycolipids	Restrepo *et al.* (2000) Haslam *et al.* (2003) Nyame *et al.* (2004)
Taenia saginata	• Glycolipids	Nyame *et al.* (2004)
Protozoa		
Trypanosoma sp	• Glycosylphosphatidylinositol Anchors (VSG) • Procyclin (PARP) • High galactose *O*-glycans – Mucin • Lipopeptideophosphoglycan (LPPG) • Phospholipids-linked GPI (GIPL) • Trans-sialidase	Previato *et al.* (1990) Ferguson *et al.* (1993) Costa *et al.* (1998) Ferguson (1999) Todeschini *et al.* (2001)
Plasmodium sp	• Glycosylphosphatidylinositol Anchors (GPI) • *N*- and *O*-linked glycans	Khan *et al.* (1997) Gowda and Davidson (1999)
Leishmania sp	• Lipophosphoglycan (LPG) • Phospholipids-linked GPI (GIPL) • Phosphoglycan • Proteophosphoglycan (PPG) • *O*-glycan, mucin type	Ilg *et al.* (1995) Descoteaux and Turco (1999) Guha-Niyogi *et al.* (2001)

(*Continued*)

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Table 1. (*Continued.*)

Parasite	Glycan name	Reference
Entamoeba histolytica	• Lectin Gal-GalNAc • LPG • LPPG • Sialyl glycoconjugates	Stanley *et al.* (1995) Petri (1996) Moody-Haupt *et al.* (2000)
Trichomonas vaginalis	• LPG • Adesins • *N*-linked glycans with specific modifications	Paschinger *et al.* (2012)
Giardia duonenalis	• Variable surface glycoprotein (VSG) • *O*-glycans • *N*-glycans	Papanastasiou *et al.* (1997) Bulik *et al.* (2000)
Toxoplasma gondii	• *O*-glycans • *N*-glycans • GPI anchors	Guha-Niyogi *et al.* (2001)

[a]Possible sample contamination.

further vaccine trials still necessary to guarantee that TS is a suitable vaccine candidate to Chagas disease.

The immune modulation observed during Chagas disease is dependent on the genetic backgrounds of both host and parasite (Terrazas *et al.*, 2010). By expressing antigens, among them glycoconjugates that target DCs receptors, *T. cruzi* has developed an important strategy for immune evasion that unbalances the host–parasite relationship at various levels depending on the virulence of the parasite strain. In general, *T. cruzi* infection is characterized by reduced levels of pro-inflammatory cytokines, such as IL-12 and TNF-α and a lack of mature DCs (Alba Soto *et al.*, 2003). This last effect is one observed on splenic and marrow-derived DCs when the cells were exposed to parasite-GIPLs, indicating that this glycoconjugate plays a primary role in modulating the host cellular response (Brodskyn *et al.*, 2002; Poncini *et al.*, 2008). *Trypanosoma cruzi* GIPLs were also shown to activate DCs through TLR signalling, which contributed to IFN-β production and clearance of infection (Campos *et al.*, 2001; Koga *et al.*, 2006).

Leishmania

The genus *Leishmania* (Euglenozoa: Kinetoplastida: Trypanosomatidae) includes over 20 species able to infect humans, causing different forms of leishmaniasis endemic in 97 countries. Forms of the disease include the potentially fatal visceral (kala-azar) leishmaniasis, the disfiguring cutaneous leishmaniasis and mucocutaneous leishmaniasis (WHO, 2017).

The complex set of glycoconjugates forming the glycocalyx covering *Leishmania* cells have been extensively studied because of their immunomodulatory properties. These molecules may include different types of mucin glycan, GIPLs, GPI-linked carbohydrate, phosphoglycans (PGs) and the group of molecules variously identified as lipophosphoglycans (LPG), lipopeptidophosphoglycan (LPPG) or proteophosphoglycan (PPG) by different authors. Moreover, phospholipids and glycoproteins are found attached to the GPI anchors, while *Leishmania* presents a LPG-containing mannose residue attached in uncommon positions, among other structural modifications (Descoteaux and Turco, 1999; Guha-Niyogi *et al.*, 2001).

Glycan metabolism in *Leishmania* species is highly complex. It has been demonstrated that both the terminal oligosaccharide motifs and the carbohydrate chains that decorate the GPIs vary among species and various life-stages of the parasites (Guha-Niyogi *et al.*, 2001). Glycoproteins and LPG also decrease in abundance in the coated surface of promastigotes as they differentiate into amastigotes, so that GIPL is the predominant glycoconjugate in the latter stage (Naderer *et al.*, 2004). Intraspecific variation in LPG has been observed from different field isolates (Coelho-Finamore *et al.*, 2011). Although still poorly understood, this variable glycan profile could be responsible for the success of *Leishmania* species in evading and modulating host responses throughout the whole life cycle. An understanding of the variation may assist in resolving specific attributes of infections among different species. For a complete review of the role of LPG in leishmaniasis infection, see Forestier *et al.* (2015).

