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Sera from Chronic Chagasic Patients and Rodents Infected with *Trypanosoma cruzi* Inhibit *trans*-Sialidase by Recognizing Its Amino-Terminal and Catalytic Domain

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We investigated whether sera from chronic Chagasic patients and animals infected with *Trypanosoma cruzi* inhibit the removal of sialic acid from human erythrocytes and the transfer of sialic acid from sialyllactose to [^{14}C]lactose in the reactions catalyzed by the parasite *trans*-sialidase. Sera from Swiss mice and *Calomys callosus* animals infected with three different *T. cruzi* strains inhibit both reactions. Inhibition increases during the infection, reaching maximal levels when the parasitemia decreases. Among 44 sera of untreated chronic Chagasic patients, 40 inhibit both reactions. Inhibition is observed with total, defatted sera or with purified immunoglobulins. Whereas most of the inhibitory antibodies from Chagasic patients react with the papain fragment of *trans*-sialidase in immunoblots, a few patients have noninhibitory antibodies that react only with the entire *trans*-sialidase. These findings may be relevant for the pathology of Chagas' disease.

Trypanosoma cruzi does not synthesize sialic acid but contains a *trans*-sialidase (TS) that removes this carbohydrate from exogenous sources and transfers it to parasite glycoprotein acceptors (13, 16, 19). Without acceptors, the TS catalyzes the hydrolysis of sialic acid bonds as a typical neuraminidase (20). The biological role of TS in *T. cruzi* is not yet understood, but several lines of evidence suggest that it may be important for the parasites' survival in their hosts (18).

The TS expressed in the parasites circulating in the blood of mammalian hosts is formed by two distinct domains: the amino terminal, containing the catalytic domain, and the carboxyl terminal, containing a tandem repeat (CTR) of 12 amino acids (10, 12). Antibodies to the CTRs aggregate the enzyme and inhibit the hydrolysis of sialic acid from cell surfaces but not from soluble substrates (14). It has been suggested by Cazzulo and Frasch that the presence of CTRs, which are immunodominant, prevents the host immune system from making antibodies to the catalytic domain and favors the survival of the parasite in the mammalian host (1, 4, 5). A prediction of this hypothesis is that *T. cruzi*-infected animals and chronic Chagasic patients will have few or no antibodies able to inhibit TS activity. Here we show that this is not the case and that *T. cruzi* infection induces antibodies to the amino-terminal and catalytic domain of TS that inhibit enzymatic activity.

MATERIALS AND METHODS

***T. cruzi* strains and experimental animals.** *T. cruzi* Y (21) and F (7) were maintained by serial passages in mice. *T. cruzi* Costalimai (9) was maintained in *Calomys callosus*, a wild rodent, by intraperitoneal inoculation of 10^5 parasites. Parasites were recovered from blood collected by cardiac puncture. To monitor the parasitemia and obtain serum samples, the animals were infected with 10^5 blood trypomastigotes, except for Y infection of Swiss mice, which were inoculated with 10^3 parasites. At appropriate times, 5 μl of blood was collected from the tail tip and the number of trypanosomes was counted in 50 microscopic fields (3).

Sera. Sera were obtained from at least five animals on different days after *T. cruzi* inoculation. Human sera were obtained by informed consent from chronic untreated Chagasic patients. The patients were all positive by xenodiagnostic and conventional serological tests for Chagas' disease. The patients came from different areas of endemicity and lived in the São Paulo area. They did not present any clinical manifestations of other infectious diseases. Sera were defatted by ultracentrifugation at 145,000 × *g* for 40 h in the presence of KBr at a density of 1.21 mg/ml and then dialyzed against phosphate-buffered saline (PBS). Immunoglobulins were purified by chromatography in protein A-Sepharose CL-4B columns equilibrated with 50 mM Tris-HCl, pH 8.0. Bound antibodies were eluted with 0.05 M sodium citrate, pH 2.9, and dialyzed against PBS. Pools of noninfected animal sera and non-Chagasic human sera were used as controls.

