**trans-Sialidase from *Trypanosoma cruzi* Binds Host T-lymphocytes in a Lectin Manner**

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*Trypanosoma cruzi*, the protozoan parasite responsible for Chagas’ disease, expresses on its surface an uncommon membrane-bound sialidase, known as *trans*-sialidase. *trans*-Sialidase is the product of a multigene family encoding both active and inactive proteins. We report here that an inactive mutant of *trans*-sialidase physically interacts with CD4⁺ T cells. Using a combination of flow cytometry and immunoprecipitation techniques, we identified the sialomucin CD43 as a counter-receptor for *trans*-sialidase on CD4⁺ T cells. Using biochemical, immunological, and spectroscopic approaches, we demonstrated that the inactive *trans*-sialidase is a sialic acid-binding protein displaying the same specificity required by active *trans*-sialidase. Taken together, these results suggest that inactive members of the *trans*-sialidase family can physically interact with sialic acid-containing molecules on host cells and could play a role in host cell/T. cruzi interaction.

The surface of the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas’ disease (American Trypanosomiasis), displays a unique enzyme known as *trans*-sialidase (TS) (1, 2). TS is a modified sialidase (3) sharing the catalytic mechanism (4) and the active site architecture (5) with other known sialidases (6–8). However, instead of releasing sialic acid, TS preferentially transfers sialic acid from β-galactopyranosyl (βGalp)-containing exogenous donor molecules to terminal βGalp-containing acceptors, attaching it in an α2–3 linkage configuration (9). *T. cruzi* TS belongs to a large family of proteins (10, 11), and several other members of this family, which lack enzymatic activity (11), are expressed. Comparison of the deduced amino acid sequences shows that enzymatic activity requires a Tyr at position 342, whereas inactive members contain a His at the same position (11). The Tyr³⁴² residue is involved in the stabilization of the transition-state sialyl carbocation formed during the hydrolysis reaction (4, 5). Indirect evidence suggests that an enzymatically inactive recombinant TS acts as a lectin, agglutinating desialylated erythrocytes (12). However, no direct evidence for a βGalp binding site or for its role in the parasite host interaction has been established.

Trypomastigote-derived TS is anchored to the membrane through a glycosylphosphatidylinositol anchor and is released to the extracellular medium during acute *T. cruzi* infection in humans (13), thus acting distant from the parasite. Besides a role in mammalian cell invasion (14), the soluble TS functions as a virulence determinant molecule. Chuenkova and Pereira (15) demonstrated that *in vivo* injection of minute amounts of purified native TS increases subsequent parasitemia and mortality in *T. cruzi*-infected mice. As TS injection into severe combined immunodeficiency mice did not affect parasitemia or mortality, it was suggested that TS acts on the host adaptive immune response (15). It is well known that T lymphocytes bearing conventional αβ T cell receptors are required for control of parasitemia and mortality in murine infection by *T. cruzi* (16). Host CD4⁺ T cells are also involved in the immunopathology of *T. cruzi* infection, and their exacerbated function can lead to mortality in susceptible hosts (17). Recently, we demonstrated that both enzymatically active and inactive TS costimulated CD4⁺ T cell activation *in vitro* and *in vivo* and blocked activation-induced cell death in CD4⁺ T cells from *T. cruzi*-infected mice through CD43 engagement (18). In the present work, we extended our studies and, using CD4³⁻/⁻ mice, we show that the major lymphocyte mucin CD43 is the counterreceptor for the inactive TS on host CD4⁺ T cells. We also employed NMR spectroscopy and immunochromatographic approaches to investigate the nature of the CD43 epitope that functions as ligand for TS, and we demonstrated that inactive TS binds to sialic acid-containing molecules with the same specificity exhibited by active TS.