To maintain infection in mammals, *Leishmania* must manipulate DC activity so as to avoid the mature DC phenotype and, therefore, the Th1 pattern of immune response (Reiner and Locksley, 1995; Chakir *et al.*, 2003). The strategies the parasites adopt to manipulate the host responses vary among species and strains of *Leishmania* and are associated with the sets of glycoconjugates expressed by different parasites. *L. major* inhibits DC motility through the interaction of LPG with cell receptors (Jebbari *et al.*, 2002), while LPGs from *L. mexicana* reduce IL-12, thus limiting DC activation (Bennett *et al.*, 2001; Argueta-Donohue *et al.*, 2008). Likewise, PGs expressed by this parasite ultimately impaired the ability of DCs to induce Th1 responses (von Stebut *et al.*, 1998; Konecny *et al.*, 1999). PG produced by *L. donovani* affects DCs maturation and migration (Tejle *et al.*, 2008).

Glycoconjugates of *Leishmania* have also been shown to interact with TLRs. Even though the effect of this interaction on DC modulation is yet to be understood, it was demonstrated that LPG binds to TLR-2 and 4 on macrophages and natural killer cells, leading to a switch in the profile of pro-inflammatory molecules in these host cells that ultimately allows the intracellular parasite to survive (De Veer *et al.*, 2003; Rojas-Bernabe *et al.*, 2014). Accordingly, it was suggested that differences in the LPGs found on *L. braziliensis* and *L. infantum* surfaces may impact host cell modulation each species is able to stimulate (Ibraim *et al.*, 2013).

Curiously, in natural infections, LPG does not seem to be highly immunogenic (Goel *et al.*, 1999), although a different study showed that it could at least in part prevent complement-mediated lysis of *Leishmania* by blocking the binding of the membrane attack complex C5b-9 to the promastigote membrane (Descoteaux and Turco, 1999). However, when preparations of

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LPG were used to vaccinate rats, the animals developed a protective immune response, verified by the presence of specific anti-LPG serum antibodies (Russell and Alexander, 1988). Parasites exposed to these antibodies were incapable of infecting the phlebotomine vectors, suggesting at the time that LPGs were promising candidates antigens for transmission-blocking vaccines (Tonui *et al*., 2001).

Further studies with the same molecule revealed that subcutaneous LPG injection failed to induce protection against *L. amazonensis* in animals, but the intranasal delivery of LPG resulted in protection (Pinheiro *et al*., 2005; 2007). Although the mechanisms of LPG-mediated protection are still unknown other vaccine formulations have been better characterized. A dual stimulation using a peptidoglycan (PGN) and the soluble leishmanial antigen (SLA) was able to stimulate DCs during experimental visceral leishmaniasis (VL). The vaccinated animals showed a significant decrease in hepatic and splenic parasite burden, and an increased production of nitric oxide (NO) and pro-inflammatory cytokines such as IL-12, IFN-γ and IL-17, that ultimately resulted on increased number of Th17 cells (Jawed *et al*., 2016).

Plasmodium

Plasmodium, a genus of parasites belonging to the phylum Apicomplexa within the SAR supergroup of eukaryotes, is the agent of malaria in humans and animals.

The intracellular apicomplexans, *Plasmodium* species synthesize GPI anchors that contain a highly conserved glycan core composed of a trimannosylglucosaminyl moiety with an additional mannose attached. These anchors are attached to several *P. falciparum* proteins and constitute 90% of the glycoconjugates identified on this parasite (Gowda and Davidson, 1999). Not surprisingly, this glycoconjugate has been strongly associated with various symptoms observed during malaria infection (Schofield *et al*., 1999; Clark and Schofield, 2000; Jaurigue and Seeberger, 2017). GPI anchors can activate macrophages and cells of the vascular endothelium through several signalling pathways that result in the production of chemical mediators, such as NO, tumour necrosis factor (TNFα) and intracellular adhesion molecules I (ICAM-I). This observation led tp exploration of this molecule as a vaccine target (Schofield *et al*., 2002), a concept later reinforced by a study that demonstrated that presence of specific antibodies to *P. falciparum* GPI could neutralize the strong inflammatory response that GPI stimulates (de Souza *et al*., 2010).

Indeed, most GPI-vaccines aim to neutralize or decrease the inflammatory response observed during infection rather than produce a sterilizing immunity. Rats immunized with a synthetic GPI based on the *P. falciparum* anchor displayed high titres of IgG antibodies that neutralized pro-inflammatory effects caused by activated macrophages, despite no significant protection being observed after challenge with *P. berghei* ANKA. Even though animals immunized did not present a reduction in parasitaemia, the results suggest that GPI anchors have a conserved structure among the different species of *Plasmodium* (Schofield *et al*., 2002). Another GPI formulation used as vaccine induced an IgG antibody response able to neutralize the parasite pathogenesis *in vitro* (Taylor *et al*., 2012). Currently, the *P. falciparum* GPI vaccine is being tested in phase II clinical trials (see Anchora Pharmaceutical, MA https://www.sbir.gov/sbirsearch/detail/89678).

