

Review

*These authors have contributed equally.

Cite this article: Valente M, Castillo-Acosta VM, Vidal AE, González-Pacanowska D (2019). Overview of the role of kinetoplastid surface carbohydrates in infection and host cell invasion: prospects for therapeutic intervention. *Parasitology* 1–12. <https://doi.org/10.1017/S0031182019001355>

Received: 23 May 2019

Revised: 11 September 2019

Accepted: 11 September 2019


Key words:

Carbohydrate-binding agents; kinetoplastids; lectins; *Leishmania*; surface glycans; *Trypanosoma brucei*; *Trypanosoma cruzi*

Author for correspondence:

Dolores González-Pacanowska,
E-mail: dgonzalez@ipb.csic.es

Overview of the role of kinetoplastid surface carbohydrates in infection and host cell invasion: prospects for therapeutic intervention

Maria Valente*, Víctor M. Castillo-Acosta*, Antonio E. Vidal*
and Dolores González-Pacanowska 

Instituto de Parasitología y Biomedicina 'López-Neyra', Consejo Superior de Investigaciones Científicas (CSIC), Parque Tecnológico de Ciencias de la Salud, Avenida del Conocimiento, 17, 18016, Armilla, Granada, Spain

Abstract

Kinetoplastid parasites are responsible for serious diseases in humans and livestock such as Chagas disease and sleeping sickness (caused by *Trypanosoma cruzi* and *Trypanosoma brucei*, respectively), and the different forms of cutaneous, mucocutaneous and visceral leishmaniasis (produced by *Leishmania* spp). The limited number of antiparasitic drugs available together with the emergence of resistance underscores the need for new therapeutic agents with novel mechanisms of action. The use of agents binding to surface glycans has been recently suggested as a new approach to antitrypanosomal design and a series of peptidic and non-peptidic carbohydrate-binding agents have been identified as antiparasitics showing efficacy in animal models of sleeping sickness. Here we provide an overview of the nature of surface glycans in three kinetoplastid parasites, *T. cruzi*, *T. brucei* and *Leishmania*. Their role in virulence and host cell invasion is highlighted with the aim of identifying specific glycan–lectin interactions and carbohydrate functions that may be the target of novel carbohydrate-binding agents with therapeutic applications.

Introduction

The occurrence of surface glycoconjugates in parasitic protozoa is of paramount importance since they are crucially involved in processes such as immune evasion, host cell invasion and endocytosis. Most parasitic protozoa undergo complex life cycles and must adapt to changing hosts and environments. Surface glycans constitute a protective barrier that contributes to adaptation and the establishment of infection by participating in the subversion of the immune response and in specific interactions with host surface molecules. Thus, specific sugar–lectin interactions are involved in the colonization of the gut of the insect vector as well as in mammalian host cell recognition and parasite internalization. In this latter process, specific glycan–lectin interactions mediate mammalian host cell recognition and parasite uptake. Pattern recognition receptors (PRRs) present on the surface of immune cells distinguish pathogen-associated molecular patterns (PAMPs). The receptors used for parasite infection vary and include complement receptors, scavenger receptors, Toll-like receptors (TLR) and mannose receptors. The understanding of these interactions will provide an insight into how protozoa implement infection and subvert the host immune response.

In relation to the immune response, several carbohydrate-binding proteins, either expressed on the surface of cells of the immune system or released, play an essential role in the control of innate and adaptive immunity. These include C-type lectin receptors, sialic acid-binding immunoglobulin (Ig)-like lectins (siglecs) and galectins that interact with distinct glycan structures (van Kooyk and Rabinovich, 2008). DC-SIGN, a C-type lectin receptor found on the surface of dendritic cells specifically binds mannose and/or fucose-terminated glycans. Siglecs are included in the group of Ig-type (I-type) lectins and interact with a wide variety of structurally distinct carbohydrate ligands (Pillai *et al.*, 2012). Galectins are a family of soluble lectins that bind β -galactose (β -Gal)-containing glycoconjugates such as glycans containing *N*-acetylglucosamine and are thought to be able to associate with host membrane glycans to form a cell-surface network for an optimal receptor spacing and signalling (Liu and Rabinovich, 2005; Nieminen *et al.*, 2007). While they can act as effector factors, inhibiting pathogen adhesion and entry or stimulating phagocytosis, parasites can also make use of host galectins to facilitate host cell invasion. Furthermore, in the case of secreted glycoproteins, such as cytokines, chemokines and antibodies, the sugar portion has been described to perform important functions. This is the case of the *N*-glycans attached to the Fc portion of IgG, that when sialylated send an inhibitory signal to the immune system (Kaneko *et al.*, 2006; Anthony *et al.*, 2011). Glycans are also central to lymphocyte development (Stanley and Okajima, 2010) and leucocyte homing (Lowe, 2003; Mitoma *et al.*, 2007). Finally, an additional immunomodulatory pathway in which surface glycans have a major role is the lectin pathway (LP) for complement activation, which requires the mannose-binding lectin and ficolins, rather than the standard components (Matsushita, 2010) necessary for the activation of the classical and alternative pathways (AP). The identification of singular aspects related to

© Cambridge University Press 2019. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution, and reproduction in any medium, provided the original work is properly cited.

CAMBRIDGE
UNIVERSITY PRESS

glycan composition in kinetoplastids and their interaction with host lectins may unveil opportunities for drug design using agents that specifically bind to carbohydrate moieties important for parasite survival within the mammalian host.

The nature of surface glycans in *Trypanosoma cruzi*

As in the case of other protozoan parasites of medical and veterinary relevance, the surface of *T. cruzi* is heavily glycosylated. The dense glycocalyx performs specific and significant functions such as protection against the host defence mechanisms and/or the interaction with changing environments (Noireau *et al.*, 2009; Romano *et al.*, 2012). The carbohydrate nature of the surface coat strongly depends on the life stage and differentiation involves unique changes in its composition (de Lederkremer and Agusti, 2009).

The most abundant components of the *T. cruzi* surface coat, especially in the epimastigote form, are glycosylphosphatidylinositol (GPI)-anchored glycoconjugates of varied nature (Ferguson, 1999). The structure of this coat has been described as a basal layer of glycoinositolphospholipids (GIPLs) and phospholipid (Previato *et al.*, 1990; de Lederkremer *et al.*, 1991; Carreira *et al.*, 1996) from which other GPI-anchored molecules protrude (Previato *et al.*, 2004). The major species are mucin-like proteins (Pereira-Chioccola *et al.*, 2000; Buscaglia *et al.*, 2006), which are heavily *O*-glycosylated, while the less abundant include *trans*-sialidase (TS) (Previato *et al.*, 1985; Schenkman and Eichinger, 1993), mucin-associated proteins (MASPs) (dos Santos *et al.*, 2012), Gp85 surface glycoproteins (Mattos *et al.*, 2014), trypanomastigote small surface antigen (TSSA) (Canepa *et al.*, 2012) and Toll-T antigens (Quanquin *et al.*, 1999). Recent studies support the idea that lipid-based domains, and particularly lipid rafts, are responsible for the fine organization of all these components (Mucci *et al.*, 2017).

GIPL was the first glycoconjugate characterized in *T. cruzi* and can be found as a free entity or anchored to proteins. GIPLs were originally defined as lipopeptidophosphoglycans (LPPGs) because of the amino acids present in the early preparations (De Lederkremer *et al.*, 1976). However, with the solution of their structure, it was established that these LPPGs are typical GPIs (Previato *et al.*, 1990; de Lederkremer *et al.*, 1991). The core of GIPLs is constituted by $\text{Man}\alpha(1,2)\text{-Man}\alpha(1,6)\text{-Man}\alpha(1,4)\text{-GlcNAc}(1,6)\text{-myo-inositol-PO}_4\text{-lipid}$, in some cases with four mannose residues, where the lipid moiety is either 1-*O*-hexadecyl-2-*O*-palmitoyl glycerol or ceramide (McConville and Ferguson, 1993). Galactofuranose (Gal_f) and aminoethylphosphonic acid are substituents that can be found attached to different positions of the main core, conferring a certain microheterogeneity to the oligosaccharide structure (McConville and Ferguson, 1993).

Mucins are the most abundant glycoproteins in the *T. cruzi* surface membrane. They are a complex and heterogeneous group of variable proteins constituted by a polypeptidic core of 50–200 amino acids, rich in serine and threonine residues many of which are *O*-glycosylated (Buscaglia *et al.*, 2006). Mucins are *O*-glycosylated with *N*-acetylglucosamine (GlcNAc), which is rather unique since the glycosyltransferases that catalyse this transfer in other organisms usually use UDP-*N*-acetylgalactosamine as a precursor (Previato *et al.*, 1995, 1998). The *O*-linked GlcNAc residues can be further elongated or remain unsubstituted. Galactose is present in all mucin oligosaccharide elongations in either the pyranosic (Gal_p) or Gal_f configuration (Acosta-Serrano *et al.*, 2001). Terminal β -Gal_p residues can be further branched with sialic acid acquired from the host through TSs present on the membrane surface (Previato *et al.*, 1994; Serrano *et al.*, 1995).

TSs are another group of GPI-anchored proteins found on the surface of *T. cruzi*, and their activity allows the parasite to bypass

its lack of *de novo* synthesis of sialic acid, that is instead salvaged from the host (Previato *et al.*, 1985). TSs catalyse the transfer of sialic acid from an $\alpha(2,3)$ -linkage in the donor to a terminal β -Gal_p acceptor of the parasite mucins (Schenkman *et al.*, 1993). It has also been shown that *T. cruzi* TS (TcTS) can efficiently transfer $\alpha(2,3)$ -linked *N*-glycolylneuraminic acid (Neu5Gc) to terminal β -Gal groups (Agusti *et al.*, 2007; Schroven *et al.*, 2007). This specific activity of TcTS is unique because of several aspects. First, TcTSs, unlike mammalian TSs, do not use cytidine monophospho (CMP)-sialic acid as the monosaccharide donor. Additionally, they appear to be located on the parasite surface and not in the Golgi apparatus, which is where they carry out their normal function in other organisms. Finally, unlike conventional sialidases, TcTSs are more efficient in transferring terminal sialic acids between glycoconjugates rather than hydrolysing them. Recent studies have also shown that sialylated mucins are present in membrane lipid-rafts far away from TS and that the sialylation process is performed by microvesicles associated with active TcTS (Lantos *et al.*, 2016).

Other GPI-anchored proteins are the Gp85 surface glycoproteins, TSSAs and MASPs. Gp85 glycoproteins are usually included in the TS superfamily as TS-like proteins yet they lack TS activity (Buscaglia *et al.*, 2006) and appear to be involved in host–parasite interactions (Alves and Colli, 2008). TSSAs are polymorphic mucin-like molecules with a conserved hydrophobic C-terminus compatible with the GPI-anchoring signal, and a variable central region responsible for their antigenicity (Di Noia *et al.*, 2002). Finally, MASPs are GPI-anchored proteins that have been found predominantly in the proteome of trypanomastigotes (Atwood *et al.*, 2005). Like mucins, they contain highly conserved N- and C-terminal domains plus a variable central region (Bartholomeu *et al.*, 2009) yet they appear to be *N*-glycosylated (Atwood *et al.*, 2006).

