Sialic acid: a sweet swing between mammalian host and Trypanosoma cruzi

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THE SIALIC ACIDS

Sialic acids (Sia) are 9-carbon backbone acidic monosaccharides found at prominent positions of the sugar chains of glycoconjugates present on cell membranes or secreted into the extracellular medium. The most common members of this family are the N-acetyllactosaminic acid (NeuAc) and its derivative the N-glycolylneuraminic acid (Neu5Gc) that differ from each other at position 5 (C-5), which is substituted with an acetamido or a hydroxyacetamido moiety respectively (Figure 1). The metabolism of Sia in mammals involves 32 genes that encode enzymes and transporters, distributed among the different compartments of the cell (Wicker, 2011). The final product of this complex biosynthetic pathway, the activated form of Sia (CMP-Sia), is transferred to the non-reducing end of newly synthesized glycan chains by a family of sialyltransferases present in the Golgi lumen. In vertebrates, Sia are commonly linked via an α2–3 linkage to another Sia (Varki et al., 2009). TcTS activity is capable of extensively remodeling host cell glycomolecules, playing a role as virulence factor. This review presents the state of the art of parasite sialobiology, highlighting how the interplay between host and parasite sialic acid helps the pathogen to evade host defense mechanisms and ensure lifetime host parasitism.

Keywords: glycoconjugate, sialic acid, sialidase, parasite, immune response

Commonly found at the outermost ends of complex carbohydrates in extracellular medium or on outer cell membranes, sialic acids play important roles in a myriad of biological processes. Mammals synthesize sialic acid through a complex pathway, but Trypanosoma cruzi, the agent of Chagas’ disease, evolved to obtain sialic acid from its host through a trans-sialidase (TcTS) reaction. Studies of the parasite cell surface architecture and biochemistry indicate that a unique system comprising sialoglycoproteins and sialyl-binding proteins assists the parasite in several functions including parasite survival, infectivity, and host–cell recognition. Additionally, TcTS activity is capable of extensively remodeling host cell glycomolecules, playing a role as virulence factor. This review presents the state of the art of parasite sialobiology, highlighting how the interplay between host and parasite sialic acid helps the pathogen to evade host defense mechanisms and ensure lifetime host parasitism.

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The sialic acid acceptors on the surface of the parasite and its role in host parasite interaction

The Sia acceptors on the surface of *T. cruzi* are mainly a family of highly *N*-glycosylated, threonine-rich mucin-like glycoproteins (Figure 3D; Buscaglia et al., 2006; Mendonça-Previo et al., 2008) which are glycosphosphatidylinositol (GPI)-anchored to the parasite membrane (Previo et al., 1995). The Tc-mucins are the major component on the surface of *T. cruzi* (2 × 10⁶ copies per parasite) and are the third most widely expanded gene family in the genome, comprising more than 1000 genes (Acosta-Serrano et al., 2001, 2009; Agrellos et al., 2003; Jones et al., 2004). These genes differ from those found in mammalian systems and their expansion and variation among different strains (Minning et al., 2011).

The glycan structure of mucin is complex and heterogeneous among different *T. cruzi* strains. The structure of the oligosaccharides O-linked to the mucins of the non-infective epimastigote forms has been described (Previo et al., 1994, 1995; Todeschini et al., 2001, 2009; Agrellos et al., 2003; Jones et al., 2004). These glycosyltransferase activities are known to be the major targets of protective antibodies found in chronic Chagasic patients (Varki et al., 2009); (iii) several strains carry a β-galactofuranosyltransferase (β-Galf) gene, which allows the parasite to interact with and respond to its external environment. Furthermore, glycoproteins expressed on the parasite surface are known to be the major targets of protective immune responses, and this selective pressure presumably drives their expansion and variation among different strains (Minning et al., 2011).

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that the presence of Sias in parasite epitopes increases T. cruzi infection (Pita et al., 1987; Schenkman et al., 1991), other groups suggest that the presence of Sias is not a requirement and/or may compromise the invasion of host cells (Araújo-Jorge and De Souza, 1988; Yoshida et al., 1997).

Finally, there are increasing evidences to support the Sia-binding Ig-like lectin (Siglecs), on the host cell surface, as the coreceptor for T. cruzi mucin (Erdmann et al., 2009; Jacobs et al., 2010). The Siglecs are a family of sialic-acid-binding immunoglobulin-like lectins that promote cell–cell interactions and regulate the functions of cells in the innate and adaptive immune systems through glycan recognition (Varki and Gagneux, 2012). It was demonstrated that T. cruzi mucin engagement with the Sia-binding protein Siglec-E promotes immunosuppression of dendritic cells (DC; Erdmann et al., 2009).

**TcTS Activity and Its Role in Host Parasite Interaction**

TcTS is part of a protein family known as trans-sialidase/trans-sialidase-like, encoded by more than 1,400 genes (El-Sayed et al., 2005; Freitas et al., 2011). Members of the TcTS gene family can be classified in five groups based on sequence similarity and functional properties (Freitas et al., 2011). Active TS, also namely SAPA (shed acute-phase antigen) expressed by the infective trypomastigote (tTS) and the epimastigote TS (eTS) are grouped into Group I. eTS and tTS have identical enzymatic activities, being highly conserved in their primary sequences (Chaves et al., 1993; Briones et al., 1995; Jager et al., 2008), except for the SAPA domain and their 3′ UTRs, which are completely different in sequence (Jager et al., 2008). Besides, eTS is a trans-membrane protein, while the tTS is associated with the membrane via a GPI linker (Agusti et al., 1997). Group II comprises members of the gp85 surface glycoproteins TSA-1, SAS5, gp90, gp82, and ASP-2, which have been implicated in host cell attachment and invasion. FL-160, a representative of group III, is a complementary regulatory protein that inhibits the alternative and classical complement pathways. TcTS13, whose function is unknown, is the representative of group IV and is included in the Ts superfamily because it contains the conserved VTVxNVxL (Alves and Colli, 2008; Yoshida and Cortez, 2008; Souza et al., 2010). Recently a sequence clustering analysis demonstrated that TS family is even more complex and may arbor more groups and subgroups (Freitas et al., 2011).
Several studies suggest that the TcTS can sialylate or desialylate host cells modulating parasite adherence and penetration. Results with Sia-deficient mutants of Chinese hamster ovary (CHO) cells support this hypothesis (Carvalho et al., 1995; Ming et al., 1993). Sia deficient cells were less infected than wild-type cells, suggesting that sialylation of glycoconjugates on CHO cells surface is necessary during T. cruzi invasion. Moreover, treatment of cells with modified Sia precursors, N-propionylmannosamine and other N-acetylmannosamines, decreased cell invasion by T. cruzi (Licke et al., 2012). Importance of TcTS enzymatic activity in host cell invasion was elegantly proven using an irreversible inhibitor (Carvalho et al., 2013a).

On the other hand, desialylation of sialoglycoproteins found in the membrane of phagolysosomes by TcTS is thought to be important for the escape of the parasite from the cytoplasm of infected cells (Hall et al., 1992; Hall and Joiner, 1993; Rubin-de-Celis et al., 2006). Glycosylphosphatidylinositol-linked trypanosomastigote-derived TcTS can be released into the extracellular medium in fairly high amounts during acute T. cruzi infection in humans, thus acting distant from the parasite as a soluble factor. Besides its role in mammalian cell invasion, the soluble form of TcTS functions as a virulence determinant molecule, and therefore, could have relevant biological effects on the host immune system. It has been suggested that sialylation of CHO cells surface is necessary during T. cruzi-infected mice. The effect observed was specific for the transfer activity of TcTS because it did not occur in mice primed with viral or bacterial sialidases. The mechanisms responsible for these effects were not determined but, since TcTS injection into deficient SCID mice did not affect parasitism or mortality in T. cruzi-infected mice. The effect observed was specific for the transfer activity of TcTS because it did not occur in mice primed with viral or bacterial sialidases. The mechanisms responsible for these effects were not determined but, since TcTS injection into deficient SCID mice did not affect parasitism or mortality, it was suggested that the enzyme acts on host lymphocytes of the acquired immune system (Chuenkova and Pereira, 1995).

Indeed, multiple effects of TcTS on host T-lymphocyte function were additionally demonstrated. TcTS engagement with α2-3-linked Sia-containing epitopes on CD43 (Todeschini et al., 2002a) from CD4+ T cells triggers costimulatory responses that increase mitogenesis and cytokine secretion, as well as promote rescue from apoptosis (Todeschini et al., 2002b). These results strongly suggest that TcTS could be a key parasite molecule induc-ing host polyvalent lymphocyte activation, seen as a condition underlying induction of immunopathology and hampering effective vaccination (Minoprio et al., 1989) in the course of T. cruzi infection.

Given that surface sialylation might be crucial to decide the final fate of the cells during interaction with thymic leucins (Gille-spie et al., 1993; Priatel et al., 2000) alteration of cell sialylation by the soluble TcTS might influence thymocyte development. In fact, alteration of the surface sialylation by TcTS (Mucci et al., 2006) leads to in vivo depletion of the CD4+CD8+ double-positive thymocytes inside the “nurse cell complex” (Leguismon et al., 1999). Interestingly, thymocyte apoptosis observed after the sialyl residue mobilization requires the presence of androgena (Mucci et al., 2005), suggesting the presence of a dimorphic glycosylation survey in the development of the T cell compartment that can be related to the observed differences in the immune response among sexes (Gui et al., 2012). However, further studies about the molecular mechanism involved in the pro-apoptotic effect of TcTS are necessary. However, we can speculate that TcTS activity can mask or expose β-Gal which is recognized by molecules of the galectin family. Corroborating this hypothesis a role was reported for galectin-3 in death of CD4+CD8+ immature thymocytes and migration of these cells away from the thymus after T. cruzi infection (Silva-Monteiro et al., 2007).

The impact of sialylation mediated by TcTS on CD8+ T cell response of mice infected with T. cruzi is an exciting example of how a parasite can manipulate host cell sialylation to favor parasitism. Following infection CD8+ T cell responses are robust and persistent. However, they are significantly delayed (Garg et al., 1997; Telepul et al., 2007). This delay contrasts with the rapid appearance of CD8+ T cell responses in other viral, bacterial and even protozoal infections (Karch et al., 2002), and suggests an operative mechanism of immune evasion. During T cell activation, down-regulation of sialyltransferases (Amado et al., 2004) renders potential Sia acceptors accessible to sialylation through TcTS activity (Figure 4). This sialylation may be advantageous to the parasite, since CD8+ T cells resialylated by TcTS present compromised Ag-specific responses and TcTS-treated mice present increased parasitism (Freire-de-Lima et al., 2010). Cell surface Sia on CD8+ T cells might increase intercellular repulsion and therefore weaken TCR/MHC class I mediated cell–cell interactions. This would be the opposite of the effect of neuraminidase treatment, which removes Sia residues from various membrane glycoproteins and enhances lymphocyte proliferation (Harrington et al., 2000). In an attempt to establish the nature of the Sia acceptor for TcTS on the CD8+ T cell surface, CD8+ T cells from mice lacking the ST3Gal-I sialyltransferase, an enzyme required for sialylation of core 1 O-glycans (Priatel et al., 2000), were infected with T. cruzi. Loss of ST3Gal-I sialyltransferase exposes the Galβ1-3GalNAcSer/Thr moiety creating an interesting model to establish CD43 as a natural receptor for native TcTS during T. cruzi infection. Indeed, infection of mice lacking ST3Gal-I sialyl-transferase restores, at least in part, binding of anti-CD43 Sia mAb, which recognizes Sia-containing epitopes on CD43 of CD8+ T cells. These findings indicated that CD43 is a target receptor for TcTS on the CD8+ T cell surface. However, resialylation by TcTS was also observed on CD8+ T cells from CD43 KO mice, suggesting that in the absence of CD43 other molecules are substrates for TcTS. Other studies using azido-modified unnatural Sia revealed that CD45 isoforms are Sia acceptors for TcTS activity as well (Muñoz et al., 10).

In infected individuals, alteration of cell surface sialylation by TcTS can also compromise host cell homeostasis. Tribulatti et al. (2005) demonstrated that the administration of TcTS into uninfected mice was able to reduce the Sia content of platelets, exposing terminal galactose residues, which may explain the severe thrombocytopenia observed in T. cruzi infected individuals. The recognition of terminal galactose moiety exposed on the platelet surface accelerates platelet clearance by asialoglycoprotein receptor-expressing liver cells (Sorensen et al., 2009). The effect of TcTS on the lifetime of other cell types and plasma glycoproteins must be further verified.
Beyond host immune response, it has been observed that TcTS alters the sialylation status of the tyrosine kinase receptor-A (TrkA) in PC12 cells, which leads to receptor internalization, activation, and neuronal differentiation (Woronowicz et al., 2004). Authors demonstrated that the effects observed are triggered by hydrolysis of Sia residues of TrkA by TcTS, as a purified recombinant α2–3-neuraminidase but not a catalytically inactive mutant of TcTS induced the receptor phosphorylation. Such enzymatic activity might be involved in the neural repair and neuroprotection mediated by the TcTS, also called T. cruzi-derived neurotrophic factor (Chuenkova and Perieraperrin, 2011).

The examples described in this section strongly suggest that T. cruzi exploits the glycosylation of molecules expressed by the host to evasion of the immune response, thus perpetuating the infection.