It is important to highlight here that many studies have shown that *P. falciparum* produces a restricted glycan repertoire. Technical limitations in isolating sufficient quantities of parasite molecules have rendered it difficult to determine with clarity whether the parasites produce *O*-glycans or complex *N*-glycans (Kimura *et al*., 1996; Cova *et al*., 2015). A recent work using a mass spectrometry technique by electron transfer dissociation was able to show different glycoforms of thrombospondin type 1 repeats-containing proteins (TSR) (Swearingen *et al*., 2019). TSRs are implicated in the invasion process of *Plasmodium* and may be modified with *O*-linked fucose (*O*-Fuc) and C-linked mannose (*C*-Man).

It seems to be certain, however, that in addition to the GPI anchors, *P. falciparum* produces truncated *N*-glycans, GlcNAc and GlcNAc2 (Cova *et al*., 2015). The relevance of these glycans in the host–parasite interplay, if any, remains to be elucidated.

Entamoeba histolytica

Entamoeba histolytica (Amoebozoa: Endamoebidae) is a pathogenic amoeba responsible for significant food-borne infections in humans. The species causes intestinal amoebiasis and may affect other organs through disseminated infection. Amoebiasis is among the leading causes of death in the world attributed to a protozoan parasite (Ralston and Petri, 2011).

The most abundant glycan found in *E. histolytica* is aggregated into caps on the surface of the cell by the *N*-glycan-specific, antiretroviral lectin cyanovirin-N. Complex *N*-glycans contain α1,2-linked Gal to both arms of small oligomannose glycans, and Gal residues are capped by one or more Glc (Magnelli *et al*., 2008). The motile trophozoite stage of the parasite has a coat of lipid-linked glycoconjugates including the LPGs. The *Entamoeba* LPG has an unusual GPI anchor on its surface, which uses an ethanolamine phosphate bridge to anchor a residue of α-Gal onto the C-terminus of the protein. Several other linear glycans [Glcα1-6(n) Glcβ1-6Gal] are found attached to the LPG *via* phosphoserine residues (Moody-Haupt *et al*., 2000).

The pathogenesis observed during amoebiasis appears to result from the cytotoxic activity of the parasite, which depends mainly on the adhesive interactions between the parasite and the glycoconjugates present on the surface of host cells, and of the interaction of the parasite glycoconjugates with the host immune system. The LPG has been strongly associated with *Entamoeba* virulence, since specific antibodies to this antigen could prevent trophozoite adhesion to cells and the consequent cytolysis *in vitro*, and liver abscess formation in experimental infections in mice (Marinets *et al*., 1997). The same LPG also appears to have a central role in the first steps of infection, when the adherent *E. histolytica* trophozoites transfer their LPG to the apical side of host enterocytes causing dysfunction of the tight junctions (Lauwaet *et al*., 2004). LPGs were demonstrated to interact with TLR receptors and stimulate the production of IL-10, IL-12 and TNF-α (Maldonado-Bernal *et al*., 2005) and, together with parasite surface Gal/GalNAc lectins, are considered fundamental for regulating host cell adhesion and cytolysis. Both LPG and Gal/GalNAc lectin were observed to activate and induce maturation of DCs, leading to a Th1 response during amoebiasis (Ivory and Chadee, 2007; Vivanco-Cid *et al*., 2007; Aguirre García *et al*., 2015).

Several LPGs have been applied in diagnosis tests and vaccines of protozoan infections. Species of *Entamoeba* and *Leishmania* can be distinguished by the type of LPG they are expressing (Coelho-Finamore *et al*., 2011). Moreover, LPG identification is suitable for differential diagnosis of the pathogenic *E. histolytica* from non-pathogenic *E. dispar*, as it was demonstrated that those LPGs expressed by pathogenic strains of *E. histolytica* are unique for this species (Moody *et al*., 1997; Bhattacharya *et al*., 2000).

Glycoconjugates in helminth parasites: phylum Nematoda

Trichinella spiralis

Trichinella spiralis (Nematoda: Adenophorea: Trichinelloidea) is the causative agent of trichinellosis in mammals, a disease leading

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to severe debilitation and pain, and sometimes death. Humans become infected by consuming raw or under-cooked meat of an infected animal. The life cycle is maintained in the wild through predation and meat-scavenging behaviours.

Trichinella spiralis produces many glycans, but one of the most remarkable is a motif containing a tyvelose (Tyv) (3,6-dideoxy-D-Arabino-hexose) residue that is found attached to the glycoprotein TSL-1 (*T. spiralis* larva-1), present at the surface of the first stage larvae of *T. spiralis* and its ES products (Reason *et al.*, 1994). Reason and colleagues claimed that a major proportion of the *N*-glycans antennae of the TSL-1 are capped with Tyv and the uniqueness of this monosaccharide encouraged further studies. This represents an excellent case where a glycan motif that appears to be restricted to an organism renders the molecule as a potentially valuable target for immunodiagnostic tests. Indeed, moved by the previous results, Forbes *et al.* (2004) used a synthetic β-Tyv antigen to develop an enzyme-linked immunosorbent assay (ELISA) that presented a sensitivity of 94.3% and a specificity of 96.7% when applied to the diagnosis of swine trichinosis.

