

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 (Man₃GlcNAc₂) is attached to an

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(β 1-3) or GlcNAc(β 1-6) is added into the first GalNAc giving rise to the core type 1 [Gal(β 1-3)GalNAc] or type 2 [GlcNAc(β 1-6)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, it is not surprising that diverse eukaryotic parasites were observed to express a wide diversity of glycans or glycoconjugates containing distinctive and unusual monosaccharide 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(Fuca1-3)GlcNAc; Le^x], LacdiNAc (GalNAc β 1,4GlcNAc; LDN), fucosylated LDN [GalNAc β 1,4(Fuca1-3)GlcNAc; LDNF], truncated *O*-glycans, known as *T* antigen (Gal β 1-3GalNAc α 1-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

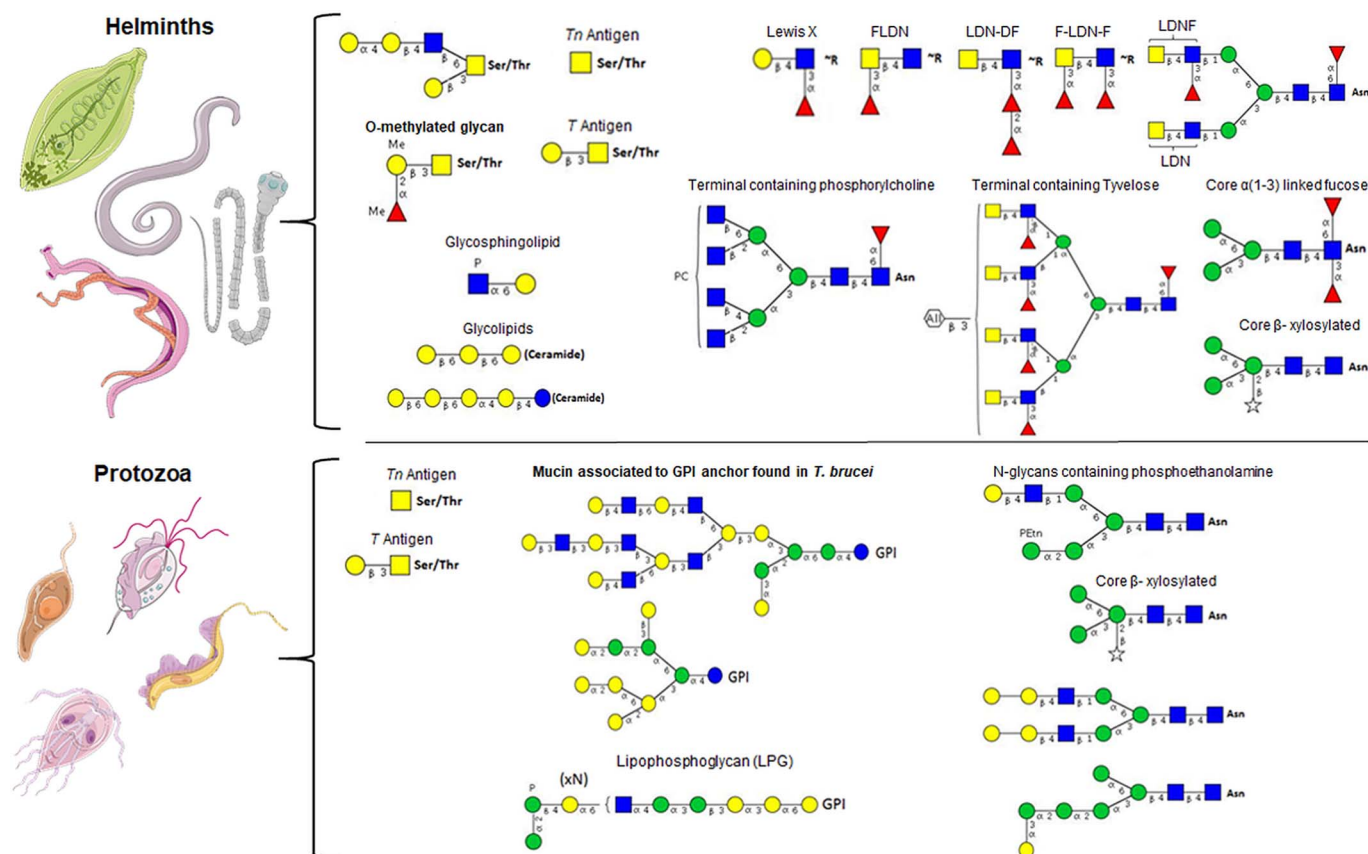


Fig. 1. Main glycan moieties found in helminth and protozoan parasites.

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-acetylglucosamine structures.

Poly-N-acetylglucosamine 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,

Table 1. Main glycan structures described in helminths and protozoa

Parasite	Glycan name	Reference
Helminths		
Trematodes		
<i>Schistosoma</i> sp	<ul style="list-style-type: none"> • 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 (Man₅₋₉GlcNAc) • N-linked glycan containing β₁₋₂ xylose • N-linked glycan containing α₃ fucose • Circulating Cathodic Antigen (CCA) • Circulating Anodic Antigen (CCA) 	Wisniewski <i>et al.</i> (1993) Bergwerff <i>et al.</i> (1994) Haslam <i>et al.</i> (1996, 1998) van Die <i>et al.</i> (1999) Cummings and Nyame (1999) Hokke <i>et al.</i> (2007) Nyame <i>et al.</i> (2002) Wuhrer <i>et al.</i> (2006) van Die and Cummings (2006) Peterson <i>et al.</i> (2009)
<i>Fasciola hepatica</i>	<ul style="list-style-type: none"> • LDN • LDNF • Truncated O-glycans T e Tn • Glycosphingolipid 	Vervelde <i>et al.</i> (2003) Freire <i>et al.</i> (2003) Wuhrer <i>et al.</i> (2004)
<i>Opisthorchis viverrini</i>	<ul style="list-style-type: none"> • Mono-fucosylated N-linked glycans • Truncated, hybrid and complex glycans with 1–4 antennas • O-glycan mucin type, containing 1–5 antennas, (Galβ₁₋₃GalNAc) 	Talabnin <i>et al.</i> (2013)
Nematodes		
<i>Ascaris suum</i>	<ul style="list-style-type: none"> • Man₅₋₉GlcNAc • N-linked glycan containing phosphorylcholine terminals • O-glycans • Glycosphingolipid with phosphorylcholine terminals • Glycosphingolipid containing 3-sulfogalactosylcerebroside 	Lochnit <i>et al.</i> (1998) Dell <i>et al.</i> (1999) Poltl <i>et al.</i> (2007)
<i>Toxocara canis</i>	<ul style="list-style-type: none"> • Truncated O-glycans T e Tn • O-Methylated glycans 	Khoo <i>et al.</i> (1991) Casaravilla <i>et al.</i> (2003)
<i>Dictyocaulus viviparus</i>	<ul style="list-style-type: none"> • Man₅₋₉GlcNAc • Lex 	Haslam <i>et al.</i> (2000)
<i>Trichinella spiralis</i>	<ul style="list-style-type: none"> • LDN e LDNF (with or without phosphorylcholine terminals) • Tyvelose β₃-linked • Man₅₋₉GlcNAc • N-linked glycans containing α₃-fucose 	Wisniewski <i>et al.</i> (1993) Haslam <i>et al.</i> (1996, 1998) van Die <i>et al.</i> (1999) Morelle <i>et al.</i> (2000) van Die and Cummings (2006)
<i>Haemonchus contortus</i>	<ul style="list-style-type: none"> • LDN • LDNF • Man₅₋₉GlcNAc • N-linked glycans containing α₃-fucose 	Wisniewski <i>et al.</i> (1993) Haslam <i>et al.</i> (1996, 1998) van Die <i>et al.</i> (1999) Geldhof <i>et al.</i> (2005) van Die and Cummings (2006)
<i>Dirofilaria immitis</i>	<ul style="list-style-type: none"> • LDN • LDNF 	Nyame <i>et al.</i> (1998)
<i>Wuchereria bancrofti</i>	<ul style="list-style-type: none"> • N-linked glycans containing phosphorylcholine terminals 	Dell <i>et al.</i> (1999)
<i>Onchocerca volvulus</i>	<ul style="list-style-type: none"> • Man₅₋₉GlcNAc 	Haslam <i>et al.</i> (1999) Wuhrer <i>et al.</i> (2000)

(Continued)

Table 1. (Continued.)