Glycans and immunomodulation during *T. cruzi* infection

The complement is the first line of defence of the innate immune system against invading microbes. *Trypanosoma cruzi* invasion generates an immediate immune response due to the interaction of the parasite with complement molecules. It has been shown that the complement can be activated by all *T. cruzi* forms: amastigote (Iida *et al.*, 1989), epimastigote (Nogueira *et al.*, 1975) and trypanomastigote (Kipnis *et al.*, 1985), but only the non-infective epimastigotes are susceptible to complement lysis. During the first seconds after *T. cruzi* infection, signal glycoproteins on the parasite surface can interact with host PRRs such as mannose-binding lectins and ficolins and lead to the activation of the LP and AP (Fig. 1) (Cestari *et al.*, 2013). However, *T. cruzi* parasites can undertake a series of strategies to escape the effects of both innate and adaptive immunity. There are at least three different mechanisms of complement system evasion by *T. cruzi*. One of such mechanisms is the translocation of calreticulin (TcCRT), a calcium binding protein normally expressed in the endoplasmic reticulum, to the surface membrane on the flagellar portion of the parasite (Ferreira *et al.*, 2004a, 2004b; Gonzalez *et al.*, 2015). This translocation allows TcCRT to interact with mannose-binding lectins and ficolins and this way interfere with the normal activation of the LP and classical pathway and enhance the rate of the internalization of parasites by host cells (Fig. 1) (Gonzalez *et al.*, 2015). Another escape mechanism from the innate immune response is the release of plasma membrane microvesicles by *T. cruzi* parasites. Extracellular vesicles contain several signal factors including glycoproteins and enzymes involved in carbohydrate metabolism, which also interfere with the LP and classical pathway activation (Geiger *et al.*, 2010; Ramirez *et al.*, 2017).

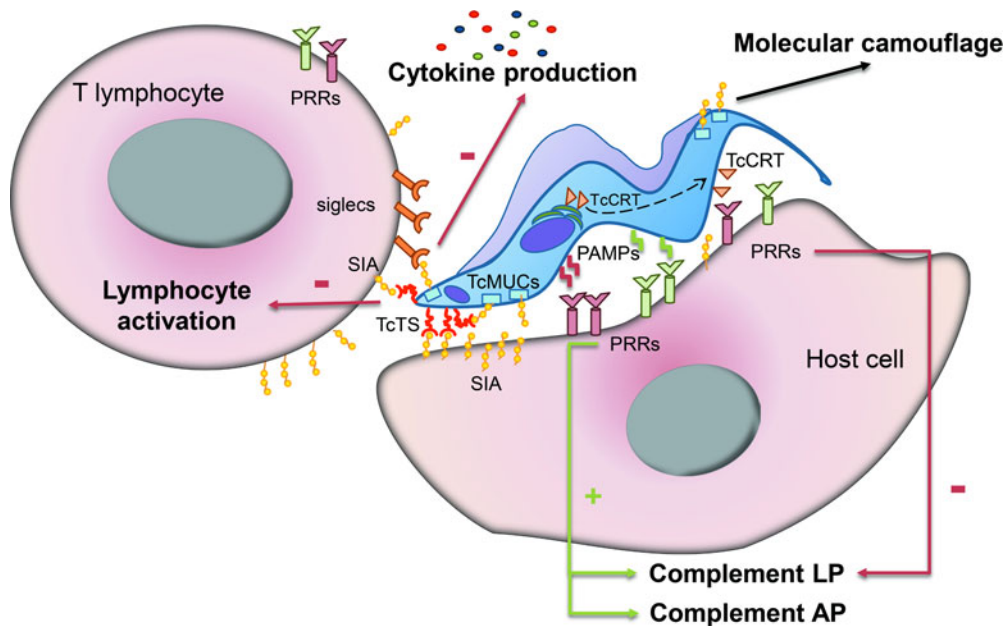


Fig. 1. Scheme of the interplay between *T. cruzi* surface glycans and mammalian host cells. Upon infection, surface glycans within PAMPs can interact with host cell (i.e. myeloid and dendritic cells) PRRs and lead to the activation of the complement LP and AP. TcCRT translocates from the endoplasmic reticulum to the surface membrane in the zone of flagellum emergence and interacts with PRRs interfering in the normal activation of the complement LP and AP. Sialic acid (SIA) is transferred from the host cell membrane to parasite surface proteins such as mucins (TcMUC), conferring this way a molecular camouflage that hinders an effective immune response. The transfer of SIA is catalysed by TcTS and leads to an inhibition of the activation of T lymphocytes. In addition, sialylated mucins may interact with siglecs expressed on the surface of T cells and inhibit cytokine production.

One of the most important carbohydrates interfering with the immune response against *T. cruzi* infection is sialic acid. *Trypanosoma cruzi* transfers sialic acid from the host to its own surface glycoproteins creating this way a perfect molecular camouflage that hinders an effective immune response (Fig. 1) (Argibay *et al.*, 2002; Gao *et al.*, 2002; Freire-de-Lima *et al.*, 2010). In addition, sialylated mucins may interact with siglecs expressed on the T cell surface and inhibit clonal expansion and cytokine production by CD4⁺ lymphocytes (Nunes *et al.*, 2013). On the other hand, TcTS interferes with the activation of T lymphocytes (Fig. 1) (Pennock *et al.*, 2013). This latter process involves the loss of sialic acid residues from O-linked oligosaccharides and the exposure of free Gal β (1,3) residues (Galvan *et al.*, 1998; Priatel *et al.*, 2000).

Host cell invasion

Trypanosoma cruzi has a quite complex life cycle that involves an obligate intracellular stage for parasite duplication. Cell invasion involves a strict and complex interaction between the parasite and the host cell. The first step of this process is the adhesion of the parasite to the target cell which involves the recognition of molecules present on the surface of both parasite and host cells. Several molecules of *T. cruzi* surface are involved, among them glycoproteins of the Gp85/TS family, and mucins are of greatest interest. β -Gal residues on surface glycoproteins have been suggested to mediate parasite attachment and entry in dendritic (Vray *et al.*, 2004) and smooth muscle cells (Kleshchenko *et al.*, 2004; Vray *et al.*, 2004). In addition, cruzipain, a major cysteine peptidase has also a role in immune evasion, host cell invasion and intracellular development. After the binding and recognition of the parasite by the host cell surface, *T. cruzi* is internalized by two possible mechanisms: phagocytosis (Vieira *et al.*, 2002) and endocytosis (Schenkman and Mortara, 1992). Once inside the host cell, parasites are confined in the parasitophorous vacuole, a membrane structure that protects them from lysosome attack while replicating. Probably one of the most

important events of host cell invasion is the 'escape' from the parasitophorous vacuole. Also in this process surface glycoproteins have a major function (Andrews and Whitlow, 1989; Stecconi-Silva *et al.*, 2003).

Surface glycans in *Trypanosoma brucei*

The *T. brucei* surface coat exhibits a dense layer of GPI-anchored glycoproteins, such as the variant surface glycoproteins (VSGs) or procyclin found in the bloodstream or procyclic forms of the parasite, respectively. In a minor amount, other glycosylated proteins are expressed in the surface membrane, such as the transmembrane invariant surface glycoproteins (ISGs) (Ziegelbauer and Overath, 1992; Ziegelbauer *et al.*, 1992), the transferrin receptor (TfR) (Grab *et al.*, 1993) and the haptoglobin-haemoglobin receptor (Vanhollebeke *et al.*, 2008) which are both GPI-anchored and located in the flagellar pocket. In addition, it has been reported that epimastigote forms found in the salivary glands of the tsetse fly present a stage-specific coat of a GPI-anchored protein named bloodstream stage alanine-rich protein or Brucei alanine-rich protein (BARP) (Nolan *et al.*, 2000; Urwyler *et al.*, 2007). The differentiation of epimastigotes to metacyclic trypanostigote forms is associated with the loss of BARP and to the expression of a new coat of metacyclic VSGs (Tetley *et al.*, 1987; Ginger *et al.*, 2002) and of a small family of metacyclic invariant surface glycoproteins which protrude and remain accessible for antibody recognition (Casas-Sánchez *et al.*, 2018). All these glycoproteins are mainly N-glycosylated with different structures containing oligomannose, paucimannose and complex-type glycans (Bangs *et al.*, 1988; Zamze *et al.*, 1990; Strang *et al.*, 1993; Treumann *et al.*, 1997; Mehlert *et al.*, 1998a, 1998b, 2012; Acosta-Serrano *et al.*, 2004).

Specifically, VSGs are homodimers susceptible to N-glycosylation with one, two or three N-linked oligosaccharides depending on the VSG class. Thus, in Class 1 VSGs only one asparagine is modified with triantennary oligomannose structures (Man₉₋₅GlcNAc₂); Class 2 VSGs have two N-glycosylation sites, one of them is

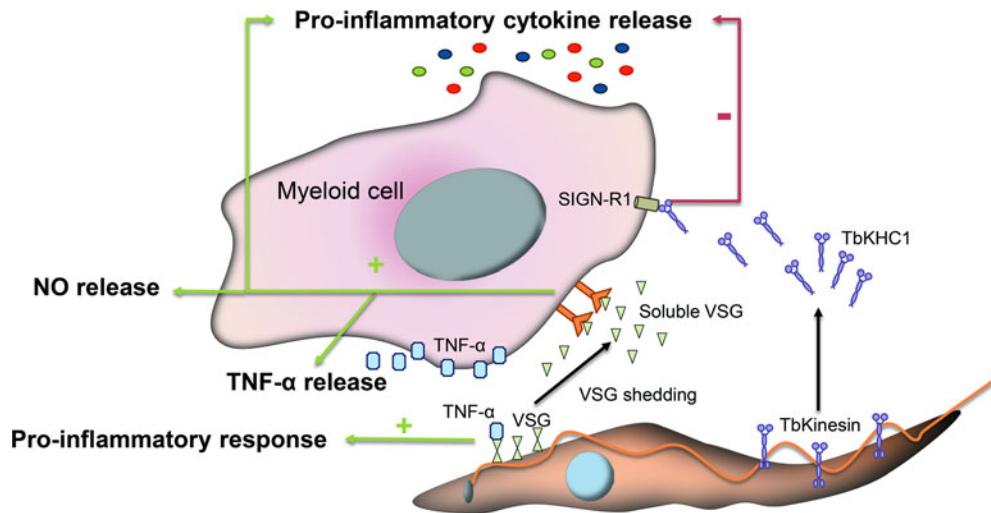


Fig. 2. Immunomodulatory events mediated by glycans during infection with *T. brucei*. VSGs interact with host immune cells and act as immunomodulatory factors. The conserved VSGs chitobiose-oligomannose moiety of VSGs binds to TNF- α , a cytokine with lectin-like properties and induces a pro-inflammatory response. Likewise, during differentiation to stumpy forms a VSG shedding process takes place allowing for the release of soluble VSG portions into the bloodstream of the mammalian host. These fragmented VSGs containing glycosylinositolphosphate induce myeloid cell activation and thereby the expression of pro-inflammatory cytokines and the release of NO and TNF- α . Other parasite-released factors interfere with the pro-inflammatory response such as the Kinesin Heavy Chain 1 (TbKHC1), which binds to the mannose-specific Intercellular Adhesion Molecule-3-Grabbing Nonintegrin-Related 1 (SIGN-R1) receptor and inhibits the host pro-inflammatory response.

occupied with oligomannose structures and the other with structure type $\text{Man}_{4-3}\text{GlcNAc}_2$ and biantennary complex glycans and Class 3 VSGs are modified with a combination of oligomannose and complex biantennary glycans (Zamze *et al.*, 1990, 1991; Mehler *et al.*, 1998b). Recently, it has been reported that VSGs can also be O-glycosylated (Pinger *et al.*, 2018).

TfR is a heterodimeric protein expressed in the bloodstream form that is encoded by expression site-associated genes (ESAGs) 6 and 7 exhibiting eight N-glycosylation sequons. Both ESAG6 and ESAG7 are heterogeneously N-glycosylated with paucimannose and oligomannose moieties such as VSGs but not with complex N-glycans (Mehler *et al.*, 2012). In contrast to the glycoproteins described above, the N-glycosylation profile of the ISGs has not been characterized so far.

On the other hand, the GPI structure built-up by $\text{NH}_2\text{CH}_2\text{CH}_2\text{-PO}_4\text{H-6-Man}\alpha(1,2)\text{-Man}\alpha(1,6)\text{-Man}\alpha(1,4)\text{-GlcNAc}(1,6)\text{-myo-inositol-1-PO}_4\text{H-3}(sn\text{-}1,2\text{-dimyristoylglycerol})$ by which the aforementioned glycoproteins, except for ISGs, are anchored to the surface membrane is further modified by N-glycosylation (Holder, 1985; Ferguson *et al.*, 1988; Redman *et al.*, 1994).