TSL-1 and Tyv have been proposed to have immunomodulatory effects. Niborski *et al.* (2004) demonstrated a Th2 pattern of response during *T. spiralis* infections. Complementary *in vitro* experiments using rat mast cells exposed to TSL-1, demonstrated an association of the glycoprotein with increased mRNA levels for IL-4, IL-5, IL-6 and TNFα. During the intestinal (adult) phase of the infection, the TSL-1 antigen is thought to stimulate mast cells in an IgE-independent manner, ultimately leading to increased levels of histamine that could contribute to worm expulsion from the intestine (Yépez-Mulia *et al.*, 2007). A different study, aiming to understand the role of the glycan portion of TSL-1, demonstrated that mice immunized with tyvelose-BSA develop high levels of IL-5 but not IFN-γ, which reinforced the idea that this glycan motif plays a central role to drive the Th2 response observed during trichinellosis (Goyal *et al.*, 2002).

More recently, however, studies focusing on the Tv antigen have become sparse, while other *Trichinella* glycans are being prospected. A *T. spiralis* glycoconjugate containing LDNF, for example, was thought to be a suitable target for immunodiagnosis of trichinellosis, showing a sensitivity of 96% and a specificity of 67% in diagnostic tests (Aranzamendi *et al.*, 2011). Interestingly, *T. spiralis* adult and L1 larvae have multiple antennae decorated with phosphorylcholine (PC)-modified LDNF that is found linked to residues of GlcNAc or GalNAc (Morelle *et al.*, 2000). The presence of this modification could explain the low specificity observed in tests using *T. spiralis* LDNF antigen since PC terminals have been associated with cross-reactivity observed in serological diagnostic tests to *Wuchereria bancrofti*, *Brugia malayi*, *Onchocerca volvulus* and *Achanthocheilonema viteae* (Dell *et al.*, 1999; Goodridge *et al.*, 2005).

Haemonchus contortus

Haemonchus contortus (Nematoda: Secernentea: Trichostrongylidae) is a highly pathogenic parasite of sheep and goats. The parasite is an avid blood-feeder in the abomasum of its ruminant hosts. *Haemonchus contortus* expresses a broad variety of glycans, including motifs containing LDN, LDNF motifs and *N*-glycans with core α1,3-fucose, which were mainly identified in ES products. A more recent and detailed mass spectrometry analysis of *H. contortus* *N*-glycans revealed a dominant trifucosylated Hex3HexNAc2Fuc3 structure containing α1,6- and α1,3-fucose on the proximal core GlcNAc, and a galactosylated distal α1,3-fucose (Table 1). Other motifs displayed galactosylation of the core α1,6-fucose, antennal fucosylation or PC modification (Haslam *et al.*, 1996; Paschinger and Wilson, 2015).

The immunogenicity of glycan motifs of *Haemonchus* was highlighted by glycan array experiments using samples from lambs vaccinated with parasite ES products. The vaccinated animals presented high levels of IgG antibodies to Gal(α1-3) GalNAc motif that was correlated with the protection observed (van Stijn *et al.*, 2010). Heim *et al.* (2015) demonstrated that larval development could be arrested *in vitro* with the lectin *Marasmius oreades* agglutinin (MOA), that specifically binds to Gal(α1-3) GalNAc. Larvae exposed to the lectin presented severe malformations in body shape and died quickly, indicating a potential application of MOA for novel chemotherapeutic strategies (Heim *et al.*, 2015). A galactose-containing glycoprotein complex (H-gal-GP), purified from the gut membrane of *Haemonchus*, inspired many studies on vaccination, leading to the first commercial vaccine available to protect sheep against the Barber's pole worm (Kearney *et al.*, 2016). The success of this vaccine is possibly related to the fact that they use a native H-gal-GP purified from worms recovered from naturally infected sheep, rather than a recombinant protein. Although successful, this native vaccine emphasizes a major limitation in vaccinology, namely, the difficulties in producing recombinant glycoproteins that preserving the glycan configuration of the native antigens.

Angiostrongylus cantonensis

Angiostrongylus cantonensis (Nematoda: Secernentea: Metastrongylidae) is the main causative agent of eosinophilic meningoencephalitis in humans in many parts of the world (Morassutti *et al.*, 2012). The glycan repertoire of *A. cantonensis* female worms includes complex (Fuc0–1Hex3–5HexNAc3–5), high mannose (Hex5–7HexNAc2) and truncated structures (Fuc0–1Hex3HexNAc2) *N*-glycans, while *O*-glycans were not identified in any analyses performed so far (Veríssimo *et al.*, 2016). Glycans with terminal containing galactose (Gal) and GalNAc are not commonly observed among nematode parasites and further investigations of the glycan repertoire of *Angiostrongylus* parasites will help to determine if such moieties identified by Veríssimo *et al.*, are indeed produced by the parasite or a result of cross-contamination with host molecules.

Behn (1997) proposed that glycans play a central role in *Angiostrongylus* biology considering their involvement with mechanisms of parasite adaptability to different temperatures of its two hosts, which vary from 36–38 °C in the mammal hosts and from 20–28 °C in mollusc hosts. Behn further proposed that a trehalose residue would promote such thermal tolerance, even though this carbohydrate has never been identified in the extracts of this parasite. While such role of trehalose is doubtful in the case of *A. cantonensis*, it is evident that other nematodes use trehalose for thermotolerance, among them the sealworm *Pseudoterranova decipiens* (Nematoda: Anisakidae), a parasite infecting animals in cold marine waters (Stormo *et al.*, 2009).

The immunogenicity of *A. cantonensis* glycans, however, is better demonstrated. Such molecules boost specific anti-glycan antibodies, observed when sera of individuals infected to the *Angiostrongylus* were tested with the 31 kDa antigen, a complex antigen containing *N*-glycosylated glycoproteins (Veríssimo *et al.*, 2016). Initially, Morassutti *et al.* (2012) demonstrated the importance of the glycan portion of the antigen by treating it with meta-periodate, which resulted in an abrogated immune recognition of the 31 kDa spots by sera from infected patients. Further, Veríssimo *et al.* (2016) confirmed that *N*-glycans associated with the 31 kDa antigen were the protagonists of this immune response by treating the antigen with the *N*-glycosidase PNGase F, which removed the carbohydrate moieties. Additionally, heterologous expression of the 31 kDa antigen in

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different prokaryotic or eukaryotic systems failed to produce an immunogenic recombinant protein (Morassutti *et al.*, 2012).

Glycoconjugates in helminth parasites: phylum Platyhelminthes

Echinococcus

Echinococcus (Platyhelminthes: Cestoda: Taeniidae) is a genus of taeniid tapeworm. Two species (or species complex) are primarily responsible for human echinococcosis, *E. granulosus* and *E. multilocularis*. The species cause unilocular and alveolar echinococcosis which are characterized by the presence of cystic lesions containing the infectious protoscoleces. Infection of the intermediate host, a mammal, occurs by the ingestion of eggs that were released with the feces of the canid definitive host.

The hydatid cyst wall of *E. granulosus* is formed of three layers, the outer pericyst, the middle-laminated layer and the inner germinal layer. The last layer produces the abundant brood capsules which in turn produce multiple protoscoleces. The crude antigen of the hydatid cyst administered at different stages prevents DCs from maturing. The inhibitory effect of the antigen operates through inhibition of CD1a and enhancement of CD86 expression. Furthermore, crude antigen stimulates IL-4 production during antigen presentation by mature DCs, which suggests that *E. granulosus* stimulates Th2 pattern responses (Rigano *et al.*, 2007).

The laminated layer of *E. granulosus* is a mucin-rich extracellular matrix composed primarily of *O*-glycan core 1 (Galβ1-3GalNAc), also known as *T* antigen, and core 2 [Galβ1-3 (GlcNAcβ1-6) GalNAc] the *Tn* antigen (Díaz *et al.*, 2009). The Galβ1-3 residue of core 1 can be decorated with additional Galβ1-3 residues, generating a linear chain (Galβ1-3Galβ1-3GalNAc), and the elongation of mucin glycans with Galβ1-3 distinguishes *E. multilocularis* from *E. granulosus* (del Puerto *et al.*, 2016). Other structures extended with Galβ1-4 are also present, although complex glycans are much less abundant than those containing a non-decorated core (Díaz *et al.*, 2009).

Khoo *et al.* (1997) analysed the glycan composition of *E. granulosus* antigen 5 (Ag5) and found small amounts of high mannose and truncated glycan structures. Subsequent analysis of the same antigen by Paschinger *et al.* (2012), identified a range of other *N*-glycans, among them a moiety containing two antennae with a core of α1,6-fucose and capped by PC. The identification of these additional glycans demonstrated that the permethylation step used by Khoo *et al.* (1997) impairs the detection of PC temini. Other important glycoconjugates have been identified in extracts of *Echinococcus*. Among these molecules, the *O*-glycosylated *Tn* antigen from *E. granulosus* protoscoleces has been proposed as a biomarker of hydatidoses, since high levels of this antigen are detected in sera from infected patients (Alvarez-Errico *et al.*, 2001). A synthetic form of Em2(11), the main glycan antigen isolated from the laminated layer of *E. multilocularis* hydatid cysts, showed high sensitivity and specificity when applied on diagnostic tests (Koizumi *et al.*, 2011). Together these results reinforce the view that the characterization of glycan produced by different organisms is necessary to improve diagnostic specificity. The benefits of glycan analysis include, for example, our current comprehension of the cross-reactivity commonly observed in diagnostic tests using sera from *E. multilocularis*, *E. granulosus* or *F. hepatica* infected patients, which is now attributed to the glycan moiety, Galβ1-6Gal, attached to molecules produced by all these parasites (Yamano *et al.*, 2009).