Parasite	Glycan name	Reference
	<ul style="list-style-type: none"> • N-linked glycans containing phosphorylcholine terminals • Glycolipids containing phosphorylcholine terminals 	
<i>Onchocerca gibsoni</i>	<ul style="list-style-type: none"> • Man₅₋₉GlcNAc • N-linked glycans containing phosphorylcholine terminals 	Haslam <i>et al.</i> (1999)
<i>Acanthocheilonema viteae</i>	<ul style="list-style-type: none"> • Man₅₋₉GlcNAc • N-linked glycans containing phosphorylcholine terminals 	Haslam <i>et al.</i> (1999)
<i>Nippostrongylus brasiliensis</i>	<ul style="list-style-type: none"> • Truncated O-glycans T e Tn 	Casaravilla <i>et al.</i> (2003)
Cestodes		
<i>Echinococcus multilocularis</i>	<ul style="list-style-type: none"> • Truncated O-glycans T e Tn • Mucin 	Ingold <i>et al.</i> (2000) Hülsmeier <i>et al.</i> (2002)
<i>Echinococcus granulosus</i>	<ul style="list-style-type: none"> • Truncated O-glycans T e Tn • Man₅₋₉GlcNAc • Truncated glycans • N-linked glycans containing phosphorylcholine terminals • Glycosphingolipid 	Khoo <i>et al.</i> (1997) Alvarez Errico <i>et al.</i> (2001) Paschinger <i>et al.</i> (2012) Wuhrer <i>et al.</i> (2004)
<i>Mesocestoides vogae</i>	<ul style="list-style-type: none"> • Truncated O-glycans T e Tn, sialyl-Tn^a 	Medeiros <i>et al.</i> (2008) Van Die and Cummings (2010)
<i>Metacestoides corti</i>	<ul style="list-style-type: none"> • Truncated O-glycans T e Tn 	Freire <i>et al.</i> (2003)
<i>Taenia hydatigena</i>	<ul style="list-style-type: none"> • Truncated O-glycans T e Tn 	Freire <i>et al.</i> (2003)
<i>Taenia crassiceps</i>	<ul style="list-style-type: none"> • N-linked glycans containing α_3-fucose • Man₅₋₉GlcNAc • Glycolipids • Glycosphingolipid 	Nyame <i>et al.</i> (2004) Lee <i>et al.</i> (2005)
<i>Taenia solium</i>	<ul style="list-style-type: none"> • Man₅₋₉GlcNAc • Complex and truncated N-linked glycans, with or without fucose • Glycolipids 	Restrepo <i>et al.</i> (2000) Haslam <i>et al.</i> (2003) Nyame <i>et al.</i> (2004)
<i>Taenia saginata</i>	<ul style="list-style-type: none"> • Glycolipids 	Nyame <i>et al.</i> (2004)
Protozoa		
<i>Trypanosoma sp</i>	<ul style="list-style-type: none"> • Glycosylphosphatidylinositol Anchors (VSG) • Procyclin (PARP) • High galactose O-glycans – Mucin • Lipopeptidephosphoglycan (LPPG) • Phospholipids-linked GPI (GIPL) • Trans-sialidase 	Previato <i>et al.</i> (1990) Ferguson <i>et al.</i> (1993) Costa <i>et al.</i> (1998) Ferguson (1999) Todeschini <i>et al.</i> (2001)
<i>Plasmodium sp</i>	<ul style="list-style-type: none"> • Glycosylphosphatidylinositol Anchors (GPI) • N- and O-linked glycans 	Khan <i>et al.</i> (1997) Gowda and Davidson (1999)
<i>Leishmania sp</i>	<ul style="list-style-type: none"> • Lipophosphoglycan (LPG) • Phospholipids-linked GPI (GIPL) • Phosphoglycan • Proteophosphoglycan (PPG) • O-glycan, mucin type 	Ilg <i>et al.</i> (1995) Descoteaux and Turco (1999) Guha-Niyogi <i>et al.</i> (2001)

(Continued)

Table 1. (Continued.)

Parasite	Glycan name	Reference
<i>Entamoeba histolytica</i>	<ul style="list-style-type: none"> • Lectin Gal-GalNAc • LPG • LPPG • Sialyl glycoconjugates 	Stanley <i>et al.</i> (1995) Petri (1996) Moody-Haupt <i>et al.</i> (2000)
<i>Trichomonas vaginalis</i>	<ul style="list-style-type: none"> • LPG • Adesins • N-linked glycans with specific modifications 	Paschinger <i>et al.</i> (2012)
<i>Giardia duodenalis</i>	<ul style="list-style-type: none"> • Variable surface glycoprotein (VSG) • O-glycans • N-glycans 	Papanastasiou <i>et al.</i> (1997) Bulik <i>et al.</i> (2000)
<i>Toxoplasma gondii</i>	<ul style="list-style-type: none"> • O-glycans • N-glycans • GPI anchors 	Guha-Niyogi <i>et al.</i> (2001)

^aPossible 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 (Brodszyn *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

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 (TNF α) and intracellular adhesion molecules I (ICAM-I). This observation led to 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, anti-retroviral lectin cyanovirin-N. Complex N-glycans contain α 1,2-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 [Glc α 1-6(n) Glc β 1-6Gal] 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

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-Arabinohexose) 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 TNF α . 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 phosphocholine (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 *Acanthocheilonema 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 α 1,3-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 α 1,6- and α 1,3-fucose on the proximal core GlcNAc, and a galactosylated distal α 1,3-fucose (Table 1). Other motifs displayed galactosylation of the core α 1,6-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(α 1-3)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(α 1-3)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 (Verissimo *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 Verissimo *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 (Verissimo *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, Verissimo *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

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 (Gal β 1-3GalNAc), also known as T antigen, and core 2 [Gal β 1-3(GlcNAc β 1-6) GalNAc] the Tn antigen (Díaz *et al.*, 2009). The Gal β 1-3 residue of core 1 can be decorated with additional Gal β 1-3 residues, generating a linear chain (Gal β 1-3Gal β 1-3GalNAc), and the elongation of mucin glycans with Gal β 1-3 distinguishes *E. multilocularis* from *E. granulosus* (del Puerto *et al.*, 2016). Other structures extended with Gal β 1-4 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 α 1,6-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, Gal β 1-6Gal, 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; Wuhler *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

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 TLR 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(β 1-6)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. Ravida *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; Verissimo *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.

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 α 3- and α 6-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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References

- Abdulla MH, Lim KC, McKerrow JH and Caffrey CR (2011) Proteomic identification of IPSE/ α -1 as a major hepatotoxin secreted by *Schistosoma mansoni* eggs. *PLoS Neglected Tropical Diseases* 5, e1368.
- Aguirre García M, Gutiérrez-Kobeh L and López Vancell R (2015) *Entamoeba histolytica*: adhesins and lectins in the trophozoite surface. *Molecules* 20, 2802–2815.
- Akira S (2006) TLR signaling. *Current Topics in Microbiology and Immunology* 311, 1–16. PMID: 17048703.
- Alba Soto CD, Mirkin GA, Solana ME and González Cappa SM (2003) Trypanosoma cruzi infection modulates in vivo expression of major histocompatibility complex class II molecules on antigen-presenting cells and T-cell stimulatory activity of dendritic cells in a strain-dependent manner. *Infection and Immunity* 71, 194–199.
- Allen S, Richardson JM, Mehlert A and Ferguson MA (2013) Structure of a complex phosphoglycan epitope from gp72 of *Trypanosoma cruzi*. *The Journal of Biological Chemistry* 288, 11093–11105.
- Alvarez Errico D, Medeiros A, Míguez M, Casaravilla C, Malgor R, Carmona C, Nieto A and Osinaga E (2001) O-glycosylation in *Echinococcus granulosus*: identification and characterization of the carcinoma-associated Tn antigen. *Experimental Parasitology* 98, 100–109.
- Aranzamendi C, Tefsen B, Jansen M, Chiumiento L, Bruschi F, Kortbeek T, Smith DF, Cummings RD, Pinelli E and Van Die I (2011) Glycan microarray profiling of parasite infection sera identifies the LDNF glycan as a potential antigen for serodiagnosis of trichinellosis. *Experimental Parasitology* 129, 221–226.
- Argueta-Donohué J, Carrillo N, Valdés-Reyes L, Zentella A, Aguirre-García M, Becker I and Gutiérrez-Kobeh L (2008) *Leishmania mexicana*: participation of NF- κ B in the differential production of IL-12 in dendritic cells and monocytes induced by lipophosphoglycan (LPG). *Experimental Parasitology* 120, 1–9.
- Bangs JD (2018) Evolution of antigenic variation in African Trypanosomes: variant surface glycoprotein expression, structure, and function. *Bioessays: News and Reviews in Molecular, Cellular and Developmental Biology* 40, e1800181.
- Behm CA (1997) The role of trehalose in the physiology of nematodes. *International Journal of Parasitology* 27, 215–229.
- Bennett CL, Misslitz A, Colledge L, Aebischer T and Blackburn CC (2001) Silent infection of bone marrow-derived dendritic cells by *Leishmania mexicana* amastigotes. *European Journal of Immunology* 31, 876–883.
- Bergwerff AA, van Dam GJ, Rotmans JP, Deelder AM, Kamerling JP and Vliegthart JF (1994) The immunologically reactive part of immunopurified circulating anodic antigen from *Schistosoma mansoni* is a threonine-linked polysaccharide consisting of \rightarrow 6)-(beta-D-GlcP-A-(1 \rightarrow 3))-beta-D-GalpNAc-(1 \rightarrow repeating units. *Journal Biology Chemistry* 269, 31510–7.
- Bhattacharya A, Arya R, Clark CG and Ackers JP (2000) Absence of lipophosphoglycan-like glycoconjugates in *Entamoeba dispar*. *Parasitology* 120, 31–35.