Glycans and interaction of *T. brucei* with the mammalian host

Trypanosoma brucei parasites dwelling in the mammalian bloodstream are exposed to innate and adaptive responses by the immune system for which they have developed sophisticated evasion strategies. An essential mechanism for effective immune evasion is the antigenic variation of VSGs whereby parasites switch to a new, immunologically distinct VSG, selected from among a huge collection of silent VSG genes. At the initial stages of the humoral immune response, when antibody levels are still low, the VSG-antibody complexes are rapidly internalized at the flagellar pocket by clathrin-dependent endocytosis, to be further dissociated in isolated VSG which is recycled to the surface, and the Ig that is directed to the lysosome to be proteolysed (O'Beirne *et al.*, 1998; Pal *et al.*, 2003; Engstler *et al.*, 2004; Overath and Engstler, 2004). Antibody internalization becomes insufficient as the titre increases, and the complement system, mediated by specific antibodies against the predominant form of VSG, promotes efficient opsonization and lysis of parasites except for those expressing

the new VSG that will spread again the infection, as they are able to escape the adaptive immune response. As the infection progresses, slender proliferative bloodstream parasites differentiate into a stumpy non-proliferative form that plays an important role in different ways. It contributes to avoid massive parasitaemia and premature host death, allows for pre-adaptation to the tsetse fly and by reducing the VSG repertoire expression it restricts antibody generation by the host, thus extending the functionality of antigenic variation (MacGregor *et al.*, 2011; Matthews, 2015).

While trypanosomes mainly rely on antigenic variation to circumvent immune detection, VSG glycosylation modulates host-parasite interactions, contributing to the formation of an efficient surface barrier, with increased antigenic variability and protective properties (Blum *et al.*, 1993). Supporting this notion, it has been shown that O-glycosylation of VSGs confers the parasite additional surface heterogeneity, impairs the functionality of the host immune response and enhances parasite virulence (Pinger *et al.*, 2018). Furthermore, specific carbohydrate branches at the trypanosome surface are involved in the process of binding and uptake of host macromolecules. The conserved VSGs chitobiose-oligomannose ($\text{GlcNAc}_2\text{-Man}_{5-9}$) moieties have been proposed to act as ligands for TNF- α , a cytokine with lectin-like properties inducing a pro-inflammatory response and parasite lysis (Fig. 2) (Magez *et al.*, 2001) although the direct induction of trypanolysis by TNF- α has been recently questioned (Vanwalleghem *et al.*, 2017).

VSGs also act as immunomodulatory factors involved in the production of TNF- α and nitric oxide (NO). During the differentiation process, slender trypanosomes suffer a VSG shedding process releasing to the bloodstream of the mammalian host soluble VSG portions from the membrane (Gruszynski *et al.*, 2003). These fragmented VSGs containing glycosylinositolphosphate induce myeloid cell activation and thereby the expression of pro-inflammatory cytokines (Fig. 2) (Leppert *et al.*, 2007). This process is amplified by T cell activation and IFN- γ release, which promotes macrophages to achieve a whole activated/M1 polarization and consequently increases TNF- α and NO secretion to control the infection (Stijlemans *et al.*, 2016).

Other parasite-released factors interfere with the pro-inflammatory response of activated macrophages thus contributing to

parasite infection. For instance, Kinesin Heavy Chain 1 (TbKHC1) is released in the blood by the parasite and interacts with the mannose-specific intercellular adhesion molecule-3-grabbing nonintegrin-related 1 (SIGN-R1) receptor, a C-type lectin expressed in the surface of immune cells to inhibit the host pro-inflammatory response and at the same time stimulates the production by the host of essential trypanosomal nutrients (Fig. 2). TbKHC1 reduces the conversion of L-arginine into NO by inducing arginase-1 activity via IL-10 of macrophages/myeloid cells. The modulation of arginase activity promotes the formation of L-ornithine and consequently of polyamines required for trypanosome growth (De Muylder *et al.*, 2013).

Other glycoproteins that play major roles in host–parasite interaction are the trypanosome-derived lymphocyte-triggering factor, which is secreted by *T. brucei* parasites promoting early IFN- γ production by CD8⁺ T lymphocytes (Vaidya *et al.*, 1997), the GPI-phospholipase C, a bloodstream stage-specific enzyme that is concentrated in the flagellar membrane and participates in VSG shedding during differentiation of bloodstream forms to procyclic forms (Grandgenett *et al.*, 2007) and the TfR, which is located in the flagellar pocket and is involved in providing iron to the parasite (Steverding *et al.*, 1994). The N-glycosylation of both TfR protein subunits has been proposed to provide a spatial localization in the plasma membrane together with the VSG coat that allows transferrin binding without significant exposure to the immune system (Mehlert *et al.*, 2012).

Surface glycans in *Leishmania*

In the case of *Leishmania*, the surface coat is covered by a dense external glycocalyx harbouring different glycoconjugates with an important role in the parasite–host interaction. Its nature varies between species and different forms of the parasite during the life cycle. Promastigote cells contain a series of glycoconjugates, which include GPI-anchored proteins such as the metalloprotease leishmanolysin/GP63, the parasite surface antigen-2 complex (PSA-2/GP46) and the mucin-like proteophosphoglycan (PPG), a complex GPI-anchored lipophosphoglycan (LPG) and low molecular weight GPIs which are not attached to either proteins or polysaccharides. *Leishmania* also secretes protein-linked phosphoglycans, such as the secreted proteophosphoglycan (sPPG) and secreted acid phosphatase (Sacks and Kamhawi, 2001).

LPG is the main cell-surface glycoconjugate in promastigotes covering the whole parasite including the flagellum. It is comprised a 1-O-alkyl-2-lyso-phosphatidyl(*myo*)inositol lipid anchor with a heptasaccharide glycan core, Gal β (1,6)-Gal β (1,3)-Gal α (1,3)(Glc β (1)-PO₄-(6))-Man β (1,3)-Man β (1,4)-GlcN, which is joined to a long polyglycosyl phosphate (PG) consisting of repeating units of a disaccharide and a phosphate (Gal β (1,4)-Man α (1)-PO₄) and terminated by an oligosaccharide cap structure consisting of Man α (1,2)-Man α (1) or as Gal β (1,4)(Man α (1,2))-Man α (1). The PG units appear to be modified by carbohydrate chains that differ markedly between species and stage (McConville *et al.*, 1995). In amastigotes, LPG expression is highly downregulated (Turco and Sacks, 1991).

PPG is the second major phosphoglycan but, unlike LPG, it contains a polypeptide backbone rich in serines to which phosphoglycans are linked via phosphodiester bonds. The PG molecules consist of three differently branched phosphodisaccharides that end with a neutral capping structure (Ilg, 2000).

GP63 is the most abundant surface glycoprotein expressed on the *Leishmania* promastigote cell membrane. GP63 is N-glycosylated with paucimannose structures such as Man₆GlcNAc₂ and GlcMan₆GlcNAc₂ (Funk *et al.*, 1997), and Man₃GlcN structure in the GPI-anchor (Cabezas *et al.*, 2015). It is a zinc-dependent

protease with a wide range of substrates including casein, haemoglobin, fibrinogen, etc. (Yao *et al.*, 2003).

Differentiation to the amastigote form involves the thinning of the glycocalyx; in addition to LPG, the levels of GP63 are also dramatically decreased and GPIs become the major surface glycoconjugate in this form (McConville and Blackwell, 1991; Schneider *et al.*, 1992; Winter *et al.*, 1994). GPIs are composed of the Man α (1,4)-GlcNAc(1,6)-*myo*-inositol unit, which is substituted with either high mannose (type-1) or Gal β -Gal α (type-2) structures or with both forming a hybrid glycoside (McConville and Ferguson, 1993; Cabezas *et al.*, 2015). Like in *T. cruzi*, *Leishmania* also presents its cell surface decorated with sialic acid-bearing glycoconjugates. *Leishmania donovani* promastigotes exhibit 9-O-acetylated sialic acid and distinct 9-O-acetylated sialoglycoproteins while the amastigote form harbours an unusual derivative of sialic acid, Neu5Gc, absent in promastigotes (Ghoshal and Mandal, 2011).

Role of *Leishmania* surface glycans in immunomodulation and parasite–host cell interactions

The presence of a cell surface glycocalyx has a critical role in host–parasite interactions and infectivity thanks to an array of well-defined epitopes of branched N-glycans that act as ligands for receptors on cells of the insect or the vertebrate host. In *Leishmania* promastigotes, the dense glycocalyx formed by LPG performs a number of functions for parasite survival within the insect and for macrophage infection within the mammalian host. LPGs confer physical protection against digestive hydrolytic enzymes of the sandfly and are involved in the attachment to the gut epithelium and migration of metacyclic parasites to the mouthparts of the insect (Ilg, 2000; Sacks and Kamhawi, 2001). In the blood stream, LPG prevents lysis by complement proteins and serves as a ligand for attachment and receptor-mediated phagocytosis by the macrophage. LPG triggers TLR signalling and interferes with pro-inflammatory and signalling pathways in host cells (Fig. 3) (Becker *et al.*, 2003; Rojas-Bernabe *et al.*, 2014). Once inside the macrophage, LPG delays the fusion of the parasitophorous vacuole with lysosomes and inhibits protein kinase C and the production of cytokines related to the microbicidal oxidative and nitrosative stress response (Fig. 3) (Descoteaux and Turco, 1993; Kavosi *et al.*, 2009; Franco *et al.*, 2012).

Besides LPG, other glycoconjugates such as GPIs and PPGs are involved in the first stages of macrophage infection (McConville and Blackwell, 1991; Piani *et al.*, 1999). Mannose-terminating GPIs interact with mannose receptors on the macrophage surface (Blackwell *et al.*, 1985) and modulate many macrophage functions such as PKC activity (Chawla and Vishwakarma, 2003), cytokine production, release of NO and differentially activate MAPK (Fig. 3) (Assis *et al.*, 2012). While the implication of GPIs in *Leishmania*–macrophages interaction is well established, their role in intramacrophage development is still unclear. On the other hand, PPGs play important biological roles in the establishment of *Leishmania* infection and virulence (Capul *et al.*, 2007; Gaur *et al.*, 2009; Olivier *et al.*, 2012). Filamentous PPGs are found in the promastigote secretory gel, a viscous mucin-like material which accumulates in sandfly gut and mouthparts and improves *Leishmania* transmission by promoting multiple insect bites and increasing the number of parasites per bite (Rogers *et al.*, 2004; Rogers and Bates, 2007). PPGs regurgitated by *Leishmania*-infected sandflies favour macrophage recruitment to the bite site and target the L-arginine metabolism of host macrophages to promote establishment of the infection (Rogers *et al.*, 2009). In a murine model, sPPG has been shown to inhibit TNF- α release to facilitate the establishment of the infection (Piani *et al.*, 1999).

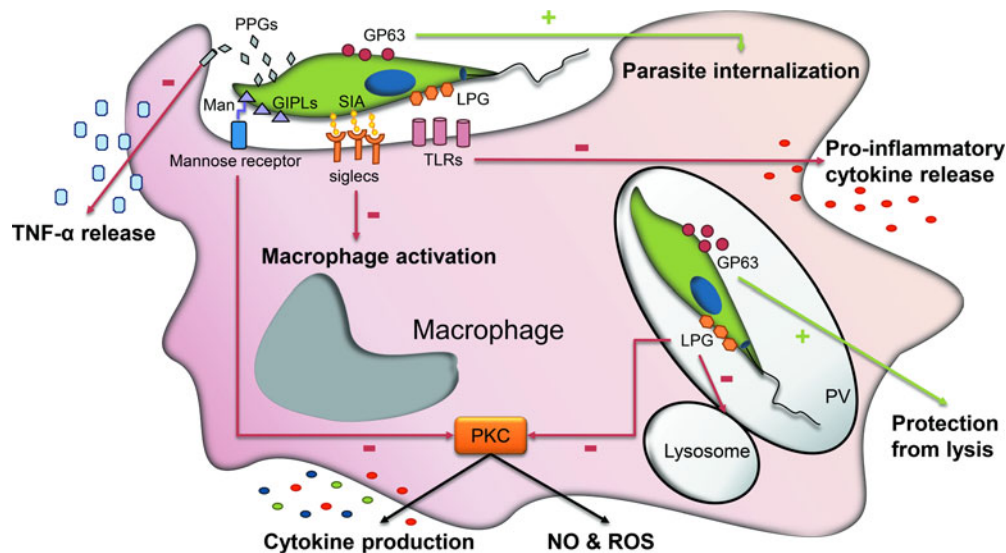


Fig. 3. Schematic representation of the major parasite-macrophage interactions mediated by surface glycans in *Leishmania*. The major glycoconjugates involved in the parasite-macrophage interplay are indicated: PPG, GPI-anchored LPG GIPLs and the metalloprotease GP63. After infection, promastigote LPG triggers TLR signalling and interferes with pro-inflammatory and signalling pathways. Once inside the macrophage, LPG delays the fusion of the parasitophorous vacuole with lysosomes and inhibits protein kinase C and therefore, the production of cytokines and the oxidative and nitrosative stress response. Likewise, mannose-terminating GIPLs interact with mannose receptors on the macrophage surface and inhibit PKC activity. sPPGs impair important macrophage functions such as the release of TNF- α . Finally, GP63 is an important virulence factor which, among other functions, promotes *Leishmania* internalization and facilitates escape from lysis by the complement pathway.

GPI-anchored glycoproteins that exhibit pivotal functions in the parasite-mammalian host interplay in *Leishmania* are GP63 and PSA-2. Metalloprotease GP63 is abundant in promastigotes but expressed to a lesser extent in amastigotes (Schneider *et al.*, 1992). GP63 is an important virulence factor which modulates a wide range of host cell signalling pathways that regulate macrophage anti-microbial and inflammatory functions. GP63 facilitates parasite escape from lysis by the complement pathway and the movement through the extracellular matrix, favours promastigote internalization into macrophages, inhibits natural killer cell responses, promotes resistance to antimicrobial peptides and seems to play a key role in protecting intracellular parasites from the hostile environment of macrophages (Fig. 3) (Olivier *et al.*, 2012; Yao *et al.*, 2003, 2010). Likewise, the PSA-2 protein is involved in the binding and invasion of parasites on macrophages and resistance to complement lysis (Kedzierski *et al.*, 2004; Lincoln *et al.*, 2004).

Another type of interaction with host cells by which parasites can establish successful infection takes place through sialic acid-siglec binding. *Leishmania* utilizes sialic acids to bind these membrane-bound receptors present in the haematopoietic cell lineages promoting parasite entry within macrophages, NO-resistance, host immunomodulation and strain virulence (Fig. 3) (Ghoshal and Mandal, 2011; Roy and Mandal, 2016).

Carbohydrate-binding agents as antimicrobials

Several studies support the therapeutic potential of carbohydrate-binding agents (CBAs). Lectins are CBAs of peptidic nature that specifically bind diverse carbohydrate structures. By acting as recognition and adhesion molecules and as signal transducers they perform a wide variety of physiological functions. Cell membrane proteins and lipids in many pathogens exhibit specific glycosylation patterns different from the mammalian host and are potential binding sites for lectins of selected specificity. Thus, lectins naturally occurring in plants, microbes, animals and humans exhibit antimicrobial activity (Petrova *et al.*, 2016; Zhang and Gallo, 2016) through the interaction with complex carbohydrates

on microbial surfaces and there is growing interest in their applicability due to the possible interference with host cell-pathogen interactions and disease development (Breitenbach Barroso Coelho *et al.*, 2018). Decreased capacity of invasion and infection, inhibition of proliferation and impairment of pathogen cell adhesion and migration has been reported to occur upon incubation with lectins from different sources (da Silva *et al.*, 2019; Hasan and Ozeki, 2019; Li *et al.*, 2019). In addition, lectins have been acknowledged as promising potential carrier molecules for directed drug delivery (Žurga *et al.*, 2017) since by binding to membrane glycan moieties, they can elicit cell internalization of molecules of therapeutic interest.

Indeed with regards to their anti-infective potential, multiple studies have demonstrated the enormous antiviral capacities of CBAs (Francois and Balzarini, 2012; Gondim *et al.*, 2019). The infectivity of several viruses requires surface glycoproteins and interference with host cell recognition has been the basis of the antiviral activity of lectins that specifically bind mannose-rich surface glycans (Dey *et al.*, 2000; Hoorelbeke *et al.*, 2010). Prolonged exposure to CBAs resulted in defects in the glycosylation status of surface glycoproteins giving rise to defective binding and increased exposure of underlying epitopes to the host immune response adding this way a new feature to their mode of action (Balzarini *et al.*, 2005). In addition, non-peptidic CBAs have been successfully used in the treatment of fungal infections both *in vitro* and *in vivo* supporting the possible employment of this class of compounds in a clinical setting (Tomita *et al.*, 1990; Walsh and Giri, 1997).

Therapeutic opportunities in kinetoplastids

In the case of kinetoplastid diseases, treatment often suffers from toxicity, side-effects and limited efficacy. New entities with novel modes of action are therefore needed to address the increasing demand for novel medicines. Despite extensive screening and *in vitro* and *in vivo* studies, only very few compounds have advanced to clinical trials. Taking into account the importance of protein glycosylation, the unique character of cell surface glycans during

the infective stages of parasitic protozoa opens exciting possibilities for the use of CBAs as antiparasitics. A mode of action can be foreseen where these agents would act directly exerting toxicity by binding to the cell surface and inducing parasite lysis and/or additionally by preventing pathogen infection in the host by impairing crucial interactions involved in the attenuation of the immune response or parasite uptake. The direct cytotoxic activity upon incubation with CBAs has been demonstrated in *T. brucei* bloodstream forms where peptidic agents such as the amaryllis lectin *Hippeastrum* hybrid (HHA) and the stinging nettle lectin (UDA) from *Urtica dioica* perturb endocytosis (Castillo-Acosta *et al.*, 2013, 2015). Likewise, a specific block in endocytosis was observed after exposure of *T. cruzi* to a Poly-LAcNAc binding lectin (Brosson *et al.*, 2016). However, while many plant peptidic CBAs exist with a wide range of glycan specificities, including mannose, galactose, glucose, fucose, sialic acid, GlcNAc and GalNAc oligomers, the approach involves major challenges. The toxicity of many peptidic lectins precludes their use as drugs and the identification of CBAs that exhibit selectivity towards parasite glycans and low toxicity towards mammalian cells are required.

Previous studies on the utility of CBAs in kinetoplastid diseases are limited. Certain plant lectins have demonstrated utility as adjuvants when studying the mouse humoral immune response to *T. cruzi* (Albuquerque *et al.*, 1999). The cramoll 1,4 lectin, is a protein that recognizes and interacts with specific glycans on the cell surface inducing mitogenic activity (Maciel *et al.*, 2004) which in *T. cruzi*, induces changes in plasma membrane permeability, production of reactive oxygen species and defects in mitochondrial function (Fernandes *et al.*, 2010). A protective effect of lectin administration in *Leishmania* infections has also been documented. Thus, lectins such as the ConBr from *Canavalia brasiliensis* and KM+ from *Artocarpus integrifolia* induce IFN- γ and IL-12 p40 production promoting a reversal of the Th2 cytokine pattern to Th1 pattern in BALB/c mice infected with *Leishmania amazonensis* and *Leishmania major*, respectively (Barral-Netto *et al.*, 1996; Panunto-Castelo *et al.*, 2001). Pretreatment of murine inflammatory peritoneal macrophages with a D-galactose-binding lectin from *Synadenium carinatum* latex (ScLL) reduced by 65.5% the association index of macrophages and *L. amazonensis* promastigotes (Afonso-Cardoso *et al.*, 2011). ScLL also reduced the growth of *L. amazonensis* amastigote intracellular forms, showing no *in vitro* cytotoxic effects in mammalian host cells (Afonso-Cardoso *et al.*, 2011).

Remarkably, evidence has been recently presented showing that the use of CBAs could involve a completely novel approach to chemotherapy of protozoan-infectious diseases in the case of sleeping sickness. Thus products from natural sources of both peptidic and non-peptidic nature have demonstrated their antitrypanosomal potential in the case of *T. brucei* both *in vitro* and *in vivo* (Castillo-Acosta *et al.*, 2013, 2016). Certain $\alpha(1,3)$ - $\alpha(1,6)$ Man-specific peptidic CBAs such as the HHA or the $\alpha(1,3)$ Man-specific snowdrop lectin from *Galanthus nivalis* (GNA) inhibit growth of bloodstream forms of *T. brucei* while exhibiting very low toxicity against mammalian cells *in vitro* (Castillo-Acosta *et al.*, 2013) although as peptides their therapeutic potential was limited. In the case of non-peptidic glycan binding agents, the identification of low molecular weight, non-toxic compounds to date has been restricted mostly to the natural products from *Actinomyces* namely pradimicin A (Walsh and Giri, 1997) and benanomycin A (Watanabe *et al.*, 1996) and their synthetic analogues although several groups have aimed at the synthesis and characterization of synthetic CBAs that bind specific oligosaccharide structures (Striegler and Dittel, 2003; Mazik *et al.*, 2005). Indeed treatment with the non-peptidic CBA pradimicin S procured parasitological cure in mouse models of acute sleeping sickness with no

evidence of toxicity or side-effects further supporting the potential of the approach (Castillo-Acosta *et al.*, 2016). Pradimicin A exhibits $\alpha(1,2)$ Man specificity and binds tightly to the parasite VSGs presumably through specific interactions with oligomannose surface glycans that are highly abundant in the bloodstream form of the parasite. Binding of pradimicin to bloodstream form trypanosomes induced defects in endocytosis and parasite lysis. Interestingly induction of resistance to pradimicin A *in vitro* resulted in parasites with defective glycosylation and a reduction in the content of mannose-rich glycans that exhibited reduced infectivity thus clearly supporting the proposed mechanism of action (Castillo-Acosta *et al.*, 2016). Binding to mannose-rich surface glycoproteins was also the basis for the potent activity of pradimicins against HIV by the interaction with the heavily mannosylated surface glycoprotein gp120 (Balzarini *et al.*, 2005).

When understanding the antiprotozoal activity of CBAs, as previously mentioned, the complement system, mediated by specific antibodies against VSGs, allows for efficient opsonization and lysis of parasites. Considering the capability of CBAs to interact with *T. brucei* membrane glycoproteins and in particular VSGs, we hypothesize that CBAs could act as opsonins *per se* and therefore increase phagocytosis by macrophages. In addition, it is possible that the endocytosis block mediated by CBAs may also interfere with VSG recycling leading to a reduced clearance of surface coat antibodies and further promoting the opsonization process. On the other hand, CBAs may bind to specific carbohydrate branches at the trypanosome surface that are crucially involved in the process of binding of host macromolecules. Thus in *T. brucei* transferrin binding by the TfR could also be compromised (Mehlert *et al.*, 2012). Furthermore, TbKHC1 interacts with the mannose-specific SIGN-R1 receptor and inhibits the pro-inflammatory response of the host (De Muylder *et al.*, 2013). Again it is possible that CBAs interfere with these or other events that may be related to its mode of action *in vivo*.

In the case of *T. cruzi*, the core of GPIs, a major surface constituent, is made up by Man $\alpha(1,2)$ -Man $\alpha(1,6)$ -Man $\alpha(1,4)$ -GlcNAc (1,6)-*myo*-inositol-PO₄-lipid (McConville and Ferguson, 1993) while galactose can be found attached to different positions. As previously mentioned TcCRT, which interacts with host PRRs mannose-binding lectins, increases host cell parasite internalization. Perturbation of this process through the use of mannose binding CBAs could reduce infection of new cells. The abundant mucins contain galactose in their oligosaccharide elongations and are O-glycosylated with GlcNAc. In addition, the exposure of free $\beta(1,3)$ Gal residues and β -Gal residues has been suggested to mediate parasite attachment and entry in dendritic cells. Indeed, human galectin-3, a member of the lectin family with affinity to β -Gal and derivatives, plays a pivotal role in controlling *T. cruzi* infection. It has been recently proposed that galectin-3 deficiency during *T. cruzi* experimental infection resulted in increased *in vivo* systemic parasitaemia, and reduced leucocyte recruitment (da Silva *et al.*, 2017). Since galactose-specific lectins are available, their possible interaction with infection mechanisms in the case of *T. cruzi* warrants investigation of the potential of this class of CBAs in the case of Chagas disease.

Finally, the use of treatment with CBAs in the case of infections caused by *Leishmania* spp. should also be further considered. *Leishmania* can target several macrophage membrane-bound receptors to subvert the inflammatory response. Mannose-terminating GPIs interact with mannose receptors on the macrophage surface (Blackwell *et al.*, 1985) modulating macrophage functions such as PKC activity (Chawla and Vishwakarma, 2003), cytokine levels and the production of NO. Sialic acids on the parasite surface interact with siglec receptors on macrophages to diminish the immune response (Roy and Mandal, 2016). Additionally, of interest is the observation that TLR-2 is involved in parasite survival in

macrophages upon activation by LPG and interactions between LPG and TLR-2 reduce anti-leishmanial responses *via* cytokine-mediated decrease of TLR-9 expression (Srivastava *et al.*, 2013). All these important mechanisms of parasite survival may be perturbed upon treatment with mannose-specific CBAs.

Concluding remarks

As highlighted in the present review, kinetoplastids interact with mammalian host cells by recognizing specific glycan ligands. Parasite surface carbohydrates are involved in parasite attachment and entry as well as in the modulation of the immune response and the progression of infection. Based on observations with lectins and non-peptidic pradimicins in the case of sleeping sickness, the use of CBAs emerges as a promising antitrypanosomal strategy. Specificity of the interactions and the unique structure of kinetoplastid surface sugars may provide a basis for future drug design. Many bioactive natural carbohydrate-binding compounds are present in nature that often exhibit exquisite specificity for binding to carbohydrates, particularly carbohydrate sequences that occur on the surface of living cells. These molecules have the potential for treatment of kinetoplastid diseases. While specificity and toxicity may constitute important issues, the possibility of identifying agents that can be used to block the attachment of the parasite to cell surfaces (or interfere with the subversion of the immune response), and thus prevent or suppress infection is appealing. Future identification of new CBAs with improved pharmacological profiles and reduced side-effects may provide novel avenues for the exploitation of this innovative concept.

Acknowledgements. The authors thank Dr Santiago Castanys Cuello for critical reading of the manuscript.

Financial support. Financial support was received from the Junta de Andalucía (BIO-199 P12-BIO-2059); the Plan Nacional, Instituto de Salud Carlos III-Subdirección General de Redes y Centros de Investigación Cooperativa-Red de Investigación Cooperativa en Enfermedades Tropicales (RICET: RD16/0027/0014) and the Plan Nacional de Investigación Científica (SAF2016-79957-R) and the FEDER funds from the EU.

Conflict of interest. None.

Ethical standards. Not applicable.

References

- Acosta-Serrano A, Almeida IC, Freitas-Junior LH, Yoshida N and Schenkman S (2001) The mucin-like glycoprotein super-family of *Trypanosoma cruzi*: structure and biological roles. *Molecular and Biochemical Parasitology* **114**, 143–150.
- Acosta-Serrano A, O'Rear J, Quellhorst G, Lee SH, Hwa KY, Krag SS and Englund PT (2004) Defects in the N-linked oligosaccharide biosynthetic pathway in a *Trypanosoma brucei* glycosylation mutant. *Eukaryotic Cell* **3**, 255–263.
- Afonso-Cardoso SR, Silva CV, Ferreira MS and Souza MA (2011) Effect of the *Synadenium carinatum* latex lectin (ScLL) on *Leishmania (leishmania) amazonensis* infection in murine macrophages. *Experimental Parasitology* **128**, 61–67.
- Agusti R, Giorgi ME and de Lederkremer RM (2007) The *trans*-sialidase from *Trypanosoma cruzi* efficiently transfers alpha-(2→3)-linked N-glycolylneuraminic acid to terminal beta-galactosyl units. *Carbohydrate Research* **342**, 2465–2469.
- Albuquerque DA, Martins GA, Campos-Neto A and Silva JS (1999) The adjuvant effect of jacalin on the mouse humoral immune response to trinitrophenyl and *Trypanosoma cruzi*. *Immunology Letters* **68**, 375–381.
- Alves MJ and Colli W (2008) Role of the gp85/*trans*-sialidase superfamily of glycoproteins in the interaction of *Trypanosoma cruzi* with host structures. *Subcellular Biochemistry* **47**, 58–69.
- Andrews NW and Whitlow MB (1989) Secretion by *Trypanosoma cruzi* of a hemolysin active at low pH. *Molecular and Biochemical Parasitology* **33**, 249–256.
- Anthony RM, Kobayashi T, Wermeling F and Ravetch JV (2011) Intravenous gammaglobulin suppresses inflammation through a novel T (H)2 pathway. *Nature* **475**, 110–113.
- Argibay PF, Di Noia JM, Hidalgo A, Mocetti E, Barbich M, Lorenti AS, Bustos D, Tambutti M, Hyon SH, Frasch AC and Sanchez DO (2002) *Trypanosoma cruzi* surface mucin TcMuc-e2 expressed on higher eukaryotic cells induces human T cell anergy, which is reversible. *Glycobiology* **12**, 25–32.
- Assis RR, Ibraim IC, Noronha FS, Turco SJ and Soares RP (2012) Glycoinositolphospholipids from *Leishmania braziliensis* and *L. infantum*: modulation of innate immune system and variations in carbohydrate structure. *PLoS Neglected Tropical Diseases* **6**, e1543.
- Atwood JA, Weatherly DB, Minning TA, Bundy B, Cavola C, Opperdoes FR, Orlando R and Tarleton RL (2005) The *Trypanosoma cruzi* proteome. *Science* **309**, 473–476.
- Atwood JA, Minning T, Ludolf F, Nuccio A, Weatherly DB, Alvarez-Manilla G, Tarleton R and Orlando R (2006) Glycoproteomics of *Trypanosoma cruzi* trypomastigotes using subcellular fractionation, lectin affinity, and stable isotope labeling. *Journal of Proteome Research* **5**, 3376–3384.
- Balzarini J, Van Laethem K, Hatse S, Froeyen M, Peumans W, Van Damme E and Schols D (2005) Carbohydrate-binding agents cause deletions of highly conserved glycosylation sites in HIV GP120: a new therapeutic concept to hit the achilles heel of HIV. *The Journal of Biological Chemistry* **280**, 41005–41014.
- Bangs JD, Doering TL, Englund PT and Hart GW (1988) Biosynthesis of a variant surface glycoprotein of *Trypanosoma brucei*. Processing of the glycolipid membrane anchor and N-linked oligosaccharides. *The Journal of Biological Chemistry* **263**, 17697–17705.
- Barral-Netto M, Von Sohsten RL, Teixeira M, dos Santos WL, Pompeu ML, Moreira RA, Oliveira JT, Cavada BS, Falcoff E and Barral A (1996) *In vivo* protective effect of the lectin from *Canavalia brasiliensis* on BALB/c mice infected by *Leishmania amazonensis*. *Acta Tropica* **60**, 237–250.
- Bartholomeu DC, Cerqueira GC, Leao AC, daRocha WD, Pais FS, Macedo C, Djikeng A, Teixeira SM and El-Sayed NM (2009) Genomic organization and expression profile of the mucin-associated surface protein (MASP) family of the human pathogen *Trypanosoma cruzi*. *Nucleic Acids Research* **37**, 3407–3417.
- Becker I, Salaiza N, Aguirre M, Delgado J, Carrillo-Carrasco N, Kobeh LG, Ruiz A, Cervantes R, Torres AP, Cabrera N, Gonzalez A, Maldonado C and Isibasi A (2003) *Leishmania* lipophosphoglycan (LPG) activates NK cells through toll-like receptor-2. *Molecular and Biochemical Parasitology* **130**, 65–74.
- Blackwell JM, Ezekowitz RA, Roberts MB, Channon JY, Sim RB and Gordon S (1985) Macrophage complement and lectin-like receptors bind *Leishmania* in the absence of serum. *The Journal of Experimental Medicine* **162**, 324–331.
- Blum ML, Down JA, Gurnett AM, Carrington M, Turner MJ and Wiley DC (1993) A structural motif in the variant surface glycoproteins of *Trypanosoma brucei*. *Nature* **362**, 603–609.
- Breitenbach Barroso Coelho LC, Marcelino Dos Santos Silva P, Felix de Oliveira W, de Moura MC, Viana Pontual E, Soares Gomes F, Guedes Paiva PM, Napoleao TH and Dos Santos Correia MT (2018) Lectins as antimicrobial agents. *Journal of Applied Microbiology* **125**, 1238–1252.
- Brosson S, Fontaine F, Vermeersch M, Perez-Morga D, Pays E, Bousbata S and Salmon D (2016) Specific endocytosis blockade of *Trypanosoma cruzi* exposed to a poly-lacnac binding lectin suggests that lectin–sugar interactions participate to receptor-mediated endocytosis. *PLoS One* **11**, e0163302.
- Buscaglia CA, Campo VA, Frasch AC and Di Noia JM (2006) *Trypanosoma cruzi* surface mucins: host-dependent coat diversity. *Nature Reviews Microbiology* **4**, 229–236.
- Cabezas Y, Legentil L, Robert-Gangneux F, Daligault F, Belaz S, Nugier-Chauvin C, Tranchimand S, Tellier C, Gangneux JP and Ferrieres V (2015) *Leishmania* cell wall as a potent target for antiparasitic drugs. A focus on the glycoconjugates. *Organic & Biomolecular Chemistry* **13**, 8393–8404.
- Canepa GE, Degese MS, Budu A, Garcia CR and Buscaglia CA (2012) Involvement of TSSA (trypomastigote small surface antigen) in

- Trypanosoma cruzi* invasion of mammalian cells. *Biochemical Journal* **444**, 211–218.
- Capul AA, Hickerson S, Barron T, Turco SJ and Beverley SM (2007) Comparisons of mutants lacking the Golgi UDP-galactose or GDP-mannose transporters establish that phosphoglycans are important for promastigote but not amastigote virulence in *Leishmania major*. *Infection and Immunity* **75**, 4629–4637.
- Carreira JC, Jones C, Wait R, Previato JO and Mendonça-Previato L (1996) Structural variation in the glycoinositolphospholipids of different strains of *Trypanosoma cruzi*. *Glycoconjugate Journal* **13**, 955–966.
- Casas-Sánchez A, Perally S, Ramaswamy R, Haines LR, Rose C, Yunta C, Aguilera-Flores M, Lehane MJ, Almeida IC, Boulanger MJ and Acosta-Serrano A (2018) The crystal structure and localization of *Trypanosoma brucei* invariant surface glycoproteins suggest a more permissive VSG coat in the tsetse-transmitted metacyclic stage. *bioRxiv*. doi: 10.1101/477737.
- Castillo-Acosta VM, Vidal AE, Ruiz-Perez LM, Van Damme EJ, Igarashi Y, Balzarini J and Gonzalez-Pacanowska D (2013) Carbohydrate-binding agents act as potent trypanocidal that elicit modifications in VSG glycosylation and reduced virulence in *Trypanosoma brucei*. *Molecular Microbiology* **90**, 665–679.
- Castillo-Acosta VM, Ruiz-Perez LM, Van Damme EJ, Balzarini J and Gonzalez-Pacanowska D (2015) Exposure of *Trypanosoma brucei* to an N-acetylglucosamine-binding lectin induces VSG switching and glycosylation defects resulting in reduced infectivity. *PLoS Neglected Tropical Diseases* **9**, e0003612.
- Castillo-Acosta VM, Ruiz-Perez LM, Etxebarria J, Reichardt NC, Navarro M, Igarashi Y, Liekens S, Balzarini J and Gonzalez-Pacanowska D (2016) Carbohydrate-binding non-peptidic pradimicins for the treatment of acute sleeping sickness in murine models. *PLoS Pathogens* **12**, e1005851.
- Cestari I, Evans-Osses I, Schlapbach LJ, de Messias-Reason I and Ramirez MI (2013) Mechanisms of complement lectin pathway activation and resistance by trypanosomatid parasites. *Molecular Immunology* **53**, 328–334.
- Chawla M and Vishwakarma RA (2003) Alkylacylglycerolipid domain of GPI molecules of *Leishmania* is responsible for inhibition of PKC-mediated c-fos expression. *The Journal of Lipid Research* **44**, 594–600.
- da Silva AA, Teixeira TL, Teixeira SC, Machado FC, Dos Santos MA, Tomioso TC, Tavares PCB, Brigido R, Martins FA, Silva NSL, Rodrigues CC, Roque-Barreira MC, Mortara RA, Lopes DS, Avila VMR and da Silva CV (2017) Galectin-3: a friend but not a foe during *Trypanosoma cruzi* experimental infection. *Frontiers in Cellular and Infection Microbiology* **7**, 463.
- da Silva JDF, da Silva SP, da Silva PM, Vieira AM, de Araujo LCC, de Albuquerque Lima T, de Oliveira APS, do Nascimento Carvalho LV, da Rocha Pitta MG, de Melo Rego MJB, Pinheiro IO, Zingali RB, do Socorro de Mendonça Cavalcanti M, Napoleão TH and Paiva PMG (2019) *Portulaca elatior* root contains a trehalose-binding lectin with antibacterial and antifungal activities. *International Journal of Biological Macromolecules* **126**, 291–297.
- de Lederkremer RM and Agustí R (2009) Glycobiology of *Trypanosoma cruzi*. *Advances in Carbohydrate Chemistry and Biochemistry* **62**, 311–366.
- De Lederkremer RM, Alves MJ, Fonseca GC and Colli W (1976) A lipopeptidophosphoglycan from *Trypanosoma cruzi* (epimastigote). Isolation, purification and carbohydrate composition. *Biochimica et Biophysica Acta* **444**, 85–96.
- de Lederkremer RM, Lima C, Ramirez MI, Ferguson MA, Homans SW and Thomas-Oates J (1991) Complete structure of the glycan of lipopeptidophosphoglycan from *Trypanosoma cruzi* epimastigotes. *The Journal of Biological Chemistry* **266**, 23670–23675.
- De Muylder G, Daulouede S, Lecordier L, Uzureau P, Morias Y, Van Den Abbeele J, Caljon G, Herin M, Holzmüller P, Semballa S, Courtois P, Vanhamme L, Stijlemans B, De Baetselier P, Barrett MP, Barlow JL, McKenzie AN, Barron L, Wynn TA, Beschin A, Vincendeau P and Pays E (2013) A *Trypanosoma brucei* kinesin heavy chain promotes parasite growth by triggering host arginase activity. *PLoS Pathogens* **9**, e1003731.
- Descoteaux A and Turco SJ (1993) The lipophosphoglycan of *Leishmania* and macrophage protein kinase C. *Parasitology Today* **9**, 468–471.
- Dey B, Lerner DL, Lusso P, Boyd MR, Elder JH and Berger EA (2000) Multiple antiviral activities of cyanovirin-N: blocking of human immunodeficiency virus type 1 gp120 interaction with CD4 and coreceptor and inhibition of diverse enveloped viruses. *Journal of Virology* **74**, 4562–4569.
- Di Noia JM, Buscaglia CA, De Marchi CR, Almeida IC and Frasch AC (2002) A *Trypanosoma cruzi* small surface molecule provides the first immunological evidence that Chagas' disease is due to a single parasite lineage. *The Journal of Experimental Medicine* **195**, 401–413.
- dos Santos SL, Freitas LM, Lobo FP, Rodrigues-Luiz GF, Mendes TA, Oliveira AC, Andrade LO, Chiari E, Gazzinelli RT, Teixeira SM, Fujiwara RT and Bartholomeu DC (2012) The MASP family of *Trypanosoma cruzi*: changes in gene expression and antigenic profile during the acute phase of experimental infection. *PLoS Neglected Tropical Diseases* **6**, e1779.
- Engstler M, Thilo L, Weise F, Grunfelder CG, Schwarz H, Boshart M and Overath P (2004) Kinetics of endocytosis and recycling of the GPI-anchored variant surface glycoprotein in *Trypanosoma brucei*. *Journal of Cell Science* **117**(Pt 7), 1105–1115.
- Ferguson MA (1999) The structure, biosynthesis and functions of glycosyl-phosphatidylinositol anchors, and the contributions of trypanosome research. *Journal of Cell Science* **112**(Pt 17), 2799–2809.
- Ferguson MA, Homans SW, Dwek RA and Rademacher TW (1988) Glycosyl-phosphatidylinositol moiety that anchors *Trypanosoma brucei* variant surface glycoprotein to the membrane. *Science* **239**(Pt 1), 753–759.
- Fernandes MP, Inada NM, Chiaratti MR, Araujo FF, Meirelles FV, Correia MT, Coelho LC, Alves MJ, Gadelha FR and Vercesi AE (2010) Mechanism of *Trypanosoma cruzi* death induced by *Cratylia mollis* seed lectin. *Journal of Bioenergetics and Biomembranes* **42**, 69–78.
- Ferreira V, Molina MC, Valck C, Rojas A, Aguilar L, Ramirez G, Schwaebel W and Ferreira A (2004a) Role of calreticulin from parasites in its interaction with vertebrate hosts. *Molecular Immunology* **40**, 1279–1291.
- Ferreira V, Valck C, Sanchez G, Gingras A, Tzima S, Molina MC, Sim R, Schwaebel W and Ferreira A (2004b) The classical activation pathway of the human complement system is specifically inhibited by calreticulin from *Trypanosoma cruzi*. *The Journal of Immunology* **172**, 3042–3050.
- Franco LH, Beverley SM and Zamboni DS (2012) Innate immune activation and subversion of Mammalian functions by *Leishmania* lipophosphoglycan. *Journal of Parasitology Research* **2012**, 165126.
- Francois KO and Balzarini J (2012) Potential of carbohydrate-binding agents as therapeutics against enveloped viruses. *Medicinal Research Reviews* **32**, 349–387.
- Freire-de-Lima L, Alisson-Silva F, Carvalho ST, Takiya CM, Rodrigues MM, DosReis GA, Mendonça-Previato L, Previato JO and Todeschini AR (2010) *Trypanosoma cruzi* subverts host cell sialylation and may compromise antigen-specific CD8⁺ T cell responses. *The Journal of Biological Chemistry* **285**, 13388–13396.
- Funk VA, Thomas-Oates JE, Kielland SL, Bates PA and Olafson RW (1997) A unique, terminally glucosylated oligosaccharide is a common feature on *Leishmania* cell surfaces. *Molecular and Biochemical Parasitology* **84**, 33–48.
- Galvan M, Murali-Krishna K, Ming LL, Baum L and Ahmed R (1998) Alterations in cell surface carbohydrates on T cells from virally infected mice can distinguish effector/memory CD8⁺ T cells from naive cells. *The Journal of Immunology* **161**, 641–648.
- Gao W, Wortis HH and Pereira MA (2002) The *Trypanosoma cruzi* trans-sialidase is a T cell-independent B cell mitogen and an inducer of non-specific Ig secretion. *International Immunology* **14**, 299–308.
- Gaur U, Showalter M, Hickerson S, Dalvi R, Turco SJ, Wilson ME and Beverley SM (2009) *Leishmania donovani* lacking the Golgi GDP-Mann transporter LPG2 exhibit attenuated virulence in mammalian hosts. *Experimental Parasitology* **122**, 182–191.
- Geiger A, Hirtz C, Becue T, Bellard E, Centeno D, Gargani D, Rossignol M, Cuny G and Peltier JB (2010) Exocytosis and protein secretion in *Trypanosoma*. *BMC Microbiology* **10**, 20.
- Ghoshal A and Mandal C (2011) A perspective on the emergence of sialic acids as potent determinants affecting *Leishmania* biology. *Molecular Biology International* **2011**, 532106.
- Ginger ML, Blundell PA, Lewis AM, Browitt A, Gunzl A and Barry JD (2002) *Ex vivo* and *in vitro* identification of a consensus promoter for VSG genes expressed by metacyclic-stage trypanosomes in the tsetse fly. *Eukaryotic Cell* **1**, 1000–1009.
- Gondim ACS, Roberta da Silva S, Mathys L, Noppen S, Liekens S, Holanda Sampaio A, Nagano CS, Renata Costa Rocha C, Nascimento KS, Cavada BS, Sadler PJ and Balzarini J (2019) Potent antiviral activity of carbohydrate-specific algal and leguminous lectins from the Brazilian biodiversity. *MedChemComm* **10**, 390–398.