Taenia

Taenia (Platyhelminthes: Cestoda: Taeniidae) is a genus of tapeworms infecting humans and some carnivores. Humans serve as definitive host of two species, *T. solium and T. saginata*, where intestinal infection by the adult parasite results in taeniasis. Additionally, humans can become infected by ingesting the eggs, commonly of *T. solium* and rarely of *T. crassiceps*. In this case, the host will develop cysticercosis in the tissues, specifically in the central nervous system (Hawk *et al.*, 2005).

Different species of *Taenia* express a species-specific glycan profile. *N*-glycans containing high mannose and truncated structures were found attached to glycoproteins secreted by *T. solium* (Haslam *et al.*, 2003), whereas *T. crassiceps*, produces *N*-glycans with fucose terminals (Lee *et al.*, 2005). *Taenia crassiceps* carbohydrates play key roles in host immune evasion, either by stimulating Th2 polarization (Gomez-Garcia *et al.*, 2006) or by inducing myeloid-suppressor innate cells (Gomez-Garcia *et al.*, 2005). Several experiments using metaperiodate helped to show that only intact glycosylated antigens can induce Th2 responses and other immunomodulatory effects such as higher expression of MHC-II. DC exposure to *Taenia* glycans resulted in abrogated response to TLR ligands, inhibition of IL-15, IL-12 and TNF-α secretion, and downregulation of a chemokine receptor (CCR7) that impairs DC migration (Montero-Barrera *et al.*, 2015).

MGL1, a lectin receptor expressed on the surface of APCs, such as mature DC and macrophages, recognizes antigens such as Le^x and other motifs containing galactose residues. MGL1 is directly involved with activation of innate immunity, antigen recognition and resistance to *T. crassiceps* infection. Animals with deficiency of MGL1 receptors are more susceptible to cysticercosis (Montero-Barrera *et al.*, 2015), indicating that glycans recognized by MGL1 are important for parasite survival in the host.

Schistosoma

Species of *Schistosoma* (Platyhelminthes: Trematoda: Schistosomatidae) are blood flukes that cause hepato-intestinal and genitourinary diseases. Glycans from schistosomes are widely studied. In every stage of their life cycles, schistosomes synthesize various glycan motifs containing LDN or its fucosylated form, LDNF (Hokke *et al.*, 2007). LDN/LDNF containing glycans are commonly attached to glycoproteins and glycolipids and are considered elementary molecules, since they form a base for further modification into more complex glycans (Khoo *et al.*, 1995; Wuhrer *et al.*, 2006; Jang-Lee *et al.*, 2007). Truncated mannose moieties also often occur, but with uncommon modifications, mainly those containing core α(1-3)-fucose and β(1-2)-xylose, similar to those found in plants and insects (van Die *et al.*, 1999; van Die and Cummings, 2006). Several glycan moieties were identified in schistosome extracts, among them the Le^x, poly-Le^x, LacdiNAc (LDN), LDN fucosylated (LDNF, LDN-diF and FLDN), lacto-*N*-fucopentaose-III (LNFP-III), *N*-glycans containing β(1-2)-xylose, *N*-glycans containing core α(1-3)-fucose, circulatory antigens (CCA and CAA) and the *T* and *Tn* types (Cummings and Nyame, 1999; Kantelhardt *et al.*, 2002).

Schistosome glycans are differentially expressed throughout the various life cycle stages. The Le^x motif is identified throughout all schistosome developmental stages. *N*-glycans containing Le^X and xylose, as well as complex *O*-glycans, rapidly disappear after cercarial transformation to schistosomula, the migratory larva, whereas LDN-motifs are predominant in adult worms (Smit *et al.*, 2015).

Schistosome females lay hundreds of eggs per day in their mammal hosts. The eggs, which embryonate within the host, are largely responsible for the pathogenicity and are central with respect to diagnosis. Therefore, a full understanding of the molecular composition of eggs through development, including their glycan profile, is key to creating efficient alternatives of diagnosis and perhaps of disease amelioration. Immature eggs only present short *O*-glycan cores, while fully developed eggs express

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several complex *N*- and *O*-glycans containing Le^X and multi-fucosylated LDN motifs.

Mature eggs secrete a complex set of proteins that may interact with the host immune system in order to help them to reach the intestine (Chuah *et al.*, 2013). The importance of the immune response generated by eggs was confirmed by evaluating HIV positive patients co-infected with either *S. mansoni* or *S. haematobium*. In these patients, the egg excretion from the host, number of eggs per gram of feces, is significantly impaired due to the depleted immune response (N'Zoukoudi-N'Doundou *et al.*, 1995; Karanja *et al.*, 1997; Mwanakasale *et al.*, 2003).

Soluble egg antigen (SEA), a crude extract of eggs used as a proxy for excreted/secreted components, is widely used for studies exploring diagnosis, immunomodulation and pathogenesis of schistosomiasis (Caldas *et al.*, 2008). SEA contains, among other proteins the glycoproteins, IL-4-inducing principle (IPSE/α-1) and the T2 ribonuclease Omega-1 glycoproteins. Both glycoproteins contain *N*- and *O*-glycan able to stimulate and modulate the host immune system (Abdulla *et al.*, 2011; Ferguson *et al.*, 2015). Glycoproteins from SEA often present terminals that are multi-fucosylated with α(1-3) and α(1-6)-linked fucose, *N*-glycans containing xylose, LDN, LDNF and Le^x (Khoo *et al.*, 1997; Jacobs *et al.*, 1999; Nyame *et al.*, 2002). When SEA components interact with DCs, different effects are observed: (1) a Th2 type response, caused by increased the expression of co-stimulatory molecules and cytokines; (2) a Th1 response, caused by upregulated IL-12 production; or (3) a suppression of immune inflammatory events through TRL ligand-induction that stimulates DC maturation or activation (Kane *et al.*, 2004; 2008; Kariuki *et al.*, 2008; Everts *et al.*, 2009). In addition, glycoconjugates containing LNFP, a component of SEA, alone can drive a Th2 response, by inducing DCs in a TLR4-dependent manner (Thomas *et al.*, 2003). Interestingly, experimental immunization of mice with *Schistosoma* eggs (Kariuki *et al.*, 2008) or derived glycoconjugates generated a non-protective immune response, making some argue that the eggs-derived glycans are actually diverting the host immune response and therefore would not be useful for vaccine formulation (Eberl *et al.*, 2001).

The glycan motif DF-LDN-DF so far has been found only in *S. mansoni* and *S. japonicum* and, therefore, became a promising diagnostic target (Khoo *et al.*, 1997; Peterson *et al.*, 2009). This motif is recognized by the monoclonal antibody 114-4D12, which was then used to identify DF-LDN-DF in eggs extract, and also used in a sandwich ELISA to capture the glycan in blood and urine samples (Robijn *et al.*, 2007). Other fucosylated antigens isolated from cercariae were shown to elicit specific IgM and IgG antibodies that recognize structures such as LDN, LDNF, Le^x, F-LDN and LDN-diF, which are fucosylated antigens considered potential diagnostic targets (Nyame *et al.*, 2003; Vermeer *et al.*, 2003).

Since the 1980s, *Schistosoma* gut-secreted glycosylated antigens, known as circulating cathodic antigen (CCA) and circulating anodic antigen (CAA), have been proposed as diagnostic targets for schistosomiasis (Deelder *et al.*, 1980). Glycan analysis of CCA revealed an exclusive *O*-glycosylation moiety formed by Le^X repeating units linked to the protein. Because of the presence of Le^X in human circulating neutrophils, it was suggested that the antigenic CCA poly-Le^X might be involved in immunomodulation of granulocytes during schistosome infection (van Dam *et al.*, 1994). In 2004, Van Dam and collaborators reported on a Reagent Strip Test they had developed. The strip containing labelled monoclonal antibody specific for detection of *Schistosoma* CCA in the urine. The authors used the strips to evaluate a group of infected school children in Tanzania and the results were compared with direct egg counts in feces by Kato-Katz. Determination of CCA and CAA levels in sera and CCA in urine by ELISA showed sufficient sensitivity and specificity to be applied in the field as a non-invasive and rapid test (van Dam *et al.*, 2004). More recent studies have shown that the sensitivity of the CCA assay ranges from 65 to 100%, largely depending on the intensity of infection of the population evaluated (Stothard *et al.*, 2006; Colley *et al.*, 2013). The commercially available point-of-care urine dipstick test POC-CCA is now receiving great attention for field studies (Corstjens *et al.*, 2014; Casacuberta *et al.*, 2016; Lindholz *et al.*, 2018) and thought to possibly replace Kato-Katz in epidemiological studies.

Fasciola species

Fasciola hepatica and *F. gigantica* (Platyhelminthes: Trematoda; Fasciolidae), the liver fluke of ruminants and humans, are listed among the neglected tropical diseases. Fascioliasis has a major impact on livestock production. With respect to human health, fascioliasis is now estimated to infect over 1 million people, with 17 million people are at risk of infection (Ravida *et al.*, 2016).

Initial analyses of the *F. hepatica* glycan repertoire revealed structures containing *Tn* antigen, mannose and glucose residues, Gal(β1-6)Gal-terminating glycolipids and truncated *N*-glycans, followed by *N*-glycans containing oligomannose and some that are core-fucosylated (Freire *et al.*, 2003; Wuhrer *et al.*, 2004; Georgieva *et al.*, 2012; Garcia-Campos *et al.*, 2016). These glycans were identified mainly in the miracidium, on the tegument of newly excysted juvenile, and on the surface of the oral and ventral suckers and the gut of adult flukes. Ravidà *et al.* (2016) showed that tegument of *Fasciola* contains complex *N*-glycans, some of which are phosphorylated. *F. hepatica* is well known for inducing a strong Th2/Treg response in its hosts. Glycans might contribute to this bias, since many, including phosphorylated oligosaccharides, associate with proteins in the tegument, and may interact with lectin receptors in APCs cells. Indeed, it was demonstrated that oligomannose motifs of *Fasciola* specifically interact with C-type lectin receptors resulting in increased IL-4 and IL-10 production and suppression of IFN-γ (Rodríguez *et al.*, 2015).

Discussion

The glycan profile expressed by different parasites dictates the way these organisms interact with their hosts. Several studies have demonstrated that glycans, as opposed to the protein backbone to which they attach, are often the main stimulating agent of the host's immune system during parasitism. Indeed, glycan molecules have been implicated in the antibody response during malaria, amoebiasis, trypanosomiasis, leishmaniasis, schistosomiasis, filariasis, angiostrongyliasis, cysticercosis and hydatidosis (Norden and Strand, 1985; Cummings and Nyame, 1996; 1999; Eberl *et al.*, 2001; Hokke and Deelder, 2001; Morassutti *et al.*, 2012; Montero-Barrera *et al.*, 2015; del Puerto *et al.*, 2016; Veríssimo *et al.*, 2016).

The literature related to glycans derived from parasites so far reflects the potential application of glycans for the development of immunological tests or vaccines, and also the necessity to improve the production of recombinant antigenic glycoproteins. The expression systems used most often are peptide-based, built around prokaryotic plasmids cloned into prokaryotes that are incapable of producing eukaryotic glycan modifications (Dell *et al.*, 1999). Furthermore, the general similarities among glycans produced by different parasites, and their hosts, represent an even bigger challenge for the development and application of such molecules in specific diagnosis (van Die and Cummings, 2010). Any immunological reagent based on glycans have to take this into consideration and must be validated extensively to ensure that it is not a cross-reactive antigen.

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In past years, deep exploration of glycan molecules has resulted in advances in the development of diagnosis, vaccines and drugs. Important diseases, such as neurocysticercosis, now have an alternative and better diagnosis as a result of glycan research (Nunes *et al.*, 2013). In this review, we discussed the involvement of glycans in important processes of recognition and interactions with hosts, which potentially can allow wider comprehension of the host–parasite relationship and important aspects of infection, including the preference for certain hosts. In this last aspect, there is evidence that glycans can be important determinants of host-specificity. For example, the pattern of glycosylation of the glycoproteins present in the haemolymph of different strains of *Biomphalaria glabrata*, the intermediate host of *S. mansoni*, revealed that those strains expressing higher amounts of fucosylated glycan moieties have a higher susceptibility to *S. mansoni* infection (Lehr *et al.*, 2010). Further understanding of these interactions, may result in a successful strategy for control of parasite through transmission blocking.

In this review, we also highlighted how parasitic glycans are being considered in the context of vaccine development. Currently, carbohydrate-based vaccines are thought to be a decisive strategy to improve vaccination, which is reinforced by the success of vaccines based on capsular polysaccharides to combat different bacterial infections (Roggelin *et al.*, 2015; Gala *et al.*, 2016). The detection and characterization of glycans or glycoconjugates are becoming more feasible since new tools and resources are constantly emerging, including genomes and transcriptomes of various parasites as well as array-based techniques. These tools open up the possibility of predicting not only a specific glycan but the whole potential glycan metabolism of an organism, from monosaccharides transporters and nucleotide sugar biosynthesis pathways to probable glycan chain extensions of *N*- and *O*-glycans. Although still limited, since only enzymes with human homologues can be predicted, such determinations followed by more functional analyses of enzymes can lead to implementation of new recombinant systems to produce specific glycans (Prasanphanich *et al.*, 2014), and to the development of new drugs and vaccines (Mickum *et al.*, 2014).

In an example of the applicability of genomic prediction, Peterson *et al.* (2013) used a genome-wide homology-based bioinformatics approach to identify α3- and α6-fucosyltransferases (FucTs) genes that contribute to the production of fucosylated glycans in *S. mansoni* revealing information regarding the genomic organization, genetic variation and stage expression of these enzymes. Moreover, difficulties with the isolation and/or recombinant production of specific glycans can now be overcome using strategies of chemical synthesis of these molecules, which for instance have revolutionized the development of vaccines as mentioned for *T. spiralis, Echinococcus*, *Schistosoma* and *P. falciparum.* The last of these may result in the first glycan-based vaccine to combat a parasitic infection. Altogether, these data demonstrate the wide application of discoveries in glycobiology and inspire further research in this field.

Conclusions

Parasites are covered by or secrete distinct glycoconjugates that have been experimentally shown to help with the parasite's survival and propagation. The improvement of methods related to glycan analysis has opened a completely new field to be exploited. In recent years, the glycan profiles of several parasites have been characterized, giving us knowledge of the range of carbohydrates produced by different organisms. In fact, these molecules were shown to vary from complex to simple structures, and their expression might change with parasite genus, species and even strains and developmental stage, highlighting the numerous applications these molecules could have in strategies to control parasitic infections.

However, the general similarities among glycans produced by different parasites, and their hosts, represent a challenge for the development of specific diagnoses, as these molecules seem to haunt immunological tests through cross-reactivity that lead to false positive reactions. Forthcoming investigations and development of more advanced technologies for glycan analyses should permit us to deal with the cross-reactivity problem and uncover the machinery behind glycan biosynthesis and allow large scale production of them to fight against a wide range of diseases including parasitic infections.

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