- Brodskyn C, Patricio J, Oliveira R, Lobo L, Arnholdt A, Mendonça-Previato L, Barral A and Barral-Netto M (2002) Glycosylphospholipids from *Trypanosoma cruzi* interfere with macrophages and dendritic cell responses. *Infection and Immunity* **70**, 3736–3743.
- Bulik DA, van Ophem P, Manning JM, Shen Z, Newburg DS and Jarroll EL (2000) UDP-N-acetylglucosamine pyrophosphorylase, a key enzyme in encysting *Giardia*, is allosterically regulated. *The Journal of Biological Chemistry* **275**, 14722–14728.
- Caldas IR, Campi-Azevedo AC, Oliveira LF, Silveira AM, Oliveira RC and Gazzinelli G (2008) Human schistosomiasis mansoni: immune responses during acute and chronic phases of the infection. *Acta Tropica* **108**, 109–117.
- Campos MAS, Almeida IC, Takeuchi O, Akira S, Valente EP, Procópio DO, Travassos LR, Smith JA, Golenbock DT and Gazzinelli RT (2001) Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. *Journal of Immunology* **167**, 416–423.
- Casacuberta M, Kinunghi S, Vennervald BJ and Olsen A (2016) Evaluation and optimization of the Circulating Cathodic Antigen (POC-CCA) cassette test for detecting *Schistosoma mansoni* infection by using image analysis in school children in Mwanza Region, Tanzania. *Parasite Epidemiology and Control* **1**, 105–115.
- Casaravilla C, Freire T, Malgor R, Medeiros A, Osinaga E and Carmona C (2003) Mucin-type O-glycosylation in helminth parasites from major taxonomic groups: evidence for widespread distribution of the Tn antigen (GalNAc-Ser/Thr) and identification of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase activity. *Journal of Parasitology* **89**, 709–714.
- Chakir H, Campos-Neto A, Mojibian M and Webb JR (2003) IL-12R β 2-deficient mice of a genetically resistant background are susceptible to *Leishmania major* infection and develop a parasite-specific Th2 immune response. *Microbes and Infection* **5**, 241–249.
- Chuah C, Jones MK, Burke ML, Owen HC, Anthony BJ, McManus DP, Ramm GA and Gobert GN (2013) Spatial and temporal transcriptomics of *Schistosoma japonicum*-induced hepatic granuloma formation reveals novel roles for neutrophils. *Journal of Leukocyte Biology* **94**, 353–365.
- Cipollo JF, Awad AM, Costello CE and Hirschberg CB (2005) N-glycans of *Caenorhabditis elegans* are specific to developmental stages. *The Journal of Biological Chemistry* **280**, 26063–26072.
- Clark IA and Schofield L (2000) Pathogenesis of malaria. *Parasitology Today* **16**, 451–454.
- Coelho-Finamore JM, Freitas VC, Assis RR, Melo MN, Novozhilova N, Secundino NF, Pimenta PF, Turco SJ and Soares RP (2011) *Leishmania infantum*: lipophosphoglycan intraspecific variation and interaction with vertebrate and invertebrate hosts. *International Journal for Parasitology* **41**, 333–342.
- Colley DG, Binder S, Campbell C, King CH, Tchuem Tchuente LA, N’Goran EK, Erko B, Karanja DM, Kabatereine NB, van Lieshout L and Rathbun S (2013) A five-country evaluation of a point-of-care circulating cathodic antigen urine assay for the prevalence of *Schistosoma mansoni*. *The American Journal of Tropical Medicine and Hygiene* **88**, 426–432.
- Corstjens PL, De Dood CJ, Kornelis D, Fat EM, Wilson RA, Kariuki TM, Nyakundi RK, Loverde PT, Abrams WR, Tanke HJ, Van Lieshout L, Deelder AM and Van Dam GJ (2014) Tools for diagnosis, monitoring and screening of *Schistosoma* infections utilizing lateral-flow based assays and upconverting phosphor labels. *Parasitology* **141**, 1841–1855.
- Costa F, Franchin G, Pereira-Chioccola VL, Ribeirão M, Schenkman S and Rodrigues MM (1998) Immunization with a plasmid DNA containing the gene of *trans*-sialidase reduces *Trypanosoma cruzi* infection in mice. *Vaccine* **16**, 768–774.
- Cova M, Rodrigues JA, Smith TK and Izquierdo L (2015) Sugar activation and glycosylation in *Plasmodium*. *Malaria Journal* **14**, 427.
- Cummings RD (2009) The repertoire of glycan determinants in the human glycome. *Molecular BioSystems* **5**, 1087–1104.
- Cummings RD and Nyame AK (1996) Glycobiology of schistosomiasis. *The FASEB Journal* **10**, 838–848.
- Cummings RD and Nyame AK (1999) Schistosome glycoconjugates. *Biochimica et Biophysica Acta* **1455**, 363–374.
- Damerow M, Rodrigues JA, Wu D, Güther ML, Mehlert A and Ferguson MA (2014) Identification and functional characterization of a highly divergent N-acetylglucosaminyltransferase I (TbGnTI) in *Trypanosoma brucei*. *The Journal of Biological Chemistry* **289**, 9328–9339.
- Damerow M, Graalfs F, Güther ML, Mehlert A, Izquierdo L and Ferguson MA (2016) A gene of the β 3-glycosyltransferase family encodes N-acetylglucosaminyltransferase II function in *Trypanosoma brucei*. *The Journal of Biological Chemistry* **291**, 13834–13845.
- Deelder AM, Kornelis D, Van Marck EA, Eveleigh PC and Van Egmond JG (1980) *Schistosoma mansoni*: characterization of two circulating polysaccharide antigens and the immunological response to these antigens in mouse, hamster, and human infections. *Experimental Parasitology* **50**, 16–32, PMID: 7389856.
- Del Puerto L, Rovetta R, Navatta M, Fontana C, Lin G, Moyna G, Dematteis S, Brehm K, Koziol U, Ferreira F and Díaz A (2016) Negligible elongation of mucin glycans with Gal β 1–3 units distinguishes the laminated layer of *Echinococcus multilocularis* from that of *Echinococcus granulosus*. *International Journal for Parasitology* **46**, 311–321.
- Dell A, Haslam SM, Morris HR and Khoo KH (1999) Immunogenic glycoconjugates implicated in parasitic nematode diseases. *Biochimica et Biophysica Acta* **1455**, 353–362.
- Descoteaux A and Turco SJ (1999) Glycoconjugates in *Leishmania* infectivity. *Biochimica et Biophysica Acta* **1455**, 341–352.
- de Souza JB, Runglall M, Corran PH, Okell LC, Kumar S, Gowda DC, Couper KN and Riley EM (2010) Neutralization of malaria glycosylphosphatidylinositol in vitro by serum IgG from malaria-exposed individuals. *Infection and Immunity* **78**, 3920–3929.
- De Veer MJ, Curtis JM, Baldwin TM, Didonato JA, Sexton A, Mcconville MJ, Handman E and Schofield L (2003) Myd88 is essential for clearance of *Leishmania* major: possible role for lipophosphoglycan and toll-like receptor 2 signaling. *European Journal of Immunology* **33**, 2822–2831.
- Díaz A, Fontana EC, Todeschini AR, Soulé S, González H, Casaravilla C, Portela M, Mohana-Borges R, Mendonça-Previato L, Previato JO and Ferreira F (2009) The major surface carbohydrates of the *Echinococcus granulosus* cyst: mucin-type O-glycans decorated by novel galactose-based structures. *Biochemistry* **48**, 11678–11691.
- Diebold SS (2009) Activation of dendritic cells by toll-like receptors and C-type lectins. *Handbook of Experimental Pharmacology* **188**, 3–30.
- Eberl M, Langermans JA, Vervenne RA, Nyame AK, Cummings RD, Thomas AW, Coulson PS and Wilson RA (2001) Antibodies to glycans dominate the host response to schistosome larvae and eggs: is their role protective or subversive? *The Journal of Infectious Diseases* **183**, 1238–1247.
- Erdmann H, Steeg C, Koch-Nolte F, Fleischer B and Jacobs T (2009) Sialylated ligands on pathogenic *Trypanosoma cruzi* interact with Siglec-E (sialic acid-binding Ig-like lectin-E). *Cellular Microbiology* **11**, 1600–1611.
- Everts B, Hussaarts L, Driessen NN, Meevissen MHJ, Schramm G, van der Ham AJ, van der Hoeven B, Scholzen T, Burgdorf S, Mohrs M, Pearce EJ, Hokke CH, Haas H, Smits HH and Yazdanbakhsh M (2012) Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. *The Journal of Experimental Medicine* **209**, 1753–1767.
- Ferguson MA (1999) The structure, biosynthesis and functions of glycosylphosphatidylinositol anchors, and the contributions of trypanosome research. *Journal of Cell Science* **112**, 2799–2809.
- Ferguson BJ, Newland SA, Gibbs SE, Tourlomis P, Fernandes dos Santos P, Patel MN, Hall SW, Walczak H, Schramm G, Haas H, Dunne DW, Cooke A and Zaccaro P (2015) The *Schistosoma mansoni* T2 ribonuclease omega-1 modulates inflammasome-dependent IL-1 β secretion in macrophages. *International Journal for Parasitology* **45**, 809–813.
- Fincher GB (2009) Exploring the evolution of (1,3; 1,4)- β -D-glucans in plant cell walls: comparative genomics can help!. *Current Opinion in Plant Biology* **12**, 140–147.
- Forbes LB, Appleyard GD and Gajadhar AA (2004) Comparison of synthetic tyvelose antigen with excretory-secretory antigen for the detection of trichinellosis in swine using enzyme-linked immunosorbent assay. *Journal of Parasitology* **90**, 835–840.
- Forestier CL, Gao Q and Boons GJ (2015) *Leishmania* lipophosphoglycan: how to establish structure-activity relationships for this highly complex and multifunctional glycoconjugate? *Frontiers in Cellular and Infection Microbiology* **4**, 193.
- Freire T, Casaravilla C, Carmona C and Osinaga E (2003) Mucin type O-glycosylation in *Fasciola hepatica*: characterization of carcinoma associated Tn and sialyl-Tn antigens and evaluation of UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase activity. *International Journal of Parasitology* **33**, 47–56.
- Gala RP, D’Souza M and Zughair SM (2016) Evaluation of various adjuvant nanoparticulate formulations for meningococcal capsular polysaccharide-based vaccine. *Vaccine* **34**, 3260–3267.