INACTIVE TcTS

Inactive TcTS (TcTSY342H) is a parasite adhesin that differs from the active TcTS due to a single mutation of catalytic residue Tyr342, which is mainly changed by a histidine (Cremona et al., 1995). In some T. cruzi strains, genes encoding TcTSY342H members are present in the same copy number as those encoding TcTS (Cremona et al., 1999). However, further studies should be performed in order to address the expression levels and the ratio of the TcTS: TcTSY342H protein on T. cruzi surface.

TcTSY342H is a unique adhesin containing two sugar binding sites: one for α2,3-Sia and other for β-Galp (Todeschini et al., 2002a, 2004; Orpezzo et al., 2011). Interestingly, the carbohydrate recognition domain for β-Galp residue is formed only after a conformational switch triggered by prior sialoside binding (Todeschini et al., 2004). The bivalent nature of TcTSY342H might promote glycan cross-linking, which is believed to be essential for cellular signal transduction.

The finding that inactive TS has two carbohydrate binding domains, may explain some apparently contradictory results on the involvement of sialyl and galactosyl epitopes in T. cruzi/host cell interaction. While Schenkman et al. (1991) have shown that sialylation of Ssp-3 epitope of mammalian cell-derived trypomastigotes is required for target cell recognition, Yoshida et al. (1997) reported that the removal of Sia from the surface of insect-derived metacyclic trypomastigotes enhances parasite-host interaction. The removal of Sia from T. cruzi glycoproteins and the concomitant exposure of cryptic β-Galp residues would favor TcTSY342H interaction with both host sialoglycoconjugates and terminal β-Galp-containing glycoproteins on the parasite surface, thus enhancing T. cruzi/host adhesion. This phenomenon was well characterized for CD22, a mammalian Sia-binding lectin (Varki and Gagneux, 2012). The removal of Sia and concomitant exposure of β-Galp residues from host cell glycans, which occurs as a result of the T. cruzi TS reaction may, therefore, be physiologically significant by promoting parasite adherence to, and penetration of host cells.

On the other hand, the parasite might use the active TS to sialylate host cell glycomolecules and generate receptors for TcTSY342H mediating trypanosome adherence to a target cells. Data showing that sialic acid-deficient cells are less infected than wild-type cells...
Activation of endothelial cells increases trypomastigotes attachment to gastric mucin and establishes a host infection in the Oximmuno-03-00356—2012/11/29—12:47—page 6—#6
TcTS active site succeeding the sialic acid donor is necessary for the transfer reaction to proceed (Hazelhurst et al., 2004). Results of TcTrylac incubated with 2-α,6-sialylactose in the presence of lacto-N-tetraose, showing that incorrect fitting of sialoside into the binding site of TS does not trigger β-Gal binding, corroborate this hypothesis. Furthermore, surface plasmon resonance results showing that lactose binds to an inactive mutant of TSsuggest in the presence of α2-3-sialylactose (Buschiazio et al., 2002).

This discussion shows that further structural data are needed to shed light into the reaction mechanism that underlies efficient sugar transfer activity rather than simple hydrolysis by TcTS. Given that genomic analysis suggests that TcTS proteins have several point mutations (Fretas et al., 2011), structural and mechanistic works must be persistent, as mutations in key amino acids (Paris et al., 2001; Carvalho et al., 2010a) would produce critical modifications in TcTS catalysis and specificity.

**INHIBITION OF SIALIC ACID TRANSFERRENCY BY TcTS**

Together, the above observations support the hypothesis that TcTS enhances T. cruzi virulence by altering host immune responses directed against the parasite. The fact that TcTS presents low homology with mammalian sialidases, and that it is the lonely protagonist for sialic acid acquisition by T. cruzi, provide a rationale for a new potential intervention strategy in chemotherapy of Chagas’ disease. Beyond the urgency of alternative drugs to treat the illness, to pursuit of TcTS inhibitors has been the target of several research groups that aim to clarify the role of TcTS in the pathogenesis of Chagas’ disease. However, compounds that effectively inhibit the catalytic activity of TcTS have not been described. The advances made in this field were somehow indirect, relying on few strategies like the use of neutralizing antibodies, given that the siRNA mechanism of gene silencing in T. cruzi -sp. -tetraose, showing that incorrect fitting of sialoside into the binding site of TS does not trigger β-Gal binding, corroborate this hypothesis. Furthermore, surface plasmon resonance results showing that lactose binds to an inactive mutant of TSsuggest in the presence of α2-3-sialylactose (Buschiazio et al., 2002).

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### Donor substrate analogues

| Compound | IC₅₀ (μM) | Notes |
|----------|-----------|-------|
| (1) GlcNAc | 0.25 | 92% TcTS inhibition at 1 μM (Woessner et al., 2006) |
| (2) Neuraminic acid (GalNAc-O-propyl) | 9.2 | More than 1000-fold decrease in enzymatic reaction by lactose (Buchini et al., 2006) |
| (3) 2,3-dideoxy-2,3-dehydroribose | 2.4 | Complete TcTS inactivation at 20 μM. Glycanspecific recovery upon removal of sialic acid. (Mehta et al., 2003; Walls et al., 2006) |
| (4) Neuraminic acid (GalNAc-O-propyl) | 9.2 | More than 1000-fold decrease in enzymatic reaction by lactose (Buchini et al., 2006) |

### Acceptor substrate analogues

| Compound | IC₅₀ (μM) | Notes |
|----------|-----------|-------|
| (9) Leukosialin | 0.01 | 92% TcTS inhibition at 1 μM (Harrington et al., 2007) |
| (12) Triacetyl galactosamine | 9.2 | Complete TcTS inhibition at 20 μM (Anjum et al., 2004) |
| (13) Sulfamethazine | 9.2 | Complete TcTS inhibition at 20 μM (Hann et al., 2007) |
| (14) Quinoline | 9.2 | Complete TcTS inhibition at 1 μM (Hann et al., 2007) |

### Unrelated substrate compounds

| Compound | IC₅₀ (μM) | Notes |
|----------|-----------|-------|
| (4) Isoquinoline | 0.52 | Complete TcTS inhibition at 1 μM (Hann et al., 2007) |
| (5) Anthraquinone | 9.2 | Complete TcTS inhibition at 1 μM (Hann et al., 2007) |
| (6) 8-Methoxsalen | 9.2 | Complete TcTS inhibition at 1 μM (Hann et al., 2007) |

**FIGURE 5** | Compounds tested as TcTS inhibitors.
triazole-substituted saccharides. Starting from galactose derivatives bearing an azide group at C1 or C6, a triazole-substituted saccharide library was made and tested against TcTS. Despite its low inhibitory activity against TcTS, the N-methyl benzylation derivative presented trypanocidal activity (Carvalho et al., 2010b).

Recently, a series of octyl galactosides and octyl N-acetyllactosamines were tested against TcTS. Results showed that the TcTS acceptor binding site is intolerant of substitution of β-Galp at positions 2 and 4, whereas substitution at position 6 of the Gal ring is well accepted, highlighting the potential of 6-substituted Gal residues as TcTS acceptor substrates (Harrison et al., 2011).

**RELATED SUBSTRATE COMPOUND (Figure 5)**

The 3-benzothiazol-2-yl-4-phenyl-but-3-enoic acid and sulfonamide scaffolds emerged, from virtual screening, as new framework for TcTS inhibition (Neres et al., 2009). Sulfonamides figured also as good substrates for chalcones used as TcTS inhibitors by Kim et al. (2009). Of the compounds tested, the tetrahydroxylated quinoline inhibited both hydrolytic and TcTS activities at millimolar concentration (Kim et al., 2009). Similar to chalcones, various flavonoids and anthraquinones were systematically screened from a large library. A highly hydroxylated anthraquinone was the best inhibitor of TcTS, with an IC_{50} of 0.58 μM (Arioka et al., 2010). Moreover, this compound did not inhibit Neu5a2, a mammalian neuraminidase, demonstrating that its inhibition is reasonably specific to TcTS (Arioka et al., 2010). Therefore, this last structure represents, to date, the most promising scaffold for TcTS inhibition.

Despite great advances made toward TcTS inhibition, works highlighting the enzyme’s plasticity (Demir and Roitberg, 2009; M( Arioka et al., 2010). Moreover, this compound did not inhibit Neu5a2, a mammalian neuraminidase, demonstrating that its inhibition is reasonably specific to TcTS (Arioka et al., 2010). Therefore, this last structure represents, to date, the most promising scaffold for a TcTS inhibitor. However, a series of octyl galactosides and octyl N-acetyllactosamines were tested against TcTS. Results showed that the TcTS acceptor binding site is intolerant of substitution of β-Galp at positions 2 and 4, whereas substitution at position 6 of the Gal ring is well accepted, highlighting the potential of 6-substituted Gal residues as TcTS acceptor substrates (Harrison et al., 2011).

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