TS purification. TS was purified from the culture supernatant of *T. cruzi* trypomastigotes, Y strain, derived from cultures of $LLCMK_2$ cells (ATCC-CCL-7; American Type Culture Collection, Rockville, Md.) grown in low-glucose Dulbecco's modified Eagle's medium with penicillin and streptomycin (Gibco, Grand Island, N.Y.), containing 10% fetal bovine serum at 37°C, 5% CO_2, as described previously (20). Briefly, Nonidet-P40 was added to a final concentration of 0.1% to pooled supernatants from cultures of *T. cruzi*-infected $LLCMK_2$ cells, and the supernatants were filtered through a 0.22-μm-pore-size filter and concentrated by precipitation with 50% NH_4SO_2. The precipitates were dialyzed against PBS and then applied into a tresyl-agarose (Schleicher & Schuell, Keene, N.H.) column containing immobilized monoclonal antibody 39, specific for TS (20). The column was washed with PBS, with 10 mM sodium phosphate, pH 6.5, and the TS was eluted with 3.5 M $MgCl_2$–10 mM sodium phosphate, pH 6.0.

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TABLE 1. Inhibition of TS and neuraminidase activities by sera from infected rodents[a]

Strain	Rodent	Day	TS activity[b]		Neuraminidase activity[c]		
			cpm	Inhibition (%)	PNA (μg/ml)	Serum dilution	Inhibition (%)
F					1	No serum	0
	Mouse	0	8,588	8	4	1/2	12
		30	5,041	46	500	1/16	81
		60	4,014	57	500	1/16	81
	C. callosus	0	7,770	17	2	1/2	6
		30	373	96	500	1/4	69
		60	1,960	79	500	1/32	87
Y			9,335	0	4	No serum	0
	Mouse	0	8,588	8	63	1/2	29
		10	1,213	87	500	1/4	64
		60	1,120	88	500	1/32	86
	C. callosus	0	7,770	17	31	1/2	21
		10	5,601	40	125	1/2	36
		60	4,947	47	500	1/4	64
Costalimai					1	No serum	0
	Mouse	0	8,588	8	4	1/2	12
		20	3,734	60	500	1/2	56
		60	1,494	84	500	1/4	69
	C. callosus	0	7,770	17	4	1/2	12
		20	5,507	41	500	1/2	56
		60	4,014	57	500	1/4	69

[a] Rodents were infected intraperitoneally with 10^5 bloodstream forms of the F and Costalimai strains, or 10^3 bloodstream forms of the Y strain of *T. cruzi*. Pooled sera from five animals were collected before (day 0) or on the indicated days after the infection and used to inhibit TS and neuraminidase activity.

[b] TS activity was measured by the formation of [^{14}C]sialyllactose by the enzyme purified from the Y strain, and the percentage of inhibition was calculated in relation to the activity in the absence of serum.

[c] Neuraminidase activity was measured by the agglutination of human erythrocytes by PNA with crude enzymes from tissue culture-derived trypomastigotes as described in Materials and Methods for each one of the strains. The PNA values are the minimal concentrations that caused agglutination at the respective serum dilutions. The percentage of inhibition was calculated from the extrapolated ratio of the minimal PNA agglutination concentration relative to the agglutination in the absence of serum.

The fractions eluted from the column were immediately filtered through a Sephadex G-25 column equilibrated in 20 mM Tris-HCl, pH 8.0, to remove $MgCl_2$. The TS was further purified by chromatography on a Mono Q column equilibrated in 20 mM Tris-HCl, pH 8.0, and eluted with a linear gradient from 0 to 1 M NaCl in the same buffer.

To generate the papain fragment of TS (TS-pap), purified TS at 20 to 100 μg/ml was incubated with 10% (vol/vol) of agarose-immobilized papain (Pierce Chemical Co., Rockford, Ill.) in 0.1 M Tris-HCl, pH 7.4, containing 10 mM EDTA and 10 mM cysteine. After 1 h, at 25°C under agitation, the resin was removed by centrifugation, and the supernatant was diluted five times with a buffer containing 20 mM Tris-HCl, pH 8.0. The diluted sample was centrifuged for 30 min, at 10,000 × *g*, and loaded into a Mono Q column equilibrated in 20 mM Tris-HCl, pH 8.0. Fractions containing activity eluted with a linear gradient of NaCl in the same buffer as described above.

Activity measurements. TS activity was measured by the transfer of sialic acid from sialyllactose to D-glucose-1-[^{14}C]lactose by using purified TS from a Y-strain trypomastigote as described previously (17). TS activity was also assayed under agitation by using the enzyme immobilized in 5-mm disks of nitrocellulose or immobilized in protein A-Sepharose containing preadsorbed immunoglