A ligand that *Trypanosoma cruzi* uses to bind to mammalian cells to initiate infection

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Abstract We purified a soluble gp83 trans-sialidase (gp83-TSA), from phospholipase C-treated *Trypanosoma cruzi* trypomastigote membranes, which binds to myoblasts, fibroblasts and macrophages to mediate trypanosome entry. Myoblasts display a single class of receptors for the gp83-TSA present at 4×10^4 per myoblast with a K_d of 8 nM. Monovalent Fab fragments of the monoclonal antibody 4A4 specific for gp83-TSA inhibit gp83-TSA binding to myoblasts, fibroblasts and macrophages, block the trypanosomes from attaching to and entering these cells and neutralize *T. cruzi* infection in BALB/c mice. This is the first demonstration that gp83-TSA is a ligand that *T. cruzi* uses to attach to cells.

Key words: Host cell receptor for *Trypanosoma cruzi*; gp83 Trans-sialidase ligand; Host cell receptor for gp83 trans-sialidase; *Trypanosoma cruzi* ligand; *Trypanosoma cruzi* attachment and entry

1. Introduction

Trypanosoma cruzi, the protozoon that causes Chagas' disease affecting millions of people, must bind to mammalian cells before entry to establish infection [1]. Chagas' disease is acquired by binding and entry of invasive trypomastigotes, which are transmitted by insect vectors, or by blood infected with trypomastigotes during blood transfusion. This organism is now viewed as an emerging human pathogen of HIV-1-infected individuals, since it induces dramatic brain pathology and earlier death when associated with HIV-1 infection [2]. Although molecules have been suggested to serve as parasite ligands [3–6], direct evidence of their involvement or of their host cell receptors in the binding process does not currently exist. The identification of *T. cruzi* surface molecules that function as ligands to mediate trypanosome attachment to phagocytic and non-phagocytic cells may be critical to the development of molecular means of intervention against this organism.

The surface of invasive *T. cruzi* trypomastigotes is covered primarily by a very large and diverse family of trans-sialidases and mucin-like molecules, with yet unknown functions in trypanosome–host cell interactions [7,8]. *T. cruzi* is unable to synthesize sialic acid and acquires this molecule from the host via surface trans-sialidases which transfer sialic acid from host glycoproteins to mucin-like molecules located on the surface of the trypanosome [7]. Current thoughts suggest that trans-sialidases will do more than just transfer sialic acid [7]. Because previous reports have shown that the *T. cruzi* surface gp83 [9,10] is expressed only in invasive trypomastigotes [11], is more expressed in highly than weakly infective trypomastigote clones [3] and that blocking this molecule with anti-gp83 antibodies reduces trypanosome binding and invasion [11], we explored the possibility that the surface gp83 is a *T. cruzi* ligand that binds to host cells in a ligand–receptor interaction manner in order to facilitate trypanosome entry.

In this paper we provide the first direct evidence that *T. cruzi* uses the surface gp83 trans-sialidase as a ligand to bind receptors on myoblasts, fibroblasts and macrophages to mediate trypanosome attachment to these cells and initiate in vitro and in vivo infection.

2. Materials and methods

2.1. Organism

The highly infective trypomastigote clone MMC 20A of the Tulahuen strain of *T. cruzi* that highly expresses the surface gp83 was used [3]. Pure culture trypomastigotes were obtained from the supernatant of heart myoblast monolayers [12].

2.2. Monovalent Fab fragments

The mouse monoclonal antibody (mAb) 4A4 that recognizes the surface gp83 of *T. cruzi* trypomastigotes [11] and inhibits trypanosome binding to heart myoblasts [11] was used. mAb 4A4 and an isotype control (I-mAb) were purified using a mannan binding column (IgM purification kit, Pierce). Monovalent Fab fragments (Fab) were obtained using an IgM fragmentation kit (Pierce). Digestion products were separated on Superose gel filtration (Pharmacia Biotechnology) using FPLC [13]. Purified Fab showed only one band when analyzed by SDS–PAGE and stained with silver stain or when analyzed by immunoblots [14]. In this paper, Fab of mAb 4A4 are abbreviated as 4A4-Fab and Fab of isotype control mAb are abbreviated I-Fab. Fab of rabbit polyclonal antibodies to gp83 (P-Fab) and of pre-immune serum (IP-Fab) were purified by isolating IgG using a protein G column followed by a monovalent Fab IgG purification kit (Pierce) and Superose gel filtration as above. Immunoblots of gp83 probed with Fab were developed by ECL [15].

2.3. Purification of soluble trypanosome gp83

Soluble gp83 was purified from glycosylphosphatidylinositol-phospholipase C (GPI-PLC)-treated trypomastigote membrane fractions as previously described [10]. Batches of trypomastigote membrane fractions (each at 5×10^{-10} cells) were treated with purified GPI-PLC (Oxford GlycoSystems) in the presence of protease inhibitors [15] followed by preparative isoelectric focusing (Bio-Rad) and Mono-Q anion exchange chromatography using FPLC [10]. GPI-PLC actively

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cleaves proteins carrying GPI anchors, releasing a soluble protein retaining the inositol glycan and diacylglycerol [16]. *T. cruzi* fractions containing soluble GPI membrane-anchored proteins reacted positively by immunoblots with anti-cross-reacting determinant (CRD) antibodies that recognize the membrane-soluble form of VSG of *Trypanosoma brucei* (Oxford GlycoSystems) [17]. Fractions or purified gp83 were analyzed by SDS–PAGE [18] followed by staining the gels with silver stain, immunoblotting with anti-CRD antibodies and mAb 4A4 [11], and testing for sialidase and trans-sialidase activities as described below. In addition, the radioiodinated purified soluble gp83 trans-sialidase was deglycosylated with *N*-glycanase and its mobility shift was analyzed by autoradiography [10].

2.4. Enzymatic activities

Trans-sialidase activity was determined in 10 μl of purified gp83 in 20 mM HEPES pH 7.2, 1 mM sialylactose and 44 000–45 000 cpm of the substrate (D-glucose-1-^{14}C]lactose (specific activity: 54 mCi/mmol, Amersham). The reaction was incubated for 1 h at 37°C, stopped and passed through a QAE-Sephadex A-25 column [19]. Radioactive oligosaccharides were eluted with 1 ml of 1 M ammonium formate and the activity was expressed as eluted cpm. Sialidase activity was determined by measuring the fluorescence of 4-methyllumbelliferone released by the hydrolysis of 2′-(4-methylumbelliferyl) α-D-*N*-acetylneuraminic acid [20]. Chromatographic fractions were incubated in the presence of 1 mM 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid with constant agitation for 4 h at 25°C. Purified sialidase from *Salmonella typhimurium* (Oxford GlycoSystems) was used as positive control. The reactions were stopped by addition of 100 mM of Tris–HCl, pH 10, 100 mM $NaCl_2$, 2 mM $MgCl_2$ and fluorescence was measured at 360 nm excitation and expressed in fluorescence units. 4A-Fab, I-Fab, P-Fab or IP-Fab were preincubated with purified gp83 for 1 h at room temperature and tested for their ability to inhibit sialidase and trans-sialidase activities.

2.5. Binding of gp83 trans-sialidase to host cells

Purified soluble gp83 trans-sialidase was radioiodinated in the presence of Iodogen at a specific activity of 4.3×10^6 cpm/μg protein as described [20,21]. Binding of soluble [^{125}I]gp83 trans-sialidase to rat heart myoblast monolayers was carried out in Teflon microwell chambers containing micromonolayers of heart myoblasts (10^4 cells/well) [3]. Increasing concentrations of soluble [^{125}I]gp83 trans-sialidase (10 μl) were added to myoblast monolayers in Hanks' balanced salt solution supplemented with bovine serum albumin (HBSS-BSA) and 10 μl of HBSS-BSA alone or containing 100-fold excess unlabeled soluble gp83 trans-sialidase to determine non-specific binding. Each point was done in triplicate. Cells were incubated at 4°C for 2 h. Unbound radioiodinated gp83 trans-sialidase was removed by three washes with cold HBSS. Cell monolayers were lysed with 20 μl of 2.5% SDS and the radioactivity was determined. Specific binding was determined [21] and binding data were analyzed using the Graphpad Prism Software. Inhibition experiments were done using soluble [^{125}I]gp83 (40 ng/ml) in the presence of 4A4-Fab, I-Fab, P-Fab or IP-Fab at 40 ng/ml. A control was also performed in the presence of phosphate-buffered saline (PBS) instead of Fab. Binding of biotinylated gp83 trans-sialidase to Vero cell fibroblast, baby hamster kidney fibroblast or mouse peritoneal macrophage micromonolayers was performed as described [23]. Specific binding was evaluated in the presence of 100-fold unlabeled excess of gp83 or in the presence of 4A4-Fab or I-Fab. The reaction was developed with fluorescein-labeled avidin [23].

2.6. Neutralization of T. cruzi binding and entry and mice infection

T. cruzi trypomastigote–cell interactions were performed using a trypanosome:cell ratio of 10. The ability of 4A4-Fab or I-Fab to inhibit the attachment of live trypomastigotes to 0.1% paraformaldehyde-fixed rat heart myoblasts, Vero cell fibroblasts and macrophages was evaluated using microassays as described [3,22,24]. The ability of

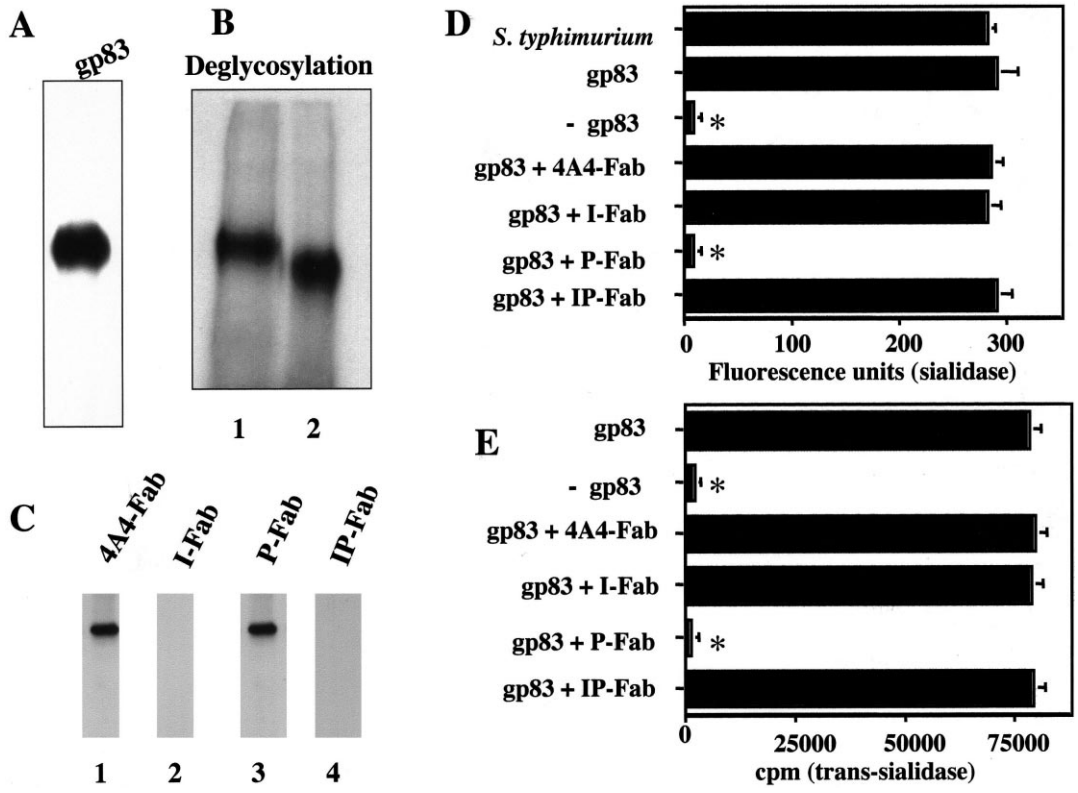

Fig. 1. Purified soluble gp83 presents sialidase and trans-sialidase activities and 4A4-Fab do not inhibit these enzymatic activities, whereas P-Fab do. A: Silver stain of purified gp83 (0.1 μg) separated by SDS–PAGE. B: Mobility shift of [^{125}I]gp83 treated with *N*-glycanase detected by autoradiography. Untreated gp83 (lane 1) and treated gp83 (lane 2). C: Immunoblot of purified soluble gp83 probed with 4A4-Fab (lane 1), I-Fab (lane 2), P-Fab (1ane 3) and IP-Fab (lane 4) developed by ECL. D: Effect of 4A4-Fab, I-Fab, P-Fab or IP-Fab on sialidase activity of purified soluble gp83. Purified *S. typhimurium* sialidase was used as a positive control for sialidase activity. E: Effect of 4A4-Fab, I-Fab, P-Fab or IP-Fab on trans-sialidase activity of purified soluble gp83. Each column represents the mean of triplicate determinations ± S.D. Differences between * and the rest, $P<0.01$.

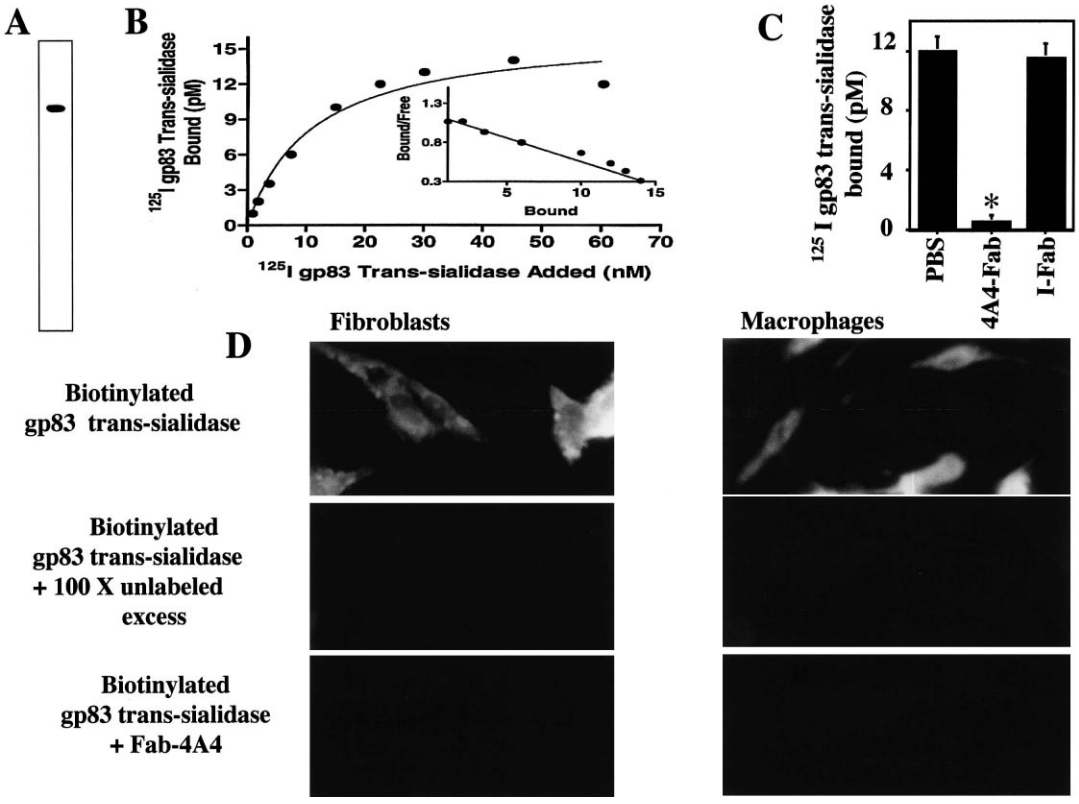

Fig. 2. Labeled purified soluble gp8 trans-sialidase binds to myoblasts, fibroblasts, and macrophages in a ligand–receptor interaction manner and these interactions are inhibited by 4A4-Fab. A: Autoradiogram of the radioiodinated purified soluble gp83 trans-sialidase subjected to SDS–PAGE used for binding studies. B: Binding of [^{125}I]gp83 trans-sialidase to heart myoblasts. Inset: Scatchard analysis of the binding data. C: Inhibition of [^{125}I]gp83 trans-sialidase binding to myoblasts by 4A4-Fab. D: Binding of biotinylated soluble gp83 trans-sialidase to Vero cell fibroblasts and macrophages developed by fluorescein-labeled avidin and inhibition of this binding by excess unlabeled gp83 or 4A4-Fab. Each column in C represents the mean of triplicate determinations ± S.D. Differences between * and the rest, $P < 0.01$.

these Fab to inhibit trypanosome internalization into myoblasts, fibroblasts and macrophages was performed as described [3,22]. The ability of 4A4-Fab to neutralize *T. cruzi* infection in mice was evaluated as follows. Either 4A4-Fab or I-Fab (20 μg in PBS) were administered i.v. into 4-week-old BALB/c female mice ($n = 10$) 2 h before i.v. challenge with 2×10^3 blood trypomastigotes and mice were given three consecutive i.p. injections of either 4A4-Fab or I-Fab (20 μg) for the next 3 days. The number of trypomastigotes in the blood was monitored in 5 μl tail blood [25].

2.7. Presentation of results and statistical analysis

Results in this work were obtained from triplicate values and represent three independent experiments with identical protocols. Results are expressed as the mean ± 1 S.D. Differences were considered to be statistically significant if $P < 0.05$ as determined by Student's *t*-test or by the Mann–Whitney *U*-test for the in vivo experiments.

3. Results

3.1. The surface T. cruzi gp83 is a trans-sialidase that binds to myoblasts, fibroblasts and macrophages in a ligand–receptor interaction manner and this binding is neutralized by 4A4-Fab

The purified soluble surface gp83 from *T. cruzi* trypomastigote membranes treated with GPI-PLC (Fig. 1A) presents *N*-linked oligosaccharides (Fig. 1B) and sialidase and trans-sialidase activities (Fig. 1D,E). Uncleaved mAb 4A4 specific for gp83 was previously shown to inhibit *T. cruzi* trypomastigote binding to myoblasts [11]. Both the mAb 4A4 and polyclonal antibodies specific for gp83 are able to immunoprecipitate the soluble gp83 (results not shown). 4A4-Fab recognize the gp83 by immunoblots (Fig. 1C) and do not inhibit either the sialidase or trans-sialidase activities of gp83 (Fig. 1D,E). However, P-Fab, which also recognize the purified soluble gp83 (Fig. 1C), neutralize both sialidase and trans-sialidase activities, whereas IP-Fab do not (Fig. 1D,E). These results indicate that 4A4-Fab do not recognize the sialidase or trans-sialidase active site of the gp83 molecule. Purified soluble radioiodinated gp83 trans-sialidase (Fig. 2A) binds to heart myoblasts in a ligand–receptor interaction manner with a K_d of 8 nM (Fig. 2B). Myoblasts display a single class of receptors for the gp83 trans-sialidase, present at 4×10^4 per myoblast (Fig. 2B). Binding of [^{125}I]gp83 trans-sialidase to myoblasts is abolished in the presence of 4A4-Fab, but not in the presence of I-Fab (Fig. 2C). Biotinylated gp83 trans-sialidase also binds to Vero cell fibroblasts and macrophages and this binding is inhibited by an excess of either unlabeled gp83 or 4A4-Fab (Fig. 2D). I-Fab do not inhibit these interactions (results not shown). Similar results were observed when baby hamster kidney fibroblasts were used (results not shown). These results indicate that the gp83 trans-sialidase binds to myoblasts, fibroblasts and macrophages in a ligand–receptor interaction manner and that these interactions do not involve the enzymatic active site of the gp83.

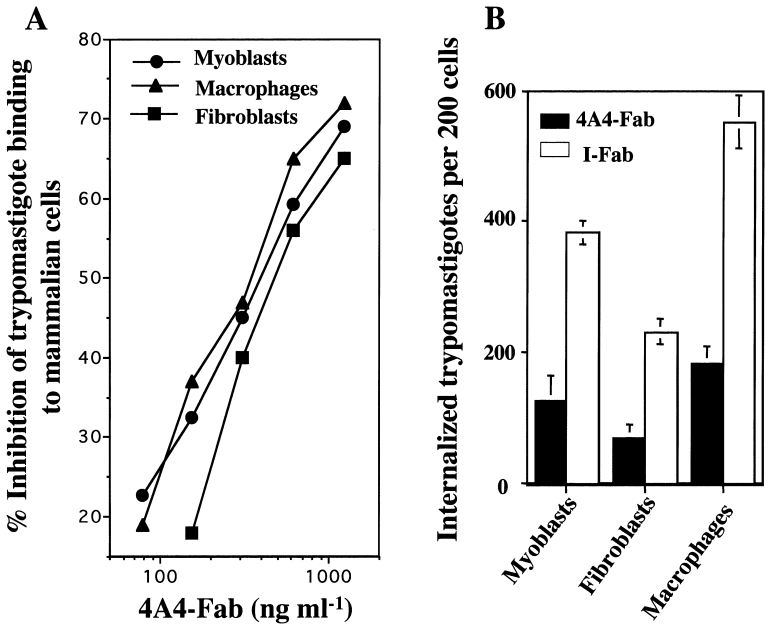

Fig. 3. 4A4-Fab neutralize *T. cruzi* trypomastigote binding to myoblasts, fibroblasts and macrophages and prevent parasite entry into these cells. A: Binding of trypomastigotes to heart myoblasts, Vero cell fibroblasts and macrophage monolayers after 2 h of interaction. B: Internalization of trypomastigotes into myoblasts, Vero cell fibroblasts and macrophages after 4 h. Each bar represents the mean of triplicate determinations ± S.D. Differences in parasite internalization between test and control, $P < 0.05$.

3.2. *4A4-Fab block the trypomastigotes from attaching to and entering myoblasts, fibroblasts and macrophages and neutralizes T. cruzi infection in BALB/c mice*

We reasoned that if trypomastigotes use gp83 trans-sialidase to bind to heart myoblasts, fibroblasts and macrophages, then 4A4-Fab, which block the binding of this molecule to these cells (Fig. 2), should inhibit the attachment and internalization of trypomastigotes into host cells. Indeed, 4A4-Fab effectively blocked trypomastigote attachment to heart myoblasts, Vero cell fibroblasts and macrophages in a concentration-dependent manner (Fig. 3A). A significant reduction in trypanosome entry these cells was detected at 4 h (Fig. 3B). 4A4-Fab effectively inhibited trypomastigote binding and internalization into mammalian cells at nanogram amounts, whereas I-Fab failed to inhibit binding and internalization of trypanosomes; values obtained with I-Fab were similar to PBS controls (data not shown). 4A4-Fab neutralize *T. cruzi* trypomastigote infection in BALB/c mice (Fig. 4). I.v. injection of 4A4-Fab into BALB/c mice 2 h before i.v. challenge with a lethal dose of blood trypomastigotes followed by three i.p. doses of 4A4-Fab dramatically reduced parasitemia compared to control mice receiving I-Fab (Fig. 4A). After 27 days, the mortality of mice receiving I-Fab was 100%, whereas no mortality was observed in the group of mice receiving 4A4-

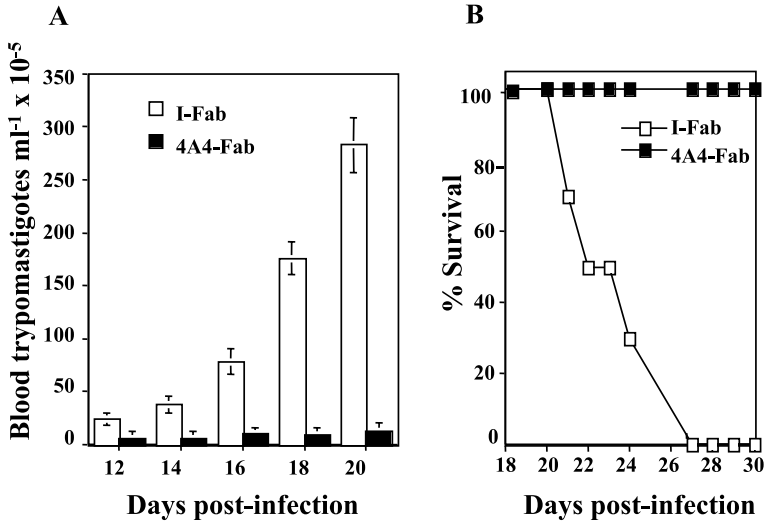

Fig. 4. Injection of 4A4-Fab into BALB/c mice neutralizes the infectivity of *T. cruzi* trypomastigotes. A: Levels of blood trypomastigotes in mice injected with either 4A4-Fab or I-Fab and infected with *T. cruzi* blood trypomastigotes as described in Section 2. B: Mortality of *T. cruzi*-infected BALB/c mice receiving either 4A4-Fab or I-Fab. Each column represents the mean of parasitemia ± S.D. Differences in parasitemia between test and control, $P < 0.05$.

Fab (Fig. 4B). At the termination of the experiment (118 days) no mortality had been observed in these mice. Parasitemia levels and mortality with I-Fab were similar to PBS controls (data not shown). Taken together these results indicate that 4A4-Fab strongly neutralize the infectivity of *T. cruzi* in vitro and in vivo by blocking the surface gp83 trans-sialidase ligand of invasive forms of this organism.

4. Discussion

These results indicate for the first time that *T. cruzi* trypomastigotes use the surface gp83 trans-sialidase to bind to host cell receptors in order to mediate attachment and initiate infection in vitro and in vivo. Binding of gp83 trans-sialidase to phagocytic and non-phagocytic cells to promote entry in the host appears not to require the enzyme active site of gp83 trans-sialidase, since 4A4-Fab, which abolish binding of labeled gp83 trans-sialidase to mammalian cells and neutralize in vitro and in vivo infection, do not affect the sialidase and trans-sialidase activities that the gp83 bears. Furthermore, these results also indicate that the epitope recognized by the mAb 4A4 on the gp83 trans-sialidase of trypomastigotes is required for trypanosome binding to phagocytic and non-phagocytic cells to initiate in vitro and in vivo infection.

To our knowledge this is the first report to determine the number of receptors on a cell for a soluble surface molecule of invasive *T. cruzi* trypomastigotes and the K_d of this interaction. Heart cells are a major target for *T. cruzi* and infection of these cells is involved in cardiac arrest followed by death [1]. Elucidation of the molecular interactions between *T. cruzi* molecules and their receptors on heart cells may be important not only to understand how *T. cruzi* enters heart cells to alter their cardiophysiology, but also to the development of molecular means of intervention against Chagas' heart disease. Current studies in our laboratory indicate that the *T. cruzi* gp83 trans-sialidase interacts with a p74 expressed on the surface of heart cells [23] and on other cells that *T. cruzi* infects and that p74 functions as a receptor for *T. cruzi* and for the gp83 trans-sialidase [26].

The fact that this molecule is also expressed in other strains of *T. cruzi* [5] and that our findings in this report indicate that the gp83 trans-sialidase binds receptors on cells other than heart cells that are able to be infected by *T. cruzi* such as macrophages and fibroblasts, strongly suggests that the gp83 trans-sialidase of *T. cruzi* is a universal ligand that this organism uses to bind to and enter phagocytic and non-phagocytic cells to initiate infection in the body. These findings raise the possibility that invasive trypomastigotes might up- and down-regulate their infectivity to mammalian cells by modulating expression and secretion of the gp83 trans-sialidase. In fact, recent reports have shown that secretion of gp83 trans-sialidase by trypomastigotes increases parasite entry in human macrophages by activating a protein kinase C (PKC)-dependent mitogen-associated protein (MAP) kinase pathway in these host cells [27,28].

Fab fragments of mAb 4A4 specific for gp83 trans-sialidase inhibit gp83 trans-sialidase binding to myoblasts, fibroblasts and macrophages, block trypanosomes from attaching to and entering myoblasts, fibroblasts and macrophages and neutralize *T. cruzi* infection in BALB/c mice. These observations also strongly support the notion that gp83 trans-sialidase is a ligand used by *T. cruzi* to attach to phagocytic and non-phagocytic cells to promote entry and initiate in vitro and in vivo infection. Since 4A4-Fab strongly but not completely neutralize *T. cruzi* infectivity in vitro and in vivo, we do not exclude the possibility that this organism may use alternative mechanisms to invade host cells. A very recent report has implicated the 85 kDa protein in host cell invasion in vitro [29]. While both the gp83 and the 85 kDa protein cover part of the surface of trypomastigotes and play roles in infection, the gp83 ligand binds to host cell receptors on mammalian cells to up-regulate trypomastigote entry by activating host tyrosine kinases in a PKC-dependent MAP kinase pathway [27,28]. The gp83 is the first *T. cruzi* ligand described that triggers host cell receptors to activate host cell signal transduction events that are necessary and essential for trypanosome entry [27,28]. The gp83 ligand binds to a p74 host cell receptor [23,26] expressed on the membrane of all non-phagocytic and phagocytic cells that *T. cruzi* can infect, whereas peptide j derived from 85 kDa protein appears to restrict its interaction with a 45 kDa cytokeratin of epithelial cells [29]. Moreover, the affinity of the gp83 ligand for host cell receptors is seven times higher than the synthetic peptide j. *T. cruzi* surface molecules that bind to host cell receptors during invasion may be of interest for developing vaccines and receptor blocking therapies. In summary, we identified a ligand on invasive forms of *T. cruzi* that is used by this organism to adhere to phagocytic and non-phagocytic cells to initiate in vitro and in vivo infection.

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References

[1] Brenner, Z. (1973) Annu. Rev. Microbiol. 27, 347–382.
[2] Rosemberg, S., Chaves, C.J., Higuchi, M.L., Lopes, M.B., Castro, L.H. and Machado, L.R. (1992) Neurology 42, 640–642.
[3] Lima, M.F. and VilIalta, F. (1989) Mol. Biochem. Parasitol. 33, 159–170.
[4] Ortega-Barria, E. (1991) Cell 67, 411–421.
[5] Colli, W. (1993) FASEB J. 7, 1257–1264.
[6] Ramirez, M.I., Ruiz, R., Araya, J.E., Da Silveira, J.F. and Yoshida, N. (1993) Infect. Immun. 61, 3636–3641.
[7] Frasch, A.C.C. (2000) Parasitol. Today 16, 282–286.
[8] Acosta-Serrano, A., Almeida, I.C., Freitas-Junior, L.H., Yoshida, N. and Schenkman, S. (2001) Mol. Biochem. Parasitol. 114, 143–150.
[9] Lima, M.F. and Villalta, F. (1988) Biochem. Biophys. Res. Commun. 155, 256–262.
[10] Villalta, F. and Lima, M.F. (1990) Biochem. Biophys. Res. Commun. 172, 925–931.
[11] Villalta, F., Lima, M.F., Zhou, L. and Ruiz-Ruano, A. (1992) Biochem. Biophys. Res. Commun. 182, 6–13.
[12] Villalta, F. and Kiersenbaum, F. (1983) Biochim. Biophys. Acta 736, 39–44.
[13] Villalta, F., Lima, M.F., Howard, S.A., Zhou, L. and Ruiz-Ruano, A. (1992) Infect. Immun. 60, 3025–3033.
[14] Lima, M.F., Zhang, Y. and Villalta, F. (1997) Cell. Mol. Biol. 43, 1067–1076.
[15] Moody, T.N., Ocheing, J. and Villalta, F. (2000) FEBS Lett. 470, 305–308.
[16] Cardoso de Almeida, M.L. and Turner, M.J. (1983) Nature 302, 349–352.
[17] Zamze, S.E., Ferguson, M.A., Collins, R., Dwek, R.A. and Rademacher, T.W. (1988) Eur. J. Biochem. 176, 527–534.
[18] Laemmli, U.K. (1970) Nature 227, 680–685.
[19] Parodi, A.J., Polleick, G.D., Mautner, M., Buschiazzo, A., Sanchez, D.O. and Frasch, A.C.C. (1992) EMBO J. 11, 1705–1710.
[20] Previato, J.0., Andrade, A.F., Pessolani, M.C.V. and Mendonca-Previato, L. (1985) Mol. Biochem. Parasitol. 16, 85–96.

[21] Lima, M.F. and Villalta, F. (1990) Mol. Biochem. Parasitol. 38, 245–252.
[22] Noisin, E.L. and Villalta, F. (1989) Infect. Immun. 57, 1030–1034.
[23] Villalta, F., Ruiz-Ruano, A., Valentine, A.A. and Lima, M.F. (1993) Mol. Biochem. Parasitol. 61, 217–230.
[24] Villalta, F. and Kierszenbaum, F. (1984) J. Immunol. 133, 3338–3343.
[25] Villalta, F. and Kierszenbaum, F. (1983) Am. J. Trop. Med. 32, 273–276.
[26] Villalta, F., Pratap, S., Nde, P., Burns, J.M. Jr. and Lima, M.F., unpublished data.
[27] Villalta, F., Zhang, Y., Bibb, K.E., Burns Jr., J.M. and Lima, M.F. (1998) Biochem. Biophys. Res. Commun. 248, 247–252.
[28] Villalta, F., Zhang, Y., Bibb, K.E., Pratap, S., Burns Jr., J.M. and Lima, M.F. (1999) Mol. Cell. Biol. Res. Commun. 2, 64–70.
[29] Magdesian, M.H., Giordano, R., Ulrich, H., Juliano, M.P., Juliano, L., Schumaker, R.I., Colli, W. and Alves, M.J.M. (2001) J. Biol. Chem. 276, 19382–19389.