- García-Campos A, Ravidà A, Nguyen DL, Cwiklinski K, Dalton JP, Hokke CH, O'Neill S and Mulcahy G (2016) Tegument glycoproteins and cathepsins of newly excysted juvenile *Fasciola hepatica* carry mannosidic and paucimannosidic N-glycans. *PLoS Neglected Tropical Diseases* **10**, e0004688.
- Geijtenbeek TB and Gringhuis SI (2009) Signalling through C-type lectin receptors: shaping immune responses. *Nature Reviews Immunology* **9**, 465–479.
- Geijtenbeek TB, Van Vliet SJ, Koppel EA, Sanchez-Hernandez M, Vandenbroucke-Grauls CM, Appelmek B and Van Kooyk Y (2003) Mycobacteria target DC-SIGN to suppress dendritic cell function. *Journal Experimental Medicine* **197**, 7–17.
- Georgieva K, Georgieva S, Mizinska Y and Stoitsova SR (2012) *Fasciola hepatica* miracidia: lectin binding and stimulation of in vitro miracidium-to-sporocyst transformation. *Acta Parasitologica* **57**, 46–52.
- Giddings OK, Eickhoff CS, Sullivan NL and Hoft df (2010) Intranasal vaccinations with the trans-sialidase antigen plus CpG adjuvant induce mucosal immunity protective against conjunctival *Trypanosoma cruzi* challenges. *Infection and Immunity* **78**, 1333–1338.
- Goel A, Vohra H and Varshney GC (1999) Strain-specific recognition of live *Leishmania donovani* promastigotes by homologous antiserum raised against a crude membrane fraction of infected macrophages. *Parasitology Research* **85**, 19–24.
- Gomez-García L, Lopez-Marin LM, Saavedra R, Reyes JL, Rodríguez-Sosa M and Terrazas LI (2005) Intact glycans from cestode antigens are involved in innate activation of myeloid suppressor cells. *Parasite Immunology* **27**, 395–405.
- Gómez-García L, Rivera-Montoya I, Rodríguez-Sosa M and Terrazas LI (2006) Carbohydrate components of *Taenia crassiceps* metacestodes display Th2-adjuvant and antiinflammatory properties when co-injected with bystander antigen. *Parasitology Research* **99**, 440–448.
- Goodridge HS, Marshall FA, Else KJ, Houston KM, Egan C, Al-Riyami L, Liew FY, Harnett W and Harnett MM (2005) Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine-containing secreted product, ES-62. *Journal of Immunology* **174**, 284–293.
- Gowda DC and Davidson EA (1999) Protein glycosylation in the malaria parasite. *Parasitology today* **15**, 147–152.
- Goyal PK, Wheatcroft J and Wakelin D (2002) Tyvelose and protective responses to the intestinal stages of *Trichinella spiralis*. *Parasitology International* **51**, 91–98.
- Guha-Niyogi A, Sullivan DR and Turco SJ (2001) Glycoconjugate structures of parasitic protozoa. *Glycobiology* **11**, 45R–59R.
- Handel TM, Johnson Z, Crown SE, Lau EK and Proudfoot AE (2005) Regulation of protein function by glycosaminoglycans as exemplified by chemokines. *Annual Review of Biochemistry* **74**, 385–410.
- Haslam SM, Coles GC, Morris HR and Dell A (2000) Structural characterization of the N-glycans of *Dictyocaulus viviparus*: discovery of the Lewis(x) structure in a nematode. *Glycobiology* **10**, 223–229.
- Haslam SM, Coles GC, Munn EA, Smith TS, Smith HF, Morris HR and Dell A (1996) *Haemonchus contortus* glycoproteins contain N-linked oligosaccharides with novel highly fucosylated core structures. *The Journal of Biological Chemistry* **271**, 30561–30570.
- Haslam SM, Coles GC, Reason AJ, Morris HR and Dell A (1998) The novel core fucosylation of *Haemonchus contortus* N-glycans is stage specific. *Molecular and Biochemical Parasitology* **93**, 143–147.
- Haslam SM, Houston KM, Harnett W, Reason AJ, Morris HR and Dell A (1999) Structural studies of N-glycans of filarial parasites. Conservation of phosphorylcholine-substituted glycans among species and discovery of novel chito-oligomers. *The Journal of Biological Chemistry* **274**, 20953–20960.
- Haslam SM, Morris HR and Dell A (2001) Mass spectrometric strategies: providing structural clues for helminth glycoproteins. *Trends in Parasitology* **17**, 231–235.
- Haslam SM, Restrepo BI, Obregón-Henao A, Teale JM, Morris HR and Dell A (2003) Structural characterization of the N-linked glycans from *Taenia solium* metacestodes. *Molecular and Biochemical Parasitology* **126**, 103–107.
- Hawk MW, Shahlai K, Kim KD and Theis JH (2005) Neurocysticercosis: a review. *Surgical Neurology* **63**, 123–132.
- Heim C, Hertzberg H, Butschli A, Bleuler-Martinez S, Aebi M, Deplazes P, Künzler M and Štefanić S (2015) Inhibition of *Haemonchus contortus* larval development by fungal lectins. *Parasites and Vectors* **19**, 425.
- Hoft df, Eickhoff CS, Giddings OK, Vasconcelos JR and Rodrigues MM (2007) Trans-sialidase recombinant protein mixed with CpG motif-containing oligodeoxynucleotide induces protective mucosal and systemic *Trypanosoma cruzi* immunity involving CD8+CTL and B cell-mediated cross-priming. *The Journal of Immunology* **179**, 6889–6900.
- Hokke CH and Deelder AM (2001) Schistosome glycoconjugates in host-parasite interplay. *Glycoconjugate Journal* **18**, 573–587.
- Hokke CH and van Diepen A (2017) Helminth glycomics – glycan repertoires and host-parasite interactions. *Molecular and Biochemical Parasitology* **215**, 47–57.
- Hokke CH, Deelder AM, Hoffmann KF and Wuhrer M (2007) Glycomics-driven discoveries in schistosome research. *Experimental Parasitology* **117**, 275–283.
- Hülsmeier AJ, Gehrig PM, Geyer R, Sack R, Gottstein B, Deplazes P and Köhler P (2002) A major *Echinococcus multilocularis* antigen is a mucin-type glycoprotein. *The Journal of Biological Chemistry* **277**, 5742–5748.
- Hwa KY and Khoo KH (2000) Structural analysis of the asparagine-linked glycans from the procyclic *Trypanosoma brucei* and its glycosylation mutants resistant to Concanavalin A killing. *Molecular Biochemical Parasitology* **111**, 173–184, PMID: 11087927.
- Ibraim IC, de Assis RR, Pessoa NL, Campos MA, Melo MN, Turco SJ and Soares RP (2013) Two biochemically distinct lipophosphoglycans from *Leishmania braziliensis* and *Leishmania infantum* trigger different innate immune responses in murine macrophages. *Parasites & Vectors* **6**, 54–65. doi: 10.1186/1756-3305-6-54.
- Ilg T, Stierhof YD, McConville MJ and Overath P (1995) Purification, partial characterization and immunolocalization of a proteophosphoglycan secreted by *Leishmania mexicana* amastigotes. *European Journal of Cell Biology* **66**, 205–215.
- Ingold K, Gottstein B and Hemphill A (2000) High molecular mass glycans are major structural elements associated with the laminated layer of in vitro cultivated *Echinococcus multilocularis* metacestodes. *International Journal of Parasitology* **30**, 207–214.
- Ivory CPA and Chadee K (2007) Activation of dendritic cells by the Gal-lectin of *Entamoeba histolytica* drives Th1 responses in vitro and in vivo. *European Journal of Immunology* **37**, 385–394.
- Jacobs W, Deelder A and Van Marck E (1999) Schistosomal granuloma modulation. II. Specific immunogenic carbohydrates can modulate schistosome-egg-antigen-induced hepatic granuloma formation. *Parasitology Research* **85**, 14–18.
- Jang-Lee J, Curwen RS, Ashton PD, Tissot B, Mathieson W, Panico M, Dell A, Wilson RA and Haslam SM (2007) Glycomics analysis of *Schistosoma mansoni* egg and cercarial secretions. *Molecular and Cellular Proteomics* **6**, 1485–1499.
- Jaurigue JA and Seeberger PH (2017) Parasite carbohydrate vaccines. *Frontiers in Cellular and Infection Microbiology* **12**, 248.
- Jawed JJ, Majumder S, Bandyopadhyay S, Biswas S, Parveen S and Majumdar S (2016) SLA-PGN-primed dendritic cell-based vaccination induces Th17-mediated protective immunity against experimental visceral leishmaniasis: a crucial role of PKC β . *Pathogens and Disease* **74**, pii: ftw041.
- Jebbari H, Stagg AJ, Davidson RN and Knight SC (2002) *Leishmania major* promastigotes inhibit dendritic cell motility in vitro. *Infection and Immunity* **70**, 1023–1026.
- Jones MK, Gobert GN, Zhang L, Sunderland P and McManus DP (2004) The cytoskeleton and motor proteins of human schistosomes and their roles in surface maintenance and host-parasite interactions. *Bioessays* **26**, 752–765.
- Kearney PE, Murray PJ, Hoy JM, Hohenhaus M and Kotze A (2016) The ‘Toolbox’ of strategies for managing *Haemonchus contortus* in goats: What’s in and what’s out. *Veterinary Parasitology* **15**, 93–107.
- Kailemia MJ, Ruhaak LR, Lebrilla CB and Amster IJ (2014) Oligosaccharide analysis by mass spectrometry: a review of recent developments. *Analytical Chemistry* **86**, 196–212.
- Kane CM, Cervi L, Sun J, et al. (2004) Helminth antigens modulate TLR-initiated dendritic cell activation. *Journal of Immunology* **173**, 7454–7461.
- Kane CM, Jung E and Pearce EJ (2008) *Schistosoma mansoni* egg antigen-mediated modulation of toll-like receptor (TLR)-induced activation occurs independently of TLR2, TLR4, and MyD88. *Infection and Immunity* **76**, 5754–5759.
- Kantelhardt SR, Wuhrer M, Dennis RD, Doenhoff MJ, Bickle Q and Geyer R (2002) Fuc(alpha1->3)GalNAc-: the major antigenic motif of *Schistosoma mansoni* glycolipids implicated in infection sera and keyhole-limpet haemocyanin cross-reactivity. *Biochemical Journal* **366**, 217–223.

- Kapsenberg ML (2003) Dendritic-cell control of pathogen-driven T-cell polarization. *Nature Reviews. Immunology* 3, 984–993.
- Karanja DM, Colley DG, Nahlen BL, Ouma JH and Secor WE (1997) Studies of schistosomiasis in Western Kenya: I. Evidence for immune-facilitated excretion of schistosome eggs from patients with *Schistosoma mansoni* and human immunodeficiency virus coinfections. *The American Journal of Tropical Medicine and Hygiene* 56, 515–521, PMID: 9180601.
- Kariuki TM, Farah IO, Wilson RA and Coulson PS (2008) Antibodies elicited by the secretions from schistosome cercariae and eggs are predominantly against glycan epitopes. *Parasite Immunology* 30, 554–562.
- Khan AH, Qazi AM, Hoessli DC, Torred-Duarte AP, Senaldi G, Qazi MH, Walker-Nasir E and Nasir-ud-Din (1997) Carbohydrate moiety of *Plasmodium falciparum* glycoproteins: the nature of the carbohydrate-peptide linkage in the MSP-2 glycoprotein. *Biochemistry and Molecular Biology International* 43, 655–668.
- Khoo KH, Maizels RM, Page AP, Taylor GW, Rendell NB and Dell A (1991) Characterization of nematode glycoproteins: the major O-glycans of *Toxocara* excretory-secretory antigens are O-methylated trisaccharides. *Glycobiology* 1, 163–171.
- Khoo KH, Sarda S, Xu X, Caulfield JP, McNeil MR, Homans SW, Morris HR and Dell A (1995) A unique multifucosylated-3GalNAc beta 1->4GlcNAc beta 1->3Gal alpha 1- motif constitutes the repeating unit of the complex O-glycans derived from the cercarial glycocalyx of *Schistosoma mansoni*. *The Journal of Biological Chemistry* 270, 17114–17123.
- Khoo KH, Nieto A, Morris HR and Dell A (1997) Structural characterization of the N-glycans from *Echinococcus granulosus* hydatid cyst membrane and protoscolices. *Molecular and Biochemical Parasitology* 86, 237–248.
- Kimura EA, Couto AS, Peres VJ, Casal OL and Katzin AM (1996) N-linked glycoproteins are related to schizogony of the intraerythrocytic stage in *Plasmodium falciparum*. *The Journal of Biological Chemistry* 271, 14452–14461, PMID: 8662869.
- Ko AI, Dräger UC and Harn DA (1990) A *Schistosoma mansoni* epitope recognized by a protective monoclonal antibody is identical to the stage-specific embryonic antigen 1. *Proceedings of the National Academy of Sciences USA* 87, 4159–4163.
- Koga R, Hamano S, Kuwata H, Atarashi K, Ogawa M, Hisaeda H, Yamamoto M, Akira S, Himeno K, Matsumoto M and Takeda K (2006) TLR-dependent induction of IFN- β mediates host defense against *Trypanosoma cruzi*. *Journal of Immunology* 177, 7059–7066.
- Koizumi A, Yamano K, Schweizer F, Takeda T, Kiuchi F and Hada N (2011) Synthesis of the carbohydrate moiety from the parasite *Echinococcus multilocularis* and their antigenicity against human sera. *European Journal of Medicinal Chemistry* 46, 1768–1778.
- Konecny P, Stagg AJ, Jebbari H, English N, Davidson RN and Knight SC (1999) Murine dendritic cells internalize *Leishmania major* promastigotes, produce IL-12 p40 and stimulate primary T cell proliferation in vitro. *European Journal of Immunology* 29, 1803–1811.
- Kusel JR, Al-Adhami BH and Doenhoff MJ (2007) The schistosome in the mammalian host: understanding the mechanisms of adaptation. *Parasitology* 134, 1477–1526.
- Lauwaet T, Oliveira MJ, De Bruyne G, Bruchhaus I, Duche ne M, Mareel M and Leroy A (2004) *Entamoeba histolytica* trophozoites transfer lipophosphopeptidoglycans to enteric cell layers. *International Journal for Parasitology* 34, 549–556.
- Lee JJ, Dissanayake S, Panico M, Morris HR, Dell A and Haslam SM (2005) Mass spectrometric characterisation of *Taenia crassiceps* metacystode N-glycans. *Molecular and Biochemical Parasitology* 143, 245–249.
- Lehr T, Frank S, Natsuka S, Geyer H, Beuerlein K, Doenhoff MJ, Hase S and Geyer R (2010) N-Glycosylation patterns of hemolymph glycoproteins from *Biomphalaria glabrata* strains expressing different susceptibility to *Schistosoma mansoni* infection. *Experimental Parasitology* 126, 592–602.
- Lindholz CG, Favero V, Verissimo CM, Candido RRF, de Souza RP, Dos Santos RR, Morassutti AL, Bittencourt HR, Jones MK, St Pierre TG and Graeff-Teixeira C (2018) Study of diagnostic accuracy of Helmintex, Kato-Katz, and POC-CCA methods for diagnosing intestinal schistosomiasis in Candeal, a low intensity transmission area in northeastern Brazil. *PLoS Neglected Tropical Diseases* 12, 1–16.
- Linehan SA, Coulson PS, Wilson RA, Mountford AP, Brombacher F, Martínez-Pomares L and Gordon S (2003) IL-4 receptor signaling is required for mannose receptor expression by macrophages recruited to granulomata but not resident cells in mice infected with *Schistosoma mansoni*. *Laboratory Investigation* 83, 1223–1231.
- Liu X, Siegrist S, Amacker M, Zurbriggen R, Pluschke G and Seeberger PH (2006) Enhancement of the immunogenicity of synthetic carbohydrates by conjugation to virosomes: a leishmaniasis vaccine candidate. *ACS Chemical Biology* 1, 161–164.
- Lochnit G, Nispel S, Dennis RD and Geyer R (1998) Structural analysis and immunohistochemical localization of two acidic glycosphingolipids from the porcine, parasitic nematode, *Ascaris suum*. *Glycobiology* 8, 891–899.
- Lorenzo C, Salinas G, Brugnini A, Wernstedt C, Hellman U and González-Sapienza G (2003) *Echinococcus granulosus* antigen 5 is closely related to proteases of the trypsin family. *Biochemical Journal* 369, 191–198.
- Magnelli P, Cipollo JF, Ratner DM, Cui J, Kelleher D, Gilmore R, Costello CE, Robbins PW and Samuelson J (2008) Unique Asn-linked oligosaccharides of the human pathogen *Entamoeba histolytica*. *The Journal of Biological Chemistry* 283, 18355–18364.
- Maizels RM, Balic A, GomezEscobar N, Nair M, Taylor MD and Allen JE (2004) Helminth parasites – masters of regulation. *Immunological Reviews* 201, 89–116.
- Maldonado-Bernal C, Kirschning CJ, Rosenstein Y, Rocha LM, Rios-Sarabia N, Espinosa-Cantellano M, Becker I, Estrada I, Salazar-González RM, López-Macías C, Wagner H, Sánchez J and Isibasi A (2005) The innate immune response to *Entamoeba histolytica* lipopeptidophosphoglycan is mediated by toll-like receptors 2 and 4. *Parasite Immunology* 27, 127–137.
- Marinets A, Zhang T, Guillen N, Gounon P, Bohle B, Vollmann U, Scheiner O, Wiedermann G, Stanley Jr SL and Duchene M (1997) Protection against invasive amebiasis by a single monoclonal antibody directed against a lipophosphoglycan antigen localized on the surface of *Entamoeba histolytica*. *The Journal of Experimental Medicine* 186, 1557–1565.
- Medeiros A, Chiribao ML, Ubillos L, Festari MF, Saldaña J, Robello C, Domínguez L, Calvete JJ and Osinaga E (2008) Mucin-type O-glycosylation in *Mesocestoides vogae* (syn. corti). *International Journal of Parasitology* 38, 265–276.
- Mickum ML, Prasanphanich NS, Heimbürg-Molinario J, Leon KE and Cummings RD (2014) Deciphering the glycogenome of schistosomes. *Frontiers in Genetics* 5, 262.
- Montero-Barrera D, Valderrama-Carvajal H, Terrazas CA, Rojas-Hernández S, Ledesma-Soto Y, Vera-Arias L, Carrasco-Yépez M, Gómez-García L, Martínez-Saucedo D, Becerra-Díaz M and Terrazas LI (2015) The macrophages galactose-type lectin-1 (MGL1) recognizes *Taenia crassiceps* antigens, triggers intracellular signaling, and is critical for resistance to this infection. *BioMed Research International* 2015, 615865.
- Mendonça-Previato L, Todeschini AR, Heise N and Previato JO (2003) Protozoan parasite-specific carbohydrate structures. *Current Opinion in Structural Biology* 15, 499–505.
- Moody S, Becker S, Nuchamowitz Y and Mirelman D (1997) Virulent and avirulent *Entamoeba histolytica* and *E. dispar* differ in their cell surface phosphorylated glycolipids. *Parasitology* 114, 95–104.
- Moody-Haupt S, Patterson JH, Mirelman D and McConville MJ (2000) The major surface antigens of *Entamoeba histolytica* trophozoites are GPI-anchored proteophosphoglycans. *Journal of Molecular Biology* 297, 409–420.
- Morassutti AL, Levert K, Perelygin A, da Silva AJ, Wilkins P and Graeff-Teixeira C (2012) The 31-kDa antigen of *Angiostrongylus cantonensis* comprises distinct antigenic glycoproteins. *Vector Borne Zoonotic Disease* 12, 961–968.
- Morelle W, Haslam SM, Olivier V, Appleton JA, Morris HR and Dell A (2000) Phosphorylcholine-containing N-glycans of *Trichinella spiralis*: identification of multiantennary lactiNAc structures. *Glycobiology* 10, 941–950.
- Mwanakasale V, Vounatsou P, Sukwa TY, Ziba M, Ernest A and Tanner M (2003) Interactions between *Schistosoma haematobium* and human immunodeficiency virus type 1: the effects of coinfection on treatment outcomes in rural Zambia. *The American Journal of Tropical Medicine and Hygiene* 69, 420–428, PMID: 14640503.
- Naderer T, Vince J and McConville MAJ (2004) Surface determinants *Leishmania* parasites and their role in infectivity in the mammalian host. *Current Molecular Medicine* 4, 649–665.
- Niborski V, Vallée I, Fonseca-Liñán R, Boireau P, Enciso A, Ortega-Pierres G and Yépez-Mulia L (2004) *Trichinella spiralis*: stimulation of mast cells by TSL-1 antigens trigger cytokine mRNA expression

- and release of IL-4 and TNF through an Ig-independent pathway. *Experimental Parasitology* **108**, 101–108.
- Norden AP and Strand M** (1985) Identification of antigenic *Schistosoma mansoni* glycoproteins during the course of infection in mice and humans. *The American Journal of Tropical Medicine and Hygiene* **34**, 495–507.
- Nunes Dda S, Gonzaga HT, Ribeiro Vda S, da Cunha Jr JP and Costa-Cruz J. M.** (2013) *Taenia saginata* metacystode antigenic fractions obtained by ion-exchange chromatography: potential source of immunodominant markers applicable in the immunodiagnosis of human neurocysticercosis. *Diagnostic Microbiology and Infectious Disease* **76**, 36–41.
- N'Zoukoudi-N'Doundou MY, Dirat I, Akouala JJ, Penchenier L, Makuwa M and Rey JL** (1995) Bilharziasis and human immunodeficiency virus infection in Congo. *Medecine Tropicale (Mars)* **55**, 249–251, PMID: 8559022.
- Nyame AK, Debose-Boyd R, Long TD, Tsang VC and Cummings RD** (1998) Expression of Lex antigen in *Schistosoma japonicum* and *S. haematobium* and immune responses to Lex in infected animals: lack of Lex expression in other trematodes and nematodes. *Glycobiology* **8**, 615–624.
- Nyame AK, Yoshino TP and Cummings RD** (2002) Differential expression of LacdiNAc, fucosylated LacdiNAc, and Lewis x glycan antigens in intramolluscan stages of *Schistosoma mansoni*. *Journal of Parasitology* **88**, 890–897.
- Nyame AK, Lewis FA, Doughty BL, Correa-Oliveira R and Cummings RD** (2003) Immunity to schistosomiasis: glycans are potential antigenic targets for immune intervention. *Experimental Parasitology* **104**, 1–13.
- Nyame AK, Kawar ZS and Cummings RD** (2004) Antigenic glycans in parasitic infections: implications for vaccines and diagnostics. *Archives of Biochemistry and Biophysics* **426**, 182–200.
- Okano M, Satoskar AR, Nishizaki K and Harn Jr DA** (2001) Lacto-N-fucopentaose III found on *Schistosoma mansoni* egg antigens functions as adjuvant for proteins by inducing Th2-type response. *Journal of Immunology* **167**, 442–450.
- Papanastasiou P, McConville MJ, Ralton J and Köhler P** (1997) The variant-specific surface protein of Giardia, VSP4A1, is a glycosylated and palmitoylated protein. *Biochemical Journal* **322**, 49–56.
- Paschinger K and Wilson IB** (2015) Two types of galactosylated fucose motifs are present on N-glycans of *Haemonchus contortus*. *Glycobiology* **25**, 585–590.
- Paschinger K, GonzalezSapienza GG and Wilson IBH** (2012) Mass spectrometric analysis of the immunodominant glycan epitope of *Echinococcus granulosus* antigen Ag5. *International Journal for Parasitology* **42**, 279–285.
- Pereira-Chioccola VL, Costa F, Ribeirão M, Soares IS, Arena F, Schenkman S and Rodrigues MM** (1999) Comparison of antibody and protective immune responses against *Trypanosoma cruzi* infection elicited by immunization with a parasite antigen delivered as naked DNA or recombinant protein. *Parasite Immunology* **21**, 103–110.
- Peterson NA, Hokke CH, Deelder AM and Yoshino TP** (2009) Glycotope analysis in miracidia and primary sporocysts of *Schistosoma mansoni*: differential expression during the miracidium-to-sporocyst transformation. *International Journal of Parasitology* **39**, 1331–1344.
- Peterson NA, Anderson TK and Yoshino TP** (2013) In silico analysis of the fucosylation-associated genome of the human blood fluke *Schistosoma mansoni*: cloning and characterization of the fucosyltransferase multigene family. *PLoS ONE* **8**, e63299.
- Petri Jr W** (1996) Amebiasis and the Entamoeba histolytica Gal/GalNAc lectin: from lab bench to bedside. *Journal of Investigative Medicine* **44**, 24–36.
- Pinheiro RO, Pinto EF, Lopes JR, Guedes HL, Fentanes RF and RossiBergmann B** (2005) TGF-beta-associated enhanced susceptibility to leishmaniasis following intramuscular vaccination of mice with *Leishmania amazonensis* antigens. *Microbes and Infection* **7**, 1317–1323.
- Pinheiro RO, Pinto EF, de Matos Guedes HL, Filho OA, de Mattos KA, Saraiva EM, de Mendonça SC and Rossi-Bergmann B** (2007) Protection against cutaneous leishmaniasis by intranasal vaccination with liposphoglycan. *Vaccine* **25**, 2716–2722.
- Pöhl G, Kerner D, Paschinger K and Wilson IB** (2007) N-glycans of the porcine nematode parasite *Ascaris suum* are modified with phosphorylcholine and core fucose residues. *The FEBS Journal* **274**, 714–726.
- Poncini CV, Alba Soto CD, Batalla E, Solana ME and Gonzalez Cappa SM** (2008) *Trypanosoma cruzi* induces regulatory dendritic cells in vitro. *Infection and Immunity* **76**, 2633–2641.
- Prasanphanich NS, Luyai AE, Song X, Heimburg-Molinari J, Mandalasi M, Mickum M, Smith DF, Nyame AK and Cummings RD** (2014) Immunization with recombinantly expressed glycan antigens from *Schistosoma mansoni* induces glycan-specific antibodies against the parasite. *Glycobiology* **24**, 619–637.
- Previate JO, Gorin PA, Mazurek M, Xavier MT, Fournet B, Wieruszkes JM and Mendonça-Previate L** (1990) Primary structure of the oligosaccharide chain of lipopeptidophosphoglycan of epimastigote forms of *Trypanosoma cruzi*. *Journal of Biological Chemistry* **265**, 2518–2526.
- Ralston KS and Petri WA** (2011) The ways of a killer: how does *Entamoeba histolytica* elicit host cell death? *Essays in Biochemistry* **51**, 193–210.
- Ravidà A, Aldridge AM, Driessen NN, Heus FA, Hokke CH and O'Neill SM** (2016) *Fasciola hepatica* surface coat glycoproteins contain mannose and phosphorylated N-glycans and exhibit immune modulatory properties independent of the mannose receptor. *PLoS Neglected Tropical Diseases* **10**, e0004601.
- Reason AJ, Ellis LA, Appleton JA, Wisniewski N, Grieve RB, McNeil M, Wassom DL, Morris HR and Dell A** (1994) Novel tyvelose-containing tri- and tetra-antennary N-glycans in the immunodominant antigens of the intracellular parasite *Trichinella spiralis*. *Glycobiology* **4**, 593–603.
- Reiner L and Locksley RM** (1995) The regulation of immunity to *Leishmania major*. *Annual Review of Immunology* **13**, 151–177.
- Restrepo BI, Obregón-Henao A, Mesa M, Gil DL, Ortiz BL, Mejía JS, Villota GE, Sanzón F and Teale JM** (2000) Characterisation of the carbohydrate components of *Taeniasolium* metacystode glycoprotein antigens. *International Journal of Parasitology* **30**, 689–696.
- Riganò R, Buttari B, Profumo E, Ortona E, Delunardo F, Margutti P, Mattei V, Teggi A, Sorice M and Siracusano A** (2007) *Echinococcus granulosus* antigen B impairs human dendritic cell differentiation and polarizes immature dendritic cell maturation towards a Th2 cell response. *Infection and Immunity* **75**, 1667–1678.
- Robijn MLM, Koeleman CAM, Hokke CH and Deelder AM** (2007) *Schistosoma mansoni* eggs excrete specific free oligosaccharides that are detectable in the urine of the human host. *Molecular and Biochemical Parasitology* **151**, 162–172.
- Rodriguez E, Noya V, Cervi L, Chiribao ML, Brossard N, Chiale C, Carmona C, Giacomini C and Freire T** (2015) Glycans from *Fasciola hepatica* modulate the host immune response and TLR-induced maturation of dendritic cells. *PLoS Neglected Tropical Diseases* **9**, e0004234.
- Roggelin L, Vinnemeier CD, Fischer-Herr J, Johnson-Weaver BT, Rolling T, Burchard GD, Staats HF and Cramer JP** (2015) Serological response following re-vaccination with *Salmonella typhi* Vi-capsular polysaccharide vaccines in healthy adult travellers. *Vaccine* **33**, 4141–4145.
- Rojas-Bernabe A, Garcia-Hernandez O, Maldonado-Bernal C, DelegadoDominguez J, Ortega E, Gutierrez-Kobeh L, Becker I and Aguirre-Garcia M** (2014) *Leishmania mexicana* lipophosphoglycan activates ERK and p38 MAP kinase and induces production of proinflammatory cytokines in human macrophages through TLR2 and TLR4. *Parasitology* **141**, 788–800.
- Russell DG and Alexander J** (1988) Effective immunization against cutaneous leishmaniasis with defined membrane antigens reconstituted into liposomes. *The Journal of Immunology* **140**, 1274–1279.
- San Francisco J, Barria I, Gutiérrez B, Neira I, Muñoz C, Sagua H, Araya JE, Andrade JC, Zailberger A, Catalán A, Remonsellez F, Vega JL and González J** (2017) Decreased cruzipain and gp85/transsialidase family protein expression contributes to loss of *Trypanosoma cruzi* trypomastigote virulence. *Microbes and Infection* **19**, 55–61.
- Schauer R, Reuter G, Muhlpfordt H, Andrade AF and Pereira ME** (1983) The occurrence of N-acetyl- and N-glycolylneuraminic acid in *Trypanosoma cruzi*. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* **364**, 1053–1057.
- Schofield L, McConville MJ, Hansen D, Campbell AS, Fraser-Reid B, Grusby MJ and Tachado SD** (1999) CD1d-restricted immunoglobulin G formation to GPI-anchored antigens mediated by NKT cells. *Science* **283**, 225–229.
- Schofield L, Hewitt MC, Evans K, Siomos MA and Seeberger PH** (2002) Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria. *Nature* **418**, 785–789.
- Sher A, Pearce E and Kaye P** (2003) Shaping the immune response to parasites: role of dendritic cells. *Current Opinion in Immunology* **15**, 421–429.
- Smit CH, van Diepen A, Nguyen DL, Wuhrer M, Hoffmann KF, Deelder AM and Hokke CH** (2015) Glycomic analysis of life stages of the human parasite *Schistosoma mansoni* reveals developmental expression profiles of functional and antigenic glycan motifs. *Molecular and Cellular Proteomics* **14**, 1750–1769.

- Spiro RG** (2002) Protein glycosylation: nature, distribution, enzymatic formation and disease implications of glycopeptides bonds. *Glycobiology* **12**, 43R–56R.
- Stanley JR Sr, Tian K, Koester JP and Li E** (1995) The serine-rich Entamoeba histolytica protein is a phosphorylated membrane protein containing O-linked terminal N-acetylglucosamine residues. *The Journal of Biological Chemistry* **270**, 4121–4126.
- Stormo SK, Praebel K and Elvevoll EO** (2009) Cold tolerance in sealworm (*Pseudoterranova decipiens*) due to heat-shock adaptations. *Parasitology* **136**, 1317–1324.
- Stothard JR, Kabatereine NB, Tukahebwa EM, Kazibwe F, Rollinson D, Mathieson W, Webster JP and Fenwick A** (2006) Use of circulation cathodic antigen (CCA) dipsticks for detection of intestinal and urinary schistosomiasis. *Acta Tropica* **97**, 219–228.
- Swearingen KE, Eng JK, Shteynberg D, Vigdorovich V, Springer TA, Mendoza L, Sather DN, Deutsch EW, Kappe SHI and Moritz RL** (2019) A tandem mass spectrometry sequence database search method for identification of O-fucosylated proteins by mass spectrometry. *Journal of Proteome Research* **18**, 652–663.
- Talabnin K, Aoki K, Saichua P, Wongkham S, Kaewkes S, Boons GJ, Sripa B and Tiemeyer M** (2013) Stage-specific expression and antigenicity of glycoprotein glycans isolated from the human liver fluke, *Opisthorchis viverrini*. *International Journal for Parasitology* **43**, 37–50.
- Taratuto AL and Venturiello SM** (1997) Echinococcosis. *Brain Pathology* **7**, 673–679, PMID: 9034573.
- Taylor CE, Cobb BA, RittenhouseOlson K, Paulson JC and Schreiber JR** (2012) Carbohydrate moieties as vaccine candidates: targeting the sweet spot in the immune response. *Vaccine* **30**, 4409–4413.
- Tejle K, Lindroth M, Magnusson K-E and Rasmusson B** (2008) Wild-type *Leishmania donovani* promastigotes block maturation, increase integrin expression and inhibit detachment of human monocyte-derived dendritic cells – the influence of phosphoglycans. *FEMS Microbiology Letters* **279**, 92–102.
- Terrazas CA, Terrazas LI and Gómez-García L** (2010) Modulation of dendritic cell responses by parasites: a common strategy to survive. *Journal of Biomedicine and Biotechnology* **2010**, 357106.
- Thaysen-Andersen M and Packer NH** (2014) Advances in LC-MS/MS based glycoproteomics: getting closer to system-wide site-specific mapping of the N- and O-glycoproteome. *Biochimica et Biophysica Acta* **1844**, 1437–1452.
- Thomas PG, Carter MR, Atochina O, Da'Dara AA, Piskorska D, McGuire E and Harn DA** (2003) Maturation of dendritic cell 2 phenotype by a helminth glycan uses a toll-like receptor 4-dependent mechanism. *Journal of Immunology* **171**, 5837–5841.
- Todeschini AR, da Silveira EX, Jones C, Wait R, Previato JO and Mendonça-Previato L** (2001) Structure of O-glycosidically linked oligosaccharides from glycoproteins of *Trypanosoma cruzi* CL-Brener strain: evidence for the presence of O-linked sialyl-oligosaccharides. *Glycobiology* **11**, 47–55.
- Tonui WK, Mbatia PA, Anjili CO, Orago AS, Turco SJ, Githure JI and Koech DK** (2001) Transmission blocking vaccine studies in leishmaniasis: II. Effect of immunisation using *Leishmania major* derived 63 kilodalton glycoprotein, lipophosphoglycan and whole parasite antigens on the course of *L. major* infection in BALB/c mice. *East African Medical Journal* **78**, 90–92.
- Tundup S, Srivastava L and Harn DA** (2012) Polarization of host immune responses by helminth-expressed glycans. *Annals of the New York Academy of Sciences* **1253**, E1–13.
- Van Dam GJ, Bergwerff AA, Thomas-Oates JE, Rotmans JP, Kamerling JP, Vliegthart JF and Deelder AM** (1994) The immunologically reactive O-linked polysaccharide chains derived from circulating cathodic antigen isolated from the human blood fluke *Schistosoma mansoni* have Lewis x as repeating unit. *European Journal of Biochemistry* **225**, 467–482.
- van Dam GJ, Wichers JH, Ferreira TMF, Ghati D, van Amerongen A and Deelder AM** (2004) Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. *Journal of Clinical Microbiology* **42**, 5458–5461.
- van Die I and Cummings RD** (2006) Glycans modulate immune responses in helminth infections and allergy. *Chemical Immunology and Allergy* **90**, 91–112.
- van Die I and Cummings RD** (2010) Glycan gimmickry by parasitic helminths: a strategy for modulating the host immune response? *Glycobiology* **20**, 2–12.
- van Die I, Gomord V, Kooyman FN, van den Berg TK, Cummings RD and Vervelde L** (1999) Core alpha1-→3-fucose is a common modification of N-glycans in parasitic helminths and constitutes an important epitope for IgE from *Haemonchus contortus* infected sheep. *FEBS Letters* **463**, 189–193.
- van Liempt E, van Vliet SJ, Engering A, García Vallejo JJ, Bank CM, Sanchez-Hernandez M, van Kooyk Y and van Die I** (2007) *Schistosoma mansoni* soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. *Molecular Immunology* **44**, 2605–2615.
- van Stijn CM, van den Broek M, Vervelde L, Alvarez RA, Cummings RD, Tefsen B and van Die I** (2010) Vaccination-induced IgG response to Galalpha1-3GalNAc glycan epitopes in lambs protected against *Haemonchus contortus* challenge infection. *International Journal for Parasitology* **40**, 215–222.
- Varki A** (2017) Biological roles of glycans. *Glycobiology* **27**, 3–49.
- Verissimo CM, Morassutti AL, von Itzstein M, Sutov G, Hartley-Tassell L, McAtamney S, Dell A, Haslam SM and Graeff-Teixeira C** (2016) Characterization of the N-glycans of female *Angiostrongylus cantonensis* worms. *Experimental Parasitology* **166**, 137–143.
- Vermeer HJ, van Dam GJ, Halkes KM, Kamerling JP, Vliegthart JF, Hokke CH and Deelder AM** (2003) Immunodiagnostically applicable monoclonal antibodies to the circulating anodic antigen of *Schistosoma mansoni* bind to small, defined oligosaccharide epitopes. *Parasitology Research* **90**, 330–336.
- Vervelde L, Bakker N, Kooyman FN, Cornelissen AW, Bank CM, Nyame AK, Cummings RD and van Die I** (2003) Vaccination induced protection of lambs against the parasitic nematode *Haemonchus contortus* correlates with high IgG antibody responses to the LDNF glycan antigen. *Glycobiology* **13**, 795–804.
- Vivanco-Cid H, Alpuche-Aranda C, Wong-Baeza I, Rocha-Ramírez LM, Rios-Sarabia N, Estrada-García I, Villasis-Keever MA, Lopez-Macias C and Isibasi A** (2007) Lipopeptidephosphoglycan from *Entamoeba histolytica* activates human macrophages and dendritic cells and reaches their late endosomes. *Parasite Immunology* **29**, 467–474.
- von Stebut E, Belkaid Y, Jakob T, Sacks DL and Udey MC** (1998) Uptake of *Leishmania major* amastigotes results in activation and interleukin 12 release from murine skinderived dendritic cells: implications for the initiation of anti-Leishmania immunity. *Journal of Experimental Medicine* **188**, 1547–1552.
- Wisniewski N, McNeil M, Grieve RB and Wassom DL** (1993) Characterization of novel fucosyl- and tyvelosyl-containing glycoconjugates from *Trichinella spiralis* muscle stage larvae. *Molecular and Biochemical Parasitology* **61**, 25–35.
- World Health Organization – Leishmaniasis.** (2017) Available at <http://www.who.int/mediacentre/factsheets/fs375/en/> (Accessed December 2017).
- Wuhrer M, Grimm C, Dennis R, Idris M and Geyer R** (2004) The parasitic trematode *Fasciola hepatica* exhibits mammalian-type glycolipids as well as Gal(beta1-6)Gal-terminating glycolipids that account for cestode serological cross-reactivity. *Glycobiology* **14**, 115–126.
- Wuhrer M, Koeleman CAM, Deelder AM and Hokke CH** (2006) Repeats of LacdiNAc and fucosylated LacdiNAc on N-glycans of the human parasite *Schistosoma mansoni*. *The FEBS Journal* **273**, 347–361.
- Wuhrer M, Rickhoff S, Dennis RD, Lochnit G, Soboslay PT, Baumeister S and Geyer R** (2000) Phosphocholine-containing, zwitterionic glycosphingolipids of adult *Onchocerca volvulus* as highly conserved antigenic structures of parasitic nematodes. *Biochemical Journal* **1**, 417–423.
- Yamano K, Goto A, Nakamura-Uchiyama F, Nawa Y, Hada N and Takeda T** (2009) Galbeta1-6Gal, antigenic epitope which accounts for serological cross-reaction in diagnosis of *Echinococcus multilocularis* infection. *Parasite Immunology* **31**, 481–487.
- Yépez-Mulia L, Hernández-Bello R, Arizmendi-Puga N, Fonseca-Liñán R and Ortega-Pierres G** (2007) Contributions to the study of *Trichinella spiralis* TSL-1 antigens in host immunity. *Parasite Immunology* **29**, 661–670.