**EXPERIMENTAL PROCEDURES**

**Materials**—Most of the chemical products used were from Sigma or Fisher. The following materials were obtained from other sources: microtiter plates were from Nunc; protein G-Sepharose, prepacked Ni²⁺-chelating HP HiTrap, Mono Q HR 10/10 and Mono S HR 5/5 columns; enhanced chemiluminescence (ECL) hyperfilm were from Amersham Biosciences; biotin-conjugated polyacrylamide (PAA) probes substituted with sialylated glycans (α2–3-sialyllactose-PAA (α2–3-SL-}

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PAA) were obtained from a sialidase from *Escherichia coli* MC1061 electro-transformed with plasmids containing either the wild-type TS insert, TSREP (11), or the inactive mutant TS insert, and grown in supplemented Terrific broth in the presence of 100 μg/ml leupeptin, 0.1 μg/ml trypsin inhibitor, and 0.1 μg/ml isocyanatide. Both rTS and irTS containing a poly-His tag were purified as described by Buschiazzo et al. (19) and modified by Todeschini et al. (4) using Ni²⁺-nichel chromatography on a HiTrap column and eluted with an imidazole gradient (0–1 M). The eluates were dialyzed against 20 mM Tris-HCl, pH 7.6, further purified by ion exchange chromatography on Mono Q and Mono S columns, applying a linear NaCl gradient (0–1 M), and stored in 20 mM Tris-HCl buffer, pH 7.6. The homogenous proteins were reacted with 10% SDS-PAGE. For flow cytometry (FCM) and Western blotting analyses, rTS was biotin-conjugated as described previously (21).

**rTS Activity Assay**—Enzyme activity was assayed by incubating rTS preparations in 5 mM cocadylate buffer, pH 7.0, in the presence of 0.25 μmol of α-2-3-sialyllactose (α2-3-SL) and 0.25 μmol of [α-glucose-1-14C]lactose (400,000 cpm/μl). After incubation for 30 min at 37°C, the reaction mixture was diluted with 1 ml of water and applied to a column containing 1 ml of Dowex 2X8 (acetate form) equilibrated with water.

To remove the excess of [α-glucose-1-14C]lactose, the column was washed with 10 ml of water, and sialylated [α-glucose-1-14C]lactose was eluted with 3 ml of 0.8 M ammonium acetate and quantitated by scintillation counting (Beckman LS 6500). One unit was defined as the amount of rTS required to catalyze the incorporation of 1 μmol of sialic acid into lactose per minute.

**Animals and T Lymphocytes**—BALB/c mice (male, aged 4–5 weeks) were obtained from Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; CD4⁻/⁻ and wild-type control mice (22) were from Universidade Federal de São Paulo, São Paulo, Brazil, animal facilities. All experiments were performed according to protocols approved by the Committee on Ethics and Regulations of Animal Use of Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brazil.

**Primary T cell-enriched suspensions** were obtained by nylon-wool filtration of fractionated splenocytes depleted of red cells by treatment with Tris-buffered ammonium chloride (23). Purified CD4⁺ T cells were nylon-nonadherent cells treated with anti-CD8 mAb for 30 min at 4°C followed by anti-rat Ig κ chain mAb MAR 18.5 plus 10% rabbit complement for 45 min at 37°C.

**Immunoprecipitation and Western Blotting**—1 × 10⁶ CD4⁺ T cells were lysed in PBS containing 1 μmol phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 0.1 μg/ml aprotinin, 1 μg/ml trypsin inhibitor, and 0.1% Triton X-100 at 4°C under vigorous shaking (10,000 × g for 30 min), the supernatant was preincubated overnight at 4°C with 100 μl of protein G-Sepharose beads and centrifuged, and the supernatant was incubated with 3 μg of anti-CD43 mAb M7 for 2 h at 4°C, under shaking. Protein G-Sepharose (50 μl) was added and incubated for 3 h. The beads were separated by centrifugation, washed three times, and incubated with SDS sample buffer for 5 min at 100°C. The supernatant was run on 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked overnight with 2% bovine serum albumin in Tris-buffered saline containing 0.2% Tween 20, incubated with anti-CD43 mAb M7 for 2 h followed by incubation with horseradish peroxidase-conjugated anti-mouse IgG. The reaction was detected using enhanced chemiluminescence (ECL) in Hyperfilm-ECL according to the manufacturer’s instructions. Western blotting, streptavidin beads and biotin-conjugated irTS were used.

**Desialylation and Resialylation of T Lymphocytes**—1 × 10⁶ T cells were resuspended in serum-free Dulbecco’s modified Eagle’s medium and incubated for 30 min at 37°C with 0.05 units of *Clostridium perfringens* (Type X) sialidase in a total volume of 500 μl. Sialidase was removed by washing three times with serum-free Dulbecco’s modified Eagle’s medium. Desialylated T cells (5 × 10⁵) were resialylated by incubation with 0.05 units of recombinant irTS in the presence of 1 μg α2-3-SL for 30 min at room temperature and washed as described above. Desialylated and resialylated T lymphocytes were resuspended in sorting buffer (10 μM PBS, pH 7.4, containing 2% bovine serum albumin, 0.02% NaN₃, and 0.1% lactate) incubated with biotin-conjugated irTS for 30 min at 4°C. The T cells were then washed (3 times with 20 μM PBS containing 1 μg/ml biotin-conjugated irTS for 30 min at 4°C, washed again, and resuspended in 0.4 ml of sorting buffer plus 2% paraformaldehyde. T lymphocytes were gated by forward scatter and side scatter parameters, and 10,000 cells were analyzed on a fluorescence-activated cell sorter Xcalibur system using Cell Quest software. For irTS inhibition assay, biotin-conjugated irTS was preincubated with α2-3- or α2-6-SL in a range of 0–1 μg for 30 min at 4°C. ELISA Analysis of irTS Binding to Sialic Acid-containing Molecules—Analysis of irTS binding to sialic acid-containing molecules was done by ELISA (24). Wells in microtiter plates were coated overnight at 4°C with a monoclonal antibody against the TS repeats (25) (500 ng/ml) in 50 mM carbonate/bicarbonate buffer, pH 9.5. The plates were washed with ELISA buffer (3% bovine serum albumin in PBS, pH 7.4) and incubated overnight at 4°C with irTS (500 ng/ml) in the same buffer. Plates were blocked with ELISA buffer containing Triton X-100 1% for 1 h at room temperature and subsequently washed. Wells were then incubated with biotin-conjugated PAA probes substituted with sialylated glycans at a final concentration of 5.0 μg/ml or a range between 0.25 and 10 μg/ml for 2 h at room temperature. Following washing, wells were incubated for 1 h at room temperature with peroxidase-conjugated streptavidin, diluted (1:500) in blocking buffer, and developed with the 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diamonium substrate system (100 μl/well). The plates were read at 405 nm in an automatic microplate reader (Bio-Tek Instruments). Control was carried out by a polyclonal antibody against TS catalytic domain (irTS) (25) (500 ng/ml) for 1 h. Both mono- and polyclonal antibodies were generously supplied by Dr. Mauricio M. Rodrigues from the Universidade Federal de São Paulo, São Paulo, Brazil.

**Carboxyl Reduction of Sialylated PAA Probes by Iodoethane and Sodium Borohydride Treatment**—Sialylated PAA probes were carboxyl-reduced with sodium borohydride treatment after esterification with iodoethane (24). Briefly, 100 μg of lyophilized sialylated PAA probes was solubilized in 350 μl of dimethyl sulfoxide, esterified with 35 μl of CH₃OH₄ for 1 h at room temperature, and then reduced by addition of 0.115 μl of PBS buffer containing 10 μM NaBH₄. The reaction products were diluted 4× with ELISA buffer and directly used in the assay. For sham treatment, the same procedures were performed without adding CH₃OH₄.

**Analysis of Relative Biotinylation Level of Sialylated PAA Probes**—Microtiter plates were coated with biotinylated PAA probes (200 μg/ml) overnight at 4°C in 50 mM carbonate/bicarbonate buffer, pH 9.5. After blocking with ELISA buffer and subsequent washing, the wells were incubated for 1 h at room temperature with peroxidase-conjugated streptavidin diluted 1:500 times and developed with 100 μl/well 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium substrate system. The plates were read at 405 nm in an automated microplate reader.

**NMR Experiments**—α2-3-SL, α2-6-SL, SLeX, or methyl α-mannoside was dissolved in deuterated PBS, pH 7.6 (not corrected for isotope effects). irTS solution in 20 mM Tris-HCl was exchanged with deuterated PBS by gel filtration on a G25 column. 20 μl of a stock solution containing 10 mg/ml of irTS was added to a solution of sialyl glycosides in PBS (20 μM for 30 min with PE-conjugated irTS). NMR spectra were obtained at a probe temperature of 20°C on a Bruker DME 600 with a 5-mm self-shielded gradient triple resonance probe or on a Bruker DRX 600 with a 5-mm triple resonance probe.
One-dimensional Saturation Transfer Difference (One-dimensional STD)—One-dimensional STD experiments were performed by low power presaturation of the methyl region of the protein during the 2-s relaxation delay. The pulse scheme was as follows: relaxation delay with or without presaturation of the protein resonances, 90 degrees pulse, and acquisition of 256 scans (16,000 points for 10 ppm of spectral width). The data were obtained with interleaved acquisition of on-resonance and control spectra to minimize the effects of temperature instability.

Saturation Transfer Difference—Total Correlation Spectroscopy (STD-TOCSY) spectra were recorded with a mixing time of 66 ms, 32 scans per t1 increment. 200 t1 increments were collected in an interfaced mode with presaturation on or off for 2 s. Prior to subtraction, both spectra were processed and phased identically. The acquisition time for the two-dimensional experiments was typically 16 h. The spectra were processed and phased identically. The acquisition time for the two-dimensional experiments was typically 16 h. The spectra were processed and phased identically. The data were obtained with interleaved acquisition of one-dimensional STD experiments were performed by low power presaturation of the methyl region of the protein during the 2-s relaxation delay. The pulse scheme was as follows: relaxation delay with or without presaturation of the protein resonances, 90 degrees pulse, and acquisition of 256 scans (16,000 points for 10 ppm of spectral width). The data were obtained with interleaved acquisition of on-resonance and control spectra to minimize the effects of temperature and magnet instability.

RESULTS

irTS Binds CD43 on T Cells—We recently demonstrated that irTS from T. cruzi binds CD4+ T cells and that this binding is abrogated by prior treatment with anti-CD43 mAb S7 (18). To prove that CD43 is the counterreceptor for TS on CD4+ T cells, we investigated the interaction between irTS and the leukosialin (CD43), using splenic CD4+ T cells from CD43-deficient mice (CD43−/−). As compared with wild type, CD4+ T cells from CD43−/− mice failed to bind either anti-CD43 mAb S7 (Fig. 1A) or biotin-conjugated irTS (Fig. 1B). To confirm that CD43 is the counterreceptor for irTS, CD4+ T cell extracts from wild-type and CD43−/− mice were immunoprecipitated with biotin-conjugated irTS or with anti-CD43 mAb S7. Precipitates were immunoblotted and revealed with anti-CD43 mAb S7 (Fig. 1C). Remarkably, both irTS and anti-CD43 mAb S7 immunoprecipitated the same 115-kDa protein band expected for CD43 (19). However, this protein was absent when CD4+ T cells from CD43−/− mice were submitted to the same treatment (Fig. 1C). These results indicate that CD43 expression is required for irTS binding on T cells, showing that soluble irTS physically interacts with CD43.

α2–3-Linked Sialic Acid Is the Epitope for irTS on T Cells—CD43 is the most abundant glycoprotein bearing α2–3- and α2–6-linked sialic acids expressed on the surface of T cells (26). To investigate whether sialic acid is the epitope for irTS, T cells were treated with C. perfringens sialidase, and the binding of biotinylated irTS was tested. Fig. 2A (a) shows that irTS binds to T cells, and this binding is abrogated by sialidase treatment (Fig. 2A (b)). irTS binding was reconstituted with high intensity by resialylation of T cells with α2–3-SL in the presence of α2–3-SL as donor substrate (Fig. 2A (d)) or α2–6-SL (Fig. 2A (e)). As shown in Fig. 2A (d), binding of irTS to T cells was abrogated by the previous incubation of irTS with α2–3-SL but not with α2–6-SL (Fig. 2A (e)). These results are consistent with the irTS bright pattern of staining observed after resialylation of T cells by irTS (Fig. 2A (c)) since these cells now must bear almost exclusively α2–3-linked sialic acid. In addition, previous treatment of irTS with α2–3-SL completely disrupts irTS binding to resialylated T cells (Fig. 2A (f)). This inhibition was dose-dependent and had an I_{50} of 0.49 mM (Fig. 2B).

Binding Preferences of irTS for Sialic Acid-containing Molecules—The irTS preference for α2–3-linked sialic acid was further investigated using sialylated PAA probes. Fig. 3 shows that irTS strongly binds to α2–3-linked sialic acid. Binding was dependent on the concentration of α2–3–SL-PAAs, being maximal at 10 μg/ml, and relies on the carbohydrate group of sialic acid since irTS binding is abrogated after carboxyl reduction (Fig. 3). Furthermore, when α2–6-SL- or SLeX-PAAs were used as ligands, low level binding or no binding to irTS was observed, respectively (Fig. 4).

Periodate treatment of sialylated PAA probes did not reduce irTS binding (Fig. 4), demonstrating that the sialic acid side chain is not required for irTS recognition. Taken together, these data suggest that irTS displays a binding site that recognizes α2–3-linked sialic acid and its 7-carbon analog and that this binding can be abolished by either fucosylation or carboxyl.

Fig. 1. CD43 is the counterreceptor for irTS on T cells. Correlation between CD43 expression and irTS binding on T cells was analyzed by FCM. Naive splenic T cells from wild-type (bold line) or CD43−/− mice (thin line) were stained with fluorescein isothiocyanate-labeled anti-CD43 mAb S7 (A) or biotin-labeled irTS followed by PE-streptavidin (B). The incubation procedures were as described under “Experimental Procedures.” As shown in C, CD4+ T cell extracts from wild-type (WT) or CD43−/− mice were immunoprecipitated with anti-CD43 mAb S7 (lane 1) or biotin-labeled irTS (lane 2). The precipitates were resolved by SDS-PAGE, electro-transferred, and revealed with anti-CD43 mAb S7 by enhanced chemiluminescence.

Fig. 2. irTS binding to T cells relies on α2–3-linked sialic acid. As shown in A, binding of biotin-labeled irTS to T cells (a) is eliminated by previous C. perfringens sialidase treatment (b), irTS binding is reconstituted with high intensity by previous resialylation of T cells with α2–3-SL (c). Binding of biotin-labeled irTS is abrogated by preincubation of irTS with soluble α2–3-SL (d) but not with soluble α2–6-SL (e). Soluble α2–3-SL abrogates irTS binding to resialylated T cells (f). Untreated, desialylated and resialylated T cells, obtained as described under “Experimental Procedures,” were incubated with biotin-conjugated irTS, stained with PE-conjugated streptavidin, and analyzed by FCM. B, concentration-dependent inhibition of irTS binding by α2–3-SL (squares) and α2–6-SL (triangles).

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interaction with α2-3-SL was further verified by two-dimensional NMR experiments, which show in detail the key structural elements of α2-3-SL involved in binding to irTS. A reference TOCSY spectrum of irTS in the presence of α2-3-SL and methyl α-mannoside was recorded without protein pre-

saturation and with protein presaturation (0.5 ppm) (Fig. 6A).

In the STD-TOCSY obtained (Fig. 6B), the on-diagonal cross-peaks identify hydrogens in close proximity with the protein in the complex. It is evident that α2-3-SL binds to irTS. As the relative signal intensities on the STD spectrum correlate with the proximity to the protein, we can conclude that NAc and the H3ax protons from the NeuAc are in close contact with irTS. In the STD-TOCSY spectrum, off-diagonal cross-peaks help identify key hydrogens involved in binding. It is clear that the H3eq, H4, and H5 from the NeuAc residue and the H1, H3, and H4 from the βGalp ring also interact with the irTS as they receive saturation from the protein (Fig. 6B). The H1 and H2 peaks from the α-anomer of Glcp are low in intensity, indicating a loose contact with the irTS binding site. Cross-peaks arising from α-Glcp or from methyl α-mannoside were not detected (Fig. 6B). Binding of irTS to α2-6-linked sialic acid was also investigated using STD. The STD-TOCSY spectrum obtained from α2-6-SL in the presence of irTS (Fig. 7) shows cross-peaks in which only NAc (2.039 ppm), H3ax (1.755 ppm), and H3eq (2.721 ppm) from NeuAc receive saturation from the protein. No signals emerging from βGalp or αGalp are visible. These results indicate that irTS binds only to the sialic acid ring of α2-6-SL, consistent with an improper positioning of the entire molecule in the irTS binding site. Furthermore, in agreement with the experiments using the SLEx-PAA probe (Fig. 4), STD-NMR does not show any binding between irTS and soluble SLEx (data not shown).
DISCUSSION

*Trypanosoma cruzi* presents in its genome hundreds of genes encoding a family of TS molecules and sialic acid acceptor glycoproteins. Combined, the molecules expressed by both sets of genes are likely to cover most of the parasite surface (13), warranting a parasite-host interface. Around 140 genes encode for the TS family, comprising enzymatically active as well as inactive members (10, 28, 29). Although no function has been assigned to the inactive TS, genes coding for inactive members are present in a similar number in the parasite genome as in those encoding active TS (12). In the present work, we show for the first time that irTS can interact with host T cells. Using wild-type and CD43−/− mice and a combination of FCM and immunoprecipitation techniques, we identified the sialomucin CD43 as the counterreceptor for irTS on the T cell surface. Furthermore, using biochemical, immunological, and spectroscopic approaches, we directly demonstrate that irTS binds to α2-3-linked sialic acid.

CD43, or leukosialin, is an abundant mucin expressed on T lymphocytes and other bone marrow-derived cells with ubiquitous physiological roles in cell-to-cell interactions. It is highly glycosylated with 80–90 O-linked glycosylation sites (26, 30). High surface density, pronounced length of protruding molecules, and abundance of sialic acid residues (26) make CD43 a candidate receptor for *T. cruzi* TS on CD4+ T cells. Natural ligands for CD43 include ICAM-1 (31), galectin-1 (32), class I major histocompatibility complex (33), E-selectin (34), and a macrophage sialoadhesin (35). Our studies indicate that TS is a parasite ligand for CD43 on CD4+ T cells. Furthermore, soluble active and inactive TS costimulate CD4+ T cell activation through CD43 engagement, being a candidate molecule for induction of immunopathology in *T. cruzi* infection (18). CD43 is a known receptor for other pathogen-associated molecules. CD43 is a neutrophil receptor for influenza A hemagglutinin (36), responsible in part for neutrophil deactivation observed during infection (37). Macrophage invasion by *Mycobacterium* also requires the extracellular domain of CD43 (38). Since the immunopathology of *T. cruzi* infection involves multiple host cell types (16), binding of irTS to other myeloid cells expressing CD43, such as macrophages, neutrophils, and dendritic cells, should be investigated.

CD43 expressed on resting T lymphocytes is highly sialylated and carries the tetrasaccharide core NeuAcα2–3Galβ1–3(NeuAcα2–3GalNAcβ1–4)-Ser/Thr (39). We therefore, tested the hypothesis that sialic acid is the epitope for irTS binding on CD43. Our results demonstrated that α2–3-linked sialic acid is the epitope for irTS binding on CD43 and that irTS recognizes its ligand with similar specificity to that described for active TS (9). Thus, irTS showed a preferential binding for α2–3-linked sialic acid and for its C7 derivative obtained by truncation of the sialic acid side chain after mild periodate oxidation (1, 9).
Another characteristic shared by both proteins is that active and inactive TS are unable to recognize α2–3-sialyllactosamine bearing a fucose residue, forming the SLex epitope (9). We converted the carboxyl group of sialic acid into an alcohol on the α2–3-SL-PAA probe to explore its role on rTS binding. Our results show that the sialic acid carboxyl group is essential for rTS binding. It is known that the catalytic domain of TS contains three arginine residues that bind the carboxyl group of sialic acid (3, 5). Therefore, the sialic acid binding site found in rTS might be the same present in active TS. Consistent with these findings, an antibody directed against an epitope present in the catalytic domain of active TS (25) was able to inhibit rTS binding to sialic acid-containing PAA probes.

NMR spectroscopy was used to investigate a physical interaction between rTS and sialic acid-containing molecules. NMR spectroscopy has been a valuable tool in studying interactions between ligands and receptors and provides detailed information about binding site and binding conformations in a noninvasive manner (40). The STD NMR technique has been used to distinguish those molecules that bind to a macromolecule from nonbinding compounds (41–44). This technique relies on selective saturation of resonances arising from the receptor protein that spin-diffuses efficiently over the entire protein and consequently is transferred to the bound ligand. This disturbance on spin states in the population is carried by the ligand when released and detected as a reduction in the intensity of resonances in the free ligand. The relative intensity of the signals present in the STD spectrum identifies those nuclei closest to the protein in the binding site, permitting a detailed mapping of the interactions involved (42, 44, 45). Using one-dimensional and two-dimensional STD experiments, we demonstrated that α2–3-SL ligand binds to rTS. From our results, it is possible to map the structural regions of α2–3-SL most involved in binding to rTS. The 5NAc, H3eq, H3ax, and H4 of the NeuAc residue and the H1, H3, and H4 from βGalp are in contact with rTS, whereas the αGlcp residue is not involved in the binding to the protein. No interaction between the glycerol side chain and rTS was found, reinforcing our finding that mild periodate treatment does not impair rTS recognition. Furthermore, only NAc, H3ax, and H3eq from the NeuAc residue of α2–6-SL receive saturation from rTS, indicating that the loose binding of α2–6-SL to rTS is due to an incorrect positioning of the entire molecule in the TS binding pocket. Recently, the involvement of the Trp312 in the T. cruzi TS activity was demonstrated (46). The single mutation Trp312→Ala rendered the TS capable of hydrolyzing both α2–3- and α2–6-linked sialyloligosaccharides. Here we verified that the α2–6-SL does not bind correctly to the rTS pocket, suggesting that Trp312 prevents α2–6-linked sialic acid binding and that a proper positioning of the sialoside is necessary in active TS for the transfer reaction to take place.

This is the first time that binding of sialic acid-containing molecules by a catalytically inactive member of the T. cruzi TS family has been unambiguously proven. Our results show that rTS conserves its binding site for sialic acid despite its lack of enzymatic activity. It has been suggested that the Tyr342→His mutation abrogates the catalytic activity due to the role of Tyr342 in stabilizing the carbonium ion transition state intermediate (4, 5). We now demonstrate that this site mutation does not abolish binding to sialic acid donor molecules and confirm the prediction that loss of the enzymatic activity might generate lectin-like molecules from TS (12). Molecules having mutually exclusive glycosidase or lectin activities have been described in mammalian cells, in a family of chitinases. Although some of their members are glycosylhydrolases, others are chitin-specific lectins that lack enzymatic activity (47).

Another example is a disulfide-bonded dimer of the Golgialactoside α2–6-sialyltransferase that is catalytically inactive and retains the ability to bind galactose (48). In addition, site-directed mutagenesis of Newcastle disease virus hemagglutinin-neuraminidase found functional and topological relationships between the neuraminidase and receptor binding activities of hemagglutinin (49).

In summary, we demonstrated that CD43 is the counterreceptor for rTS on CD4+ T cells. Using different approaches, we directly demonstrated that rTS is a sialic acid-binding protein. Taken together, our results suggest that inactive molecules of the trans-sialidase family still retain sialic acid binding activity and could behave as lectins that bind and transmit signals to cells of the host immune system during T. cruzi infection.

**Acknowledgments**—We thank Dr. A. C. C. Frasch from Universidade Nacional de General San Martin, Argentina, for the gift of both rTS and irTS-expressing plasmid, Dr. M. M. Rodrigues and Dr. M. Correa from Universidade Federal de São Paulo, Brasil, for the TS antibodies, and for the CD43−/− and wild-type mice, respectively. We also thank Dr. G. Lippens from Laboratoire de RMN Synthese, Structure et Fonction des Biomolecules, Institut Pasteur, Lille, France, and Centro Nacional de Ressonância Magnética Nuclear, UPFRJ, Brasil, for the NMR facilities.
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J. Biol. Chem. 2002, 277:45962-45968.
doi: 10.1074/jbc.M203185200 originally published online September 16, 2002

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