The trans-sialidase, the major Trypanosoma cruzi virulence factor: Three decades of studies

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Received 2 June 2015; Revised 9 July 2015; Accepted 19 July 2015

Abstract

Chagas’ disease is a potentially life-threatening disease caused by the protozoan parasite Trypanosoma cruzi. Since the description of Chagas’ disease in 1909 extensive research has identified important events in the disease in order to understand the biochemical mechanism that modulates T. cruzi-host cell interactions and the ability of the parasite to ensure its survival in the infected host. Exactly 30 years ago, we presented evidence for the first time of a trans-sialidase activity in T. cruzi (T. cruzi-TS). This enzyme transfers sialic acid from the host glycoconjugates to the terminal β-galactopyranosyl residues of mucin-like molecules on the parasite’s cell surface. Thenceforth, many articles have provided convincing data showing that T. cruzi-TS is able to govern relevant mechanisms involved in the parasite’s survival in the mammalian host, such as invasion, escape from the phagolysosomal vacuole, differentiation, down-modulation of host immune responses, among others. The aim of this review is to cover the history of the discovery of T. cruzi-TS, as well as some well-documented biological effects encompassed by this parasite’s virulence factor, an enzyme with potential attributes to become a drug target against Chagas disease.

Key words: chagas disease, glycoimmunology, sialic acid, trans-sialidase, Trypanosoma cruzi

Sialic acid-containing molecules

Sialylated glycoconjugates decorate the surface of all mammalian cells. Given their ubiquitous presence and abundance, sialic acids (Sia) have many important biophysical effects (Crespo et al. 2009; Angata and Fukuda 2010; Varki and Gagneux 2012). Sia is a group of structurally diverse 9-carbon monosaccharides with heterocyclic ring structures (Varki and Schauer 2009). Sia bears a negative charge via a carboxylic acid group attached to the ring, as well as other chemical groups, including N-acetyl and N-glycolyl groups. The two main types of Sia found in mammals are N-acetyleneuraminic acid (NeuAc) and N-glycolyneuraminic acid (NeuGc) (Traving and Schauer 1998). Neu5Ac and its hydroxylated derivative Neu5Gc differ by one oxygen atom in the N-glycolyl group (Figure 1). These usually occur as terminal structures attached to β-galactopyranosyl (βGalp) residues at the non-reducing termini of both N- and O-linked glycans (Chen and Varki 2010). The typical cell exhibits tens of millions of Sia molecules, and it is estimated that the local concentration on the cell-surface glycocalyx can approach 100 mM (Varki and Gagneux 2012). Sia thus provides a large component of negative charge repulsion between cells, which could alter the biophysical properties of cellular interactions. Although Sia are the major cell-surface sugars in the Deuterostome lineage of animals, they are also found in other branches of life (Cohen and Varki 2010). It is important to point out that the “sialoglycophenotype” of many host cell types is exploited by numerous microorganisms, such as viruses (Neu et al. 2011), bacteria (Chang and Nizet 2014) and protozoa (Nishikawa et al. 2013). One of the most elegant mechanisms of cell-surface sialylation found in nature is the one utilized by parasite protozoan Trypanosoma cruzi (Previato et al. 1985).

In T. cruzi, Sia was first described in epimastigote forms by using lectins and colorimetric methods (Pereira et al. 1980). Further, the existence of Sia in the epimastigote form was confirmed by thin-layer
chromatography and gas-liquid chromatography coupled to mass spectrometry. Interestingly, the presence of Sia on the parasite cell surface was not detected when the epimastigote form was incubated with radioactive precursors of the biosynthetic pathway of Sia (Schauer et al. 1983), suggesting that an alternative mechanism for Sia acquisition was present in the parasite (Previato et al. 1985). Also in 1983, noting the ability of *T. cruzi* trypomastigotes to hydrolyze Sia from human erythrocytes and plasma glycoproteins, Pereira (1983) suggested that the parasite would express a stage-specific neuraminidase activity.

Thirty years ago our group described, for the first time, the *T. cruzi*’s ability to incorporate Sia from exogenous sialoglycoconjugates (Figure 2A). This innovative observation suggested that the incorporation of Sia onto the parasite cell surface did not occur by the conventional route; i.e. CMP-Neu5Ac as the sugar donor, but rather by a new metabolic route involving a trans-glycosylation reaction to Sia (Previato et al. 1985). Later, Sia incorporation into trypomastigote forms was demonstrated in vitro by Zingales and collaborators (Zingales et al. 1987) and then in vivo by Previato and colleagues (Previato et al. 1990). In the Previato study, it was shown that during the acute phase of Chagas disease in mice, the parasite’s trypomastigote forms incorporate Neu5Gc onto its own cell-surface glycoconjugates. Finally, the trans-glycosylase activity for Sia was characterized as *T. cruzi*-TS, which is specific for alpha(2→3) Sia and is responsible for the transfer of Sia from the host’s sialoglycoconjugates to acceptor mucin-like molecules expressed on the parasite cell surface (Schenkman et al. 1991; Parodi et al. 1992; Mendonça-Previo et al. 2013). Following the identification of the gene coding for both neuraminidase (Pereira et al. 1991) and *T. cruzi*-TS (Parodi et al. 1992; Uemura et al. 1992), it became clear that both activities were related to the same enzyme. These pioneering studies were critical to elucidate the biochemical parameters of *T. cruzi*-TS, as well as to understand how this Sia-dependent enzyme might be able to act as a virulence factor to ensure parasite survival in infected hosts. The next sections address the significance of this biological system, covered by both *T. cruzi*-TS and molecules containing alpha(2→3)-linked Sia. In addition, we will examine how the parasite exploits both normal and unusual sialoglycophenotypes of host cells.

**trans-Sialidase: a major virulence factor released to the extracellular milieu during the early stages of T. cruzi infection**

Several studies have shown that the trypomastigote form of *T. cruzi* sheds proteins which are able to modulate the host immune response during the early stages of infection (DosReis 2011). Amongst all known molecules, TS proteins have been considered as a major virulence factor, either by their capability to dampen host cell immunity or by its ability to mediate the interaction between the parasite and host cells (Frasch 1994; Chuenkova and Pereira 1995; Mendonça-Previo et al. 2010; Buschiazzo et al. 2012; Mendonça-Previo et al. 2013). It
is a well established fact that in trypomastigote forms of *T. cruzi*, TS is a GPI-anchored membrane protein, which is dynamically released to the extracellular milieu. This leads to a systemic distribution of the enzyme through the bloodstream (Figure 2B). The half-life of TS proteins in the bloodstream is considerably extended due to the occurrence of a C-terminal repetitive domain termed Shed Acute Phase Antigen (SAPA) (Alvarez et al. 2004). SAPA is believed to be an evolutionary strategy adopted by the parasite to prevent the early production of antibodies against the N-terminal domain, which is responsible for its catalytic activity (Ribeirao et al. 1997). Over the last few years, many studies have shed light on the relationship between TS proteins and numerous pathologies observed during the early stages of *T. cruzi* infection. The administration of recombinant TS in non-infected mice is capable of inducing apoptosis in thymocytes (Mucci et al. 2002) and mature T cells (Mucci et al. 2005) and causes lowering of platelet numbers (Tribulatti et al. 2005). In addition, studies from several groups have shown that the administration of recombinant TS is able to modulate the functionality of immune cells, such as T and B lymphocytes (Chuenkova and Pereira 1995; Gao and Pereira 2001; Gao et al. 2002; Freire-de-Lima et al. 2010; Bermejo et al. 2013; Ruiz Diaz et al. 2015). Interestingly, some of these events are abrogated by the passive transfer of TS-neutralizing antibodies (Tribulatti et al. 2005; Buschiazzo et al. 2012), reinforcing the possibility that increased shedding of the enzyme correlates with the increased virulence of corresponding parasite strains (Risso et al. 2004).

**Parasite-host cell contact**

*Trypanosoma cruzi* trypomastigotes are very promiscuous. Excluding the red blood cells, the parasite is able to invade any kind of nucleated cells, e.g., phagocytic and non-phagocytic cells. Host cell invasion involves multiple steps, one of which involves primary contact between parasite surface molecules and host cell glycoconjugates (Caradonna and Burleigh 2011; Barrias et al. 2013; de Souza and de Carvalho 2013). Although the enzymatic activity displayed by TS was proposed almost 30 years ago as a key factor for *T. cruzi* pathogenesis (Previo et al. 1985), it is now known that enzymatically inactive TS (iT5) may play important roles in the biology of the parasite (Todeschini et al. 2002a). In 1995, studies carried out by Cremona and colleagues demonstrated that the difference between active TS (aTS) and iT5 was restricted to a single amino acid. While aTS has a tyrosine residue at position 342 (Tyr342), iT5 displays a histidine residue (His342) in the same position. This naturally occurring Tyr342 → His substitution was enough to entirely abolish the *T. cruzi*-TS activity (Cremona et al. 1995). The importance of the adhesin property of iT5 initially was demonstrated in our laboratory when we reported that iT5 binds sialyl and β-Galp residues in a sequential-ordered mechanism (Todeschini et al. 2004). This finding could have important implications in the life cycle of the parasite, since inactive members of TS proteins may play key roles in *T. cruzi*-host cell interactions.

The significance of Sia-containing glycoconjugates on cell communication and invasion by *T. cruzi* was proposed several years ago following the demonstration that the invasion of Sia-deficient cells (CHO-Lec 2) was reduced when compared with wild-type cells (Schenkman et al. 1993). In addition, studies from our group involving the use of a *Vibrio cholerae* neuraminidase suicide-type inhibitor (Hinou et al. 2003) reinforced the importance of Sia on parasite-host cell communication. Besides inhibiting both neuraminidase and TS activities, in a time- and dose-dependent fashion, the inhibitor was also able to reduce the level of infection in cultured mammalian cells (Carvalho et al. 2010). In fact, some compounds have been synthesized and tested as specific inactivators (Buchini et al. 2008) or inhibitors (Lieke et al. 2011) of *T. cruzi*-TS activity, aiming towards the discovery of new agents for the cure of Chagas’ disease.

Given the importance of sialidases and TS as virulence factors in several infections (Frasch 2000; Wiggins et al. 2001; Hedlund et al. 2010; De-Rubin and Schenkmam 2012), Frasch and collaborators carried out crucial works on the 3D structure and mechanistic properties of the *T. rangeli* sialidase (Buschiazzo et al. 2000) and *T. cruzi*-TS activities (Buschiazzo et al. 2002). The comparison between the structural domains of these enzymes was fundamental to determine the catalytic mechanism of *T. cruzi*-TS (Watts et al. 2003; Amaya et al. 2004). At the same time, the construction and analysis of TS mutants have defined the amino acid residues relevant for the trans-sialidase activity (Paris et al. 2005). These findings are relevant to the rational design of inhibitors of *T. cruzi*-TS. However, to this date, an effective inhibitor is yet to be identified.

Exhaustive studies carried out by Pereira-Perrin’s group have shown that Sia-containing molecules may also exert control on cell signaling mediated by tyrosine kinase receptor-A (TrkA) (Chuenkova and Pereira-Perrin 2004, 2005; de Melo-Jorge and Pereira-Perrin 2007; Ardigides et al. 2013). In these studies it was clearly demonstrated that de-sialylation of TrkA by *T. cruzi*-TS leads to the rapid receptor internalization, activation and neuronal differentiation (Woronowicz et al. 2004). The authors suggested that such enzymatic activity may participate in the neural repair and neuroprotection mediated by the *T. cruzi*-TS, which is also known as parasite-derived neurotrophic factor (Chuenkova and Pereira-Perrin 2011). On the other hand, studies published by Rubin-de-Celis and colleagues suggested that Sia content, which is incorporated by *T. cruzi*-TS is not a limiting factor for host cell invasion, since trypomastigote and both control and TS-transfected metacyclic forms showed equal efficiency in mammalian cell invasion (Rubin-de-Celis et al. 2006).

Recently, Butler and colleagues demonstrated that the interaction of TS proteins with host sialoglycans was able to generate an “eat me” signal in epithelial cells, an event that may depend on G protein activation. Epithelium represents a natural barrier to infection, and the cells that compose them are considered not likely to be involved in phagocytosis of large bodies such as parasites. These authors speculated that the action of *T. cruzi*-TS on sialylated epitopes might facilitate the parasite entry into non-phagocytic cells (Butler et al. 2013). The examples described in this section strongly suggest that *T. cruzi* exploits the host cell sialoglycophenotype to set up parasite-host cell communication, which is essential for a successful infection.

**Trypanosoma cruzi-TS modulates host immune system**

As mentioned above, *T. cruzi* does not synthesize Sia. The parasite acquires Sia from host sialoconjugates, through the trans-glycosylation reaction catalyzed by *T. cruzi*-TS (Previato et al. 1985). Since Sia plays essential functions on both innate and adaptive immunity (Amon et al. 2014; Chen et al. 2014) it is not surprising that TS proteins are able to dampen the host’s immune response. Over the last years, several papers have demonstrated that TS proteins might act on distant sites from the parasite as a soluble factor. Since its discovery, many laboratories have tried to elucidate how this Sia-dependent parasitic enzyme can modulate the host immunity in order to ensure the parasite’s survival in the infected host. Initial studies reported by Chuenkova and Pereira (1995) demonstrated sensitization of mice with minute amounts of the native TS prior to infection with *T. cruzi* led to increased parasitemia and mortality. It is important to
note that such effects are specific for the transferase activity of TS, since the results could not be replicated in mice primed with viral or bacterial sialidases. The mechanisms responsible for those effects were not determined. However, since TS injection into severe combined immunodeficiency mice did not affect parasitemia or mortality, it was proposed that TS may act on host lymphocytes which are essential components of the acquired immune system (Chuenkova and Pereira 1995).

Thymocyte and T-cell apoptosis are a common feature in acute parasite infections (Begum-Haque et al. 2009; Francelin et al. 2011). In the majority of infectious diseases with thymic atrophy, the most important biological event associated with thymocyte loss is the cell death by apoptosis. This is also observed in experimental Chagas disease (Savino 2006; Morrot et al. 2012). Several articles published by Campetella’s group have pointed out the pro-apoptotic effects of aTS both on thymocytes and mature T cells (Leguizamon et al. 1999; Mucci et al. 2002; Tribulatti et al. 2007). Several hematological alterations may be the result of desialylation by the neuraminidase activity of T. cruzi-TS (Pereira 1983; de Titto and Araujo 1988; Tribulatti et al. 2005). However, further studies regarding this aspect have proposed that the pro-apoptotic effect of T. cruzi-TS on immature and mature T cells may be induced by the incorporation of sialyl residues onto the cell surface that is mediated by its trans-sialidase activity (Mucci et al. 2006). It has also been shown that there is a correlation between apoptotic cell death and an imbalance in the hypothalamus-pituitary-adrenal of T. cruzi-infected mice and GH3 cells. This later effect correlates with an increased production of glucocorticoids and a reduction in prolactin levels (Lepletier et al. 2012).

In 2002, our group demonstrated that T. cruzi-TS was able to activate CD4+ T cells through engagement of CD43 (Todeschini et al. 2002b), an integral membrane glycoprotein typically expressed at high levels on all leucocytes, except most resting B lymphocytes (Ostberg et al. 1998). To promote such activation, it was necessary to combine T. cruzi-TS with anti-CD3 or anti-TCR monoclonal antibodies. By itself T. cruzi-TS was not able to induce the activation of mouse purified CD4+ T cells in vitro (Todeschini et al. 2002b).

T-cell activation is accompanied by loss of Sia from core 1 O-glycans (Siaα2,3Galβ1,4GlcNAc-Ser/Thr), leading to enhanced expression of asialylated Galβ1,3GlcNAc glycan species which results in the induction of core 2 O-linked glycans (Galvan et al. 1998; Priatel et al. 2000). The exposed asialylated core 1 O-glycans on activated T cells may be detected with peanut agglutinin (PNA), a plant lectin that recognizes Galβ1,3GlcNAc sequences on several glycoproteins, including CD43 and CD45 (Wu et al. 1996). Since glycan structures bearing terminal βGalp units are potential substrates for T. cruzi-TS, it is plausible that T. cruzi-TS would be able to re-sialylate cell-surface asialoglycoconjugates in activated T cells (showing a PNA-high phenotype), and therefore modulate crucial physiological events, such as proliferation, cytotoxic activity and T-cell half-life. On the other hand, in T cells from non-infected mice, which express a PNA-low glyco phenotype, it is more likely that a TS exerts its neuraminidase activity. In this scenario, we would expect a stronger cell-to-cell interaction between desialylated non-activated T cells, an episode able to induce cell death triggered through the Fas-FasL pathway (Azuma et al. 2000; Tringali et al. 2007; Ghirici et al. 2013). In addition, asialoglycans generated by the hydrolytic action of aTS may be a potential target for the action of pro-apoptotic proteins belonging to the galec tin family (Brandt et al. 2010; Lee et al. 2013). Although it is a reasonable speculation, such hypothesis still needs confirmation.

Since glycoconjugates bearing terminal Sia residues promote cell-cell repulsion, it is reasonable to suggest that the PNA-high glyco phenotype adopted by activated T cells may favor building an effective immune response since Sia reduces the binding between CD8+ T and class MHC I. This is supported by data showing that cells from ST3GAL1 −/− mice show increased CD8-MHC I binding when compared with wild type (Moody et al. 2001). Besides the polyclonal activation of T cells and the massive apoptosis of immature and mature T cells, hypergammaglobulinaemia is also a typical event in the acute phase of Chagas disease (el Bouhidi et al. 1994). In 2002, Gao and colleagues demonstrated that T. cruzi-TS, through its C-terminal (long tandem repeat) domain (SAPA), is capable of activating mouse B cells in a T-cell-independent manner. The SAPA as well as the whole enzyme is capable of inducing IL-6 (in bone marrow and splenocytes) and γ-FN (in splenocytes). These authors were also able to correlate this T-cell-independent B-cell activation with polyclonal Ig secretion in vivo, which could explain, in part, its virulence-enhancing activity (Gao et al. 2002).

In a recent study, Bermejo and colleagues were able to show that T. cruzi-TS is capable of inducing the production of IL-17 by B cells in both murine and human models. The effect was dependent on the sialylation of molecules on the cell surface, particularly CD45 (or another molecule that interacts with it), since its presence is essential for IL-17 production. It is also worth noting that TS triggers a different signaling pathway from the one classically associate with IL-17 and bypasses the activation of RORγt- and RORα. (Bermejo et al. 2013).

### Sialoglycophenotypes adopted by CD8+ T cells during T. cruzi infection

As cited above, the multifunctional properties of T. cruzi-TS are essential for its ability to modulate not only the sialoglycophenotype of the parasite but also of host cells. The fact that the sialylation of T. cruzi’s surface mucin-like molecules enhances its invasive capacity is no longer a question (Burleigh and Andrews 1993; de Souza et al. 2010). TS allows the protozoan to avoid immune responses through molecular mimicry, making it possible for T. cruzi to escape from the antibodies and proteins of the complement system directed against the parasite’s epitopes (Argibay et al. 2002; Gao et al. 2002).

Several studies have confirmed the significance of differential sialylation for CD8+ T cells with respect to their development (Moody et al. 2001), activation via the TCR (Pappu and Shrikant 2004), and cytotoxic responses (Sadighi Akha et al. 2006). However, so far few studies have provided insights into the impact of surface sialylation of CD8+ T cells on the responses to pathogenic microorganisms. Infection with T. cruzi is of particular interest in this context because the parasite releases into the host plasma large amounts of aTS (Alcantara-Neves and Pontes-de-Carvalho 1995). Recently, we demonstrated that in a T. cruzi-TS-free system, such as the Plasmodium vivax infection, the activated CD8+ T cells predominantly exhibit the glyco phenotype PNA-low. However, in T. cruzi-infected mice, the glyco phenotype PNA of CD8+ T cells can be converted into PNA-high after intravenous administration of aTS. Since the Ag-specific CD8+ T cell responses in T. cruzi-infected and TS-treated mice were impaired when compared with the T. cruzi-infected and TS-untreated group, it is reasonable to suggest that atypical acquired sialoglycophenotype (PNA-high) may contribute to increased parasite half-life in the infected host (Freire-de-Lima et al. 2010). Further studies are necessary to confirm that hypothesis, but it is plausible to speculate that the manipulation of Sia by T. cruzi-TS on CD8+ T cells from T. cruzi-infected individuals might be an evasion mechanism employed by the parasite to assure its replication in the infected host cells. Figure 3 illustrates a hypothetical molecular
mechanism of how *T. cruzi*-TS might dampen the building of an effective CD8+ T cell response.

**Conclusion and perspectives**

In this review, we have focused on the history of the discovery of *T. cruzi*-TS, as well as how this Sia-dependent enzyme might be able to interfere in many biological parameters to guarantee the persistence of the parasite in the infected host. Over the last 30 years, there have been significant efforts directed towards understanding the relevance of TS on the biology of *T. cruzi*. However, the lack of TS-specific inhibitors and TS knockout strains has hampered progress in the area. Although hundreds of convincing works reinforce the idea that *T. cruzi*-TS acts as a virulence factor during the acute phase of Chagas disease, there are still open questions that need to be addressed, such as the number and the genomic organization of the genes encoding iTS and aTS in different parasite strains (Burgos et al. 2013). Another issue that requires further investigation is the impact of the altered sialoglycophenotype of cells belonging to the host immune system. Since the blood-trypanomastigote releases significant quantities of TS into the host plasma, infection with *T. cruzi* is of particular interest in this context.

Some works have proposed that in B and T lymphocytes, cell-surface glycoproteins such as CD43 and/or CD45 may act as potential acceptors for TS activity (Todeschini et al. 2002a; Freire-de-Lima et al. 2010; Muia et al. 2010; Bermejo et al. 2013). The assumption is that such a phenomenon is able to disturb the content of Sia on those cells which may enable the parasite to evade the host immune response. In vitro and in vivo experiments suggest that CD43 is a possible ligand/acceptor molecule for *T. cruzi*-TS. Initial studies revealed that the parasite’s enzyme is able to costimulate host T cells through leucosialin (CD43) engagement (Todeschini et al. 2002a). Further, Freire-de-Lima et al. (2010) showed in vivo by using ST3Gal-I KO mice that CD43 may act as a potential Sia acceptor in activated CD8+ T cells. CD43 is another glycoprotein expressed in all leukocytes that has been identified as a possible target for the parasite’s enzyme. In 2010, Muia and colleagues demonstrated by using chemical tags in Sia to show that different isoforms of CD45 may act as possible Sia acceptors for *T. cruzi*-TS. More recently, Bermejo et al. (2013) showed that CD45 is essential for the activation and production of IL-17 by B cells. Although this is a very interesting hypothesis with some supporting preliminary evidence, further studies with CD43 KO and CD45 KO mice are necessary to confirm this possibility. On a different note, since *T. cruzi*-TS plays such an important role in the parasite’s biology, TS is a tempting target for the development of new therapeutic agents. There are several groups investigating new alternatives to replace the highly toxic drugs currently used in the treatment the Chagas disease. Over the last 10 years, many potential inhibitors of TS have been described. However, due to no specificity for the parasite’s enzyme, many of them have yet to be tested in tissue-cultured cells or animal models. Because TS is such an appealing target for drug design and therapeutic intervention in Chagas’ disease substantial efforts in this area will, without doubt, increase in the coming years.

**Funding**

This work was supported by the Fundação de Apoio à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

**Conflict of interest statement**

None declared.

**Abbreviations**

aTS, active TS; iTS, inactive TS; NeuAc, N-acetyleneuraminic acid; NeuGc, N-glycolylneuraminic acid; PNA, peanut agglutinin; SAPA, Shed Acute Phase Antigen; Sia, sialic acids; TrkA, tyrosine kinase receptor-A; βGalp, β-galactopyranosyl.