- Gonzalez A, Valck C, Sanchez G, Hartel S, Mansilla J, Ramirez G, Fernandez MS, Arias JL, Galanti N and Ferreira A (2015) *Trypanosoma cruzi* calreticulin topographical variations in parasites infecting murine macrophages. *The American Journal of Tropical Medicine and Hygiene* **92**, 887–897.
- Grab DJ, Shaw MK, Wells CW, Verjee Y, Russo DC, Webster P, Naessens J and Fish WR (1993) The transferrin receptor in African trypanosomes: identification, partial characterization and subcellular localization. *European Journal of Cell Biology* **62**, 114–126.
- Grandgenett PM, Otsu K, Wilson HR, Wilson ME and Donelson JE (2007) A function for a specific zinc metalloprotease of African trypanosomes. *PLoS Pathogens* **3**, 1432–1445.
- Gruszynski AE, DeMaster A, Hooper NM and Bangs JD (2003) Surface coat remodeling during differentiation of *Trypanosoma brucei*. *The Journal of Biological Chemistry* **278**, 24665–24672.
- Hasan I and Ozeki Y (2019) Histochemical localization of N-acetylhexosamine-binding lectin HOL-18 in *Halichondria okadai* (Japanese black sponge), and its antimicrobial and cytotoxic anticancer effects. *International Journal of Biological Macromolecules* **124**, 819–827.
- Holder AA (1985) Glycosylation of the variant surface antigens of *Trypanosoma brucei*. *Current Topics in Microbiology and Immunology* **117**, 57–74.
- Hoorelbeke B, Huskens D, Ferir G, Francois KO, Takahashi A, Van Laethem K, Schols D, Tanaka H and Balzarini J (2010) Actinohivin, a broadly neutralizing prokaryotic lectin, inhibits HIV-1 infection by specifically targeting high-mannose-type glycans on the gp120 envelope. *Antimicrobial Agents and Chemotherapy* **54**, 3287–3301.
- Iida K, Whitlow MB and Nussenzweig V (1989) Amastigotes of *Trypanosoma cruzi* escape destruction by the terminal complement components. *The Journal of Experimental Medicine* **169**, 881–891.
- Ilg T (2000) Proteophosphoglycans of *Leishmania*. *Parasitology Today* **16**, 489–497.
- Kaneko Y, Nimmerjahn F and Ravetch JV (2006) Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* **313**, 670–673.
- Kavoosi G, Ardestani SK and Kariminia A (2009) The involvement of TLR2 in cytokine and reactive oxygen species (ROS) production by PBMCs in response to *Leishmania major* phosphoglycans (PGs). *Parasitology* **136**, 1193–1199.
- Kedzierski L, Montgomery J, Bullen D, Curtis J, Gardiner E, Jimenez-Ruiz A and Handman E (2004) A leucine-rich repeat motif of *Leishmania* parasite surface antigen 2 binds to macrophages through the complement receptor 3. *The Journal of Immunology* **172**, 4902–4906.
- Kipnis TL, Krettli AU and Dias da Silva W (1985) Transformation of trypanomastigote forms of *Trypanosoma cruzi* into activators of alternative complement pathway by immune IgG fragments. *Scandinavian Journal of Immunology* **22**, 217–226.
- Kleshchenko YY, Moody TN, Furtak VA, Ochieng J, Lima MF and Villalta F (2004) Human galectin-3 promotes *Trypanosoma cruzi* adhesion to human coronary artery smooth muscle cells. *Infection and Immunity* **72**, 6717–6721.
- Lantos AB, Carlevaro G, Araoz B, Ruiz Diaz P, Camara Mde L, Buscaglia CA, Bossi M, Yu H, Chen X, Bertozzi CR, Mucci J and Campetella O (2016) Sialic acid glycobiology unveils *Trypanosoma cruzi* trypanomastigote membrane physiology. *PLoS Pathogens* **12**, e1005559.
- Leppert BJ, Mansfield JM and Paulnock DM (2007) The soluble variant surface glycoprotein of African trypanosomes is recognized by a macrophage scavenger receptor and induces I kappa B alpha degradation independently of TRAF6-mediated TLR signaling. *The Journal of Immunology* **179**, 548–556.
- Li L, Yu X, Zhang H, Cheng H, Hou L, Zheng Q and Hou J (2019) *In vitro* antiviral activity of Griffithsin against porcine epidemic diarrhea virus. *Virus Genes* **55**, 174–181.
- Lincoln LM, Ozaki M, Donelson JE and Beetham JK (2004) Genetic complementation of *Leishmania* deficient in PSA (GP46) restores their resistance to lysis by complement. *Molecular and Biochemical Parasitology* **137**, 185–189.
- Liu FT and Rabinovich GA (2005) Galectins as modulators of tumour progression. *Nature Reviews Cancer* **5**, 29–41.
- Lowe JB (2003) Glycan-dependent leukocyte adhesion and recruitment in inflammation. *Current Opinion in Cell Biology* **15**, 531–538.
- MacGregor P, Savill NJ, Hall D and Matthews KR (2011) Transmission stages dominate trypanosome within-host dynamics during chronic infections. *Cell Host & Microbe* **9**, 310–318.
- Maciel EV, Araujo-Filho VS, Nakazawa M, Gomes YM, Coelho LC and Correia MT (2004) Mitogenic activity of *Cratylia mollis* lectin on human lymphocytes. *Biologicals* **32**, 57–60.
- Magaz S, Radwanska M, Stijlemans B, Xong HV, Pays E and De Baetselier P (2001) A conserved flagellar pocket exposed high mannose moiety is used by African trypanosomes as a host cytokine binding molecule. *The Journal of Biological Chemistry* **276**, 33458–33464.
- Matsushita M (2010) Ficolins: complement-activating lectins involved in innate immunity. *Journal of Innate Immunity* **2**, 24–32.
- Matthews KR (2015) 25 years of African trypanosome research: from description to molecular dissection and new drug discovery. *Molecular and Biochemical Parasitology* **200**, 30–40.
- Mattos EC, Tonelli RR, Colli W and Alves MJ (2014) The Gp85 surface glycoproteins from *Trypanosoma cruzi*. *Subcellular Biochemistry* **74**, 151–180.
- Mazik M, Cavga H and Jones PG (2005) Molecular recognition of carbohydrates with artificial receptors: mimicking the binding motifs found in the crystal structures of protein-carbohydrate complexes. *Journal of the American Chemical Society* **127**, 9045–9052.
- McConville MJ and Blackwell JM (1991) Developmental changes in the glycosylated phosphatidylinositols of *Leishmania donovani*. Characterization of the promastigote and amastigote glycolipids. *The Journal of Biological Chemistry* **266**, 15170–15179.
- McConville MJ and Ferguson MA (1993) The structure, biosynthesis and function of glycosylated phosphatidylinositols in the parasitic protozoa and higher eukaryotes. *Biochemical Journal* **294**(Pt 2), 305–324.
- McConville MJ, Schnur LF, Jaffe C and Schneider P (1995) Structure of *Leishmania* lipophosphoglycan: inter- and intra-specific polymorphism in Old World species. *Biochemical Journal* **310**(Pt 3), 807–818.
- Mehlert A, Richardson JM and Ferguson MA (1998a) Structure of the glycosylphosphatidylinositol membrane anchor glycan of a class-2 variant surface glycoprotein from *Trypanosoma brucei*. *Journal of Molecular Biology* **277**, 379–392.
- Mehlert A, Zitzmann N, Richardson JM, Treumann A and Ferguson MA (1998b) The glycosylation of the variant surface glycoproteins and procyclic acidic repetitive proteins of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology* **91**, 145–152.
- Mehlert A, Wormald MR and Ferguson MA (2012) Modeling of the N-glycosylated transferrin receptor suggests how transferrin binding can occur within the surface coat of *Trypanosoma brucei*. *PLoS Pathogens* **8**, e1002618.
- Mitoma J, Bao X, Petryanik B, Schaerli P, Gauguet JM, Yu SY, Kawashima H, Saito H, Ohtsubo K, Marth JD, Khoo KH, von Andrian UH, Lowe JB and Fukuda M (2007) Critical functions of N-glycans in L-selectin-mediated lymphocyte homing and recruitment. *Nature Immunology* **8**, 409–418.
- Mucci J, Lantos AB, Buscaglia CA, Leguizamón MS and Campetella O (2017) The *Trypanosoma cruzi* surface, a nanoscale patchwork quilt. *Trends in Parasitology* **33**, 102–112.
- Nieminen J, Kuno A, Hirabayashi J and Sato S (2007) Visualization of galectin-3 oligomerization on the surface of neutrophils and endothelial cells using fluorescence resonance energy transfer. *The Journal of Biological Chemistry* **282**, 1374–1383.
- Nogueira N, Bianco C and Cohn Z (1975) Studies on the selective lysis and purification of *Trypanosoma cruzi*. *The Journal of Experimental Medicine* **142**, 224–229.
- Noireau F, Diosque P and Jansen AM (2009) *Trypanosoma cruzi*: adaptation to its vectors and its hosts. *Veterinary Research* **40**, 26.
- Nolan DP, Jackson DG, Biggs MJ, Brabazon ED, Pays A, Van Laethem F, Paturiaux-Hanocq F, Elliott JF, Voorheis HP and Pays E (2000) Characterization of a novel alanine-rich protein located in surface microdomains in *Trypanosoma brucei*. *The Journal of Biological Chemistry* **275**, 4072–4080.
- Nunes MP, Fortes B, Silva-Filho JL, Terra-Granado E, Santos L, Conde L, de Araujo Oliveira I, Freire-de-Lima L, Martins MV, Pinheiro AA, Takyia CM, Freire-de-Lima CG, Todeschini AR, Dosreis GA and Morrot A (2013) Inhibitory effects of *Trypanosoma cruzi* sialoglycoproteins on CD4⁺ T cells are associated with increased susceptibility to infection. *PLoS One* **8**, e77568.
- O'Beirne C, Lowry CM and Voorheis HP (1998) Both IgM and IgG anti-VSG antibodies initiate a cycle of aggregation-disaggregation of bloodstream forms of *Trypanosoma brucei* without damage to the parasite. *Molecular and Biochemical Parasitology* **91**, 165–193.

- Olivier M, Atayde VD, Isnard A, Hassani K and Shio MT (2012) *Leishmania* virulence factors: focus on the metalloprotease GP63. *Microbes and Infection* **14**, 1377–1389.
- Overath P and Engstler M (2004) Endocytosis, membrane recycling and sorting of GPI-anchored proteins: *Trypanosoma brucei* as a model system. *Molecular Microbiology* **53**, 735–744.
- Pal A, Hall BS, Jeffries TR and Field MC (2003) Rab5 and Rab11 mediate transferrin and anti-variant surface glycoprotein antibody recycling in *Trypanosoma brucei*. *Biochemical Journal* **374**(Pt 2), 443–451.
- Panunto-Castelo A, Souza MA, Roque-Barreira MC and Silva JS (2001) KM (+), a lectin from *Artocarpus integrifolia*, induces IL-12 p40 production by macrophages and switches from type 2 to type 1 cell-mediated immunity against *Leishmania major* antigens, resulting in BALB/c mice resistance to infection. *Glycobiology* **11**, 1035–1042.
- Pennock ND, White JT, Cross EW, Cheney EE, Tamburini BA and Kedl RM (2013) T cell responses: naive to memory and everything in between. *Advances in Physiology Education* **37**, 273–283.
- Pereira-Chioccola VL, Acosta-Serrano A, Correia de Almeida I, Ferguson MA, Souto-Padron T, Rodrigues MM, Travassos LR and Schenkman S (2000) Mucin-like molecules form a negatively charged coat that protects *Trypanosoma cruzi* trypomastigotes from killing by human anti-alpha-galactosyl antibodies. *Journal of Cell Science* **113**(Pt 7), 1299–1307.
- Petrova MI, Imholz NC, Verhoeven TL, Balzarini J, Van Damme EJ, Schols D, Vanderleyden J and Lebeer S (2016) Lectin-like molecules of *Lactobacillus rhamnosus* GG inhibit pathogenic *Escherichia coli* and Salmonella biofilm formation. *PLoS One* **11**, e0161337.
- Piani A, Ilg T, Elefanty AG, Curtis J and Handman E (1999) *Leishmania major* proteophosphoglycan is expressed by amastigotes and has an immunomodulatory effect on macrophage function. *Microbes and Infection* **1**, 589–599.
- Pillai S, Netravali IA, Cariappa A and Mattoo H (2012) Siglecs and immune regulation. *Annual Review of Immunology* **30**, 357–392.
- Pinger J, Nestic D, Ali L, Aresta-Branco F, Lilic M, Chowdhury S, Kim HS, Verdi J, Raper J, Ferguson MAJ, Papavasiliou FN and Stebbins CE (2018) African trypanosomes evade immune clearance by O-glycosylation of the VSG surface coat. *Nature Microbiology* **3**, 932–938.
- Previato JO, Andrade AF, Pessolani MC and Mendonca-Previato L (1985) Incorporation of sialic acid into *Trypanosoma cruzi* macromolecules. A proposal for a new metabolic route. *Molecular and Biochemical Parasitology* **16**, 85–96.
- Previato JO, Gorin PA, Mazurek M, Xavier MT, Fournet B, Wieruszek JM and Mendonca-Previato L (1990) Primary structure of the oligosaccharide chain of lipopeptidophosphoglycan of epimastigote forms of *Trypanosoma cruzi*. *The Journal of Biological Chemistry* **265**, 2518–2526.
- Previato JO, Jones C, Goncalves LP, Wait R, Travassos LR and Mendonca-Previato L (1994) O-glycosidically linked N-acetylglucosamine-bound oligosaccharides from glycoproteins of *Trypanosoma cruzi*. *Biochemical Journal* **301**(Pt 1), 151–159.
- Previato JO, Jones C, Xavier MT, Wait R, Travassos LR, Parodi AJ and Mendonca-Previato L (1995) Structural characterization of the major glycosylphosphatidylinositol membrane-anchored glycoprotein from epimastigote forms of *Trypanosoma cruzi* Y-strain. *The Journal of Biological Chemistry* **270**, 7241–7250.
- Previato JO, Sola-Penna M, Agrellos OA, Jones C, Oeltmann T, Travassos LR and Mendonca-Previato L (1998) Biosynthesis of O-N-acetylglucosamine-linked glycans in *Trypanosoma cruzi*. Characterization of the novel uridine diphospho-N-acetylglucosamine:polypeptide N-acetylglucosaminyltransferase-catalyzing formation of N-acetylglucosamine alpha1→O-threonine. *The Journal of Biological Chemistry* **273**, 14982–14988.
- Previato JO, Wait R, Jones C, DosReis GA, Todeschini AR, Heise N and Previato LM (2004) Glycoinositolphospholipid from *Trypanosoma cruzi*: structure, biosynthesis and immunobiology. *Advances in Parasitology* **56**, 1–41.
- Priatel JJ, Chui D, Hiraoka N, Simmons CJ, Richardson KB, Page DM, Fukuda M, Varki NM and Marth JD (2000) The ST3Gal-I sialyltransferase controls CD8⁺ T lymphocyte homeostasis by modulating O-glycan biosynthesis. *Immunity* **12**, 273–283.
- Quanquin NM, Galaviz C, Fouts DL, Wrightsman RA and Manning JE (1999) Immunization of mice with a Tola-like surface protein of *Trypanosoma cruzi* generates CD4(+) T-cell-dependent parasiticidal activity. *Infection and Immunity* **67**, 4603–4612.
- Ramirez MI, Deolindo P, de Messias-Reason IJ, Arigi EA, Choi H, Almeida IC and Evans-Osses I (2017) Dynamic flux of microvesicles modulate parasite–host cell interaction of *Trypanosoma cruzi* in eukaryotic cells. *Cell Microbiology* **19**, 1–15.
- Redman CA, Green BN, Thomas-Oates JE, Reinhold VN and Ferguson MA (1994) Analysis of glycosylphosphatidylinositol membrane anchors by electrospray ionization-mass spectrometry and collision induced dissociation. *Glycoconjugate Journal* **11**, 187–193.
- Rogers ME and Bates PA (2007) *Leishmania* manipulation of sand fly feeding behavior results in enhanced transmission. *PLoS Pathogens* **3**, e91.
- Rogers ME, Ilg T, Nikolaev AV, Ferguson MA and Bates PA (2004) Transmission of cutaneous leishmaniasis by sand flies is enhanced by regurgitation of iPPG. *Nature* **430**, 463–467.
- Rogers M, Kropf P, Choi BS, Dillon R, Podinovskaia M, Bates P and Muller I (2009) Proteophosphoglycans regurgitated by *Leishmania*-infected sand flies target the L-arginine metabolism of host macrophages to promote parasite survival. *PLoS Pathogens* **5**, e1000555.
- Rojas-Bernabe A, Garcia-Hernandez O, Maldonado-Bernal C, Delegado-Dominguez 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.
- Romano PS, Cueto JA, Casassa AF, Vanrell MC, Gottlieb RA and Colombo MI (2012) Molecular and cellular mechanisms involved in the *Trypanosoma cruzi*/host cell interplay. *IUBMB Life* **64**, 387–396.
- Roy S and Mandal C (2016) *Leishmania donovani* utilize sialic acids for binding and phagocytosis in the macrophages through selective utilization of siglecs and impair the innate immune arm. *PLoS Neglected Tropical Diseases* **10**, e0004904.
- Sacks D and Kamhawi S (2001) Molecular aspects of parasite–vector and vector–host interactions in leishmaniasis. *Annual Review of Microbiology* **55**, 453–483.
- Schenkman S and Mortara RA (1992) Hela cells extend and internalize pseudopodia during active invasion by *Trypanosoma cruzi* trypomastigotes. *Journal of Cell Science* **101**(Pt 4), 895–905.
- Schenkman S and Eichinger D (1993) *Trypanosoma cruzi* trans-sialidase and cell invasion. *Parasitology Today* **9**, 218–222.
- Schenkman S, Ferguson MA, Heise N, de Almeida ML, Mortara RA and Yoshida N (1993) Mucin-like glycoproteins linked to the membrane by glycosylphosphatidylinositol anchor are the major acceptors of sialic acid in a reaction catalyzed by trans-sialidase in metacyclic forms of *Trypanosoma cruzi*. *Molecular and Biochemical Parasitology* **59**, 293–303.
- Schneider P, Rosat JP, Bouvier J, Louis J and Bordier C (1992) *Leishmania major*. Differential regulation of the surface metalloprotease in amastigote and promastigote stages. *Experimental Parasitology* **75**, 196–206.
- Schroven A, Meinke S, Ziegelmueller P and Thiem J (2007) Transsialidase from *Trypanosoma cruzi* for regio- and stereoselective synthesis of N-acyl-modified sialylated oligosaccharides and measurement of transfer rates. *Chemistry* **13**, 9012–9021.
- Serrano AA, Schenkman S, Yoshida N, Mehlert A, Richardson JM and Ferguson MA (1995) The lipid structure of the glycosylphosphatidylinositol-anchored mucin-like sialic acid acceptors of *Trypanosoma cruzi* changes during parasite differentiation from epimastigotes to infective metacyclic trypomastigote forms. *The Journal of Biological Chemistry* **270**, 27244–27253.
- Srivastava S, Pandey SP, Jha MK, Chandel HS and Saha B (2013) *Leishmania* expressed lipophosphoglycan interacts with Toll-like receptor (TLR)-2 to decrease TLR-9 expression and reduce anti-leishmanial responses. *Clinical & Experimental Immunology* **172**, 403–409.
- Stanley P and Okajima T (2010) Roles of glycosylation in Notch signaling. *Current Topics in Developmental Biology* **92**, 131–164.
- Steconi-Silva RB, Andreoli WK and Mortara RA (2003) Parameters affecting cellular invasion and escape from the parasitophorous vacuole by different infective forms of *Trypanosoma cruzi*. *The Memórias do Instituto Oswaldo Cruz* **98**, 953–958.
- Steverding D, Stierhof YD, Chaudhri M, Ligtenberg M, Schell D, Beck-Sickinger AG and Overath P (1994) ESAG 6 and 7 products of *Trypanosoma brucei* form a transferrin binding protein complex. *European Journal of Cell Biology* **64**, 78–87.
- Stijlemans B, Caljon G, Van Den Abbeele J, Van Ginderachter JA, Magez S and De Trez C (2016) Immune evasion strategies of *Trypanosoma brucei*

- within the mammalian host: progression to pathogenicity. *Frontiers in Immunology* 7, 233.
- Strang AM, Allen AK, Holder AA and van Halbeek H** (1993) The carbohydrate structures of *Trypanosoma brucei brucei* MITat 1.6 variant surface glycoprotein. A re-investigation of the C-terminal glycan. *Biochemical and Biophysical Research Communications* 196, 1430–1439.
- Striegler S and Dittel M** (2003) A sugar discriminating binuclear copper(II) complex. *Journal of the American Chemical Society* 125, 11518–11524.
- Tetley L, Turner CM, Barry JD, Crowe JS and Vickerman K** (1987) Onset of expression of the variant surface glycoproteins of *Trypanosoma brucei* in the tsetse fly studied using immunoelectron microscopy. *Journal of Cell Science* 87(Pt 2), 363–372.
- Tomita K, Nishio M, Saitoh K, Yamamoto H, Hoshino Y, Ohkuma H, Konishi M, Miyaki T and Oki T** (1990) Pradimicins A, B and C: new antifungal antibiotics. I. Taxonomy, production, isolation and physico-chemical properties. *The Journal of Antibiotics (Tokyo)* 43, 755–762.
- Treumann A, Zitzmann N, Hulsmeier A, Prescott AR, Almond A, Sheehan J and Ferguson MA** (1997) Structural characterisation of two forms of procyclic acidic repetitive protein expressed by procyclic forms of *Trypanosoma brucei*. *Journal of Molecular Biology* 269, 529–547.
- Turco SJ and Sacks DL** (1991) Expression of a stage-specific lipophosphoglycan in *Leishmania major* amastigotes. *Molecular and Biochemical Parasitology* 45, 91–99.
- Urwyler S, Studer E, Renggli CK and Roditi I** (2007) A family of stage-specific alanine-rich proteins on the surface of epimastigote forms of *Trypanosoma brucei*. *Molecular Microbiology* 63, 218–228.
- Vaidya T, Bakhiet M, Hill KL, Olsson T, Kristensson K and Donelson JE** (1997) The gene for a T lymphocyte triggering factor from African trypanosomes. *The Journal of Experimental Medicine* 186, 433–438.
- van Kooyk Y and Rabinovich GA** (2008) Protein–glycan interactions in the control of innate and adaptive immune responses. *Nature Immunology* 9, 593–601.
- Vanhollebeke B, De Muylder G, Nielsen MJ, Pays A, Tebabi P, Dieu M, Raes M, Moestrup SK and Pays E** (2008) A haptoglobin-hemoglobin receptor conveys innate immunity to *Trypanosoma brucei* in humans. *Science* 320, 677–681.
- Vanwalleghem G, Morias Y, Beschin A, Szymkowski DE and Pays E** (2017) *Trypanosoma brucei* growth control by TNF in mammalian host is independent of the soluble form of the cytokine. *Scientific Reports* 7, 6165.
- Vieira M, Dutra JM, Carvalho TM, Cunha-e-Silva NL, Souto-Padron T and Souza W** (2002) Cellular signaling during the macrophage invasion by *Trypanosoma cruzi*. *Histochemistry and Cell Biology* 118, 491–500.
- Vray B, Camby I, Vercruyse V, Mijatovic T, Bovin NV, Ricciardi-Castagnoli P, Kaltner H, Salmon I, Gabius HJ and Kiss R** (2004) Up-regulation of galectin-3 and its ligands by *Trypanosoma cruzi* infection with modulation of adhesion and migration of murine dendritic cells. *Glycobiology* 14, 647–657.
- Walsh TJ and Giri N** (1997) Pradimicins: a novel class of broad-spectrum antifungal compounds. *European Journal of Clinical Microbiology & Infectious Diseases* 16, 93–97.
- Watanabe M, Hiratani T, Uchida K, Ohtsuka K, Watabe H, Inouye S, Kondo S, Takeuchi T and Yamaguchi H** (1996) The *in-vitro* activity of an antifungal antibiotic benanomycin A in comparison with amphotericin B. *Journal of Antimicrobial Chemotherapy* 38, 1073–1077.
- Winter G, Fuchs M, McConville MJ, Stierhof YD and Overath P** (1994) Surface antigens of *Leishmania mexicana* amastigotes: characterization of glycoinositol phospholipids and a macrophage-derived glycosphingolipid. *Journal of Cell Science* 107(Pt 9), 2471–2482.
- Yao C, Donelson JE and Wilson ME** (2003) The major surface protease (MSP or GP63) of *Leishmania* sp. Biosynthesis, regulation of expression, and function. *Molecular and Biochemical Parasitology* 132, 1–16.
- Yao C, Li Y, Donelson JE and Wilson ME** (2010) Proteomic examination of *Leishmania chagasi* plasma membrane proteins: Contrast between avirulent and virulent (metacyclic) parasite forms. *Proteomics – Clinical Applications* 4, 4–16.
- Zamze SE, Wooten EW, Ashford DA, Ferguson MA, Dwek RA and Rademacher TW** (1990) Characterisation of the asparagine-linked oligosaccharides from *Trypanosoma brucei* type-I variant surface glycoproteins. *European Journal of Biochemistry* 187, 657–663.
- Zamze SE, Ashford DA, Wooten EW, Rademacher TW and Dwek RA** (1991) Structural characterization of the asparagine-linked oligosaccharides from *Trypanosoma brucei* type II and type III variant surface glycoproteins. *The Journal of Biological Chemistry* 266, 20244–20261.
- Zhang LJ and Gallo RL** (2016) Antimicrobial peptides. *Current Biology* 26, R14–R19.
- Ziegelbauer K and Overath P** (1992) Identification of invariant surface glycoproteins in the bloodstream stage of *Trypanosoma brucei*. *The Journal of Biological Chemistry* 267, 10791–10796.
- Ziegelbauer K, Multhaup G and Overath P** (1992) Molecular characterization of two invariant surface glycoproteins specific for the bloodstream stage of *Trypanosoma brucei*. *The Journal of Biological Chemistry* 267, 10797–10803.
- Žurga S, Nanut MP, Kos J and Sabotic J** (2017) Fungal lectin MpL enables entry of protein drugs into cancer cells and their subcellular targeting. *Oncotarget* 8, 26896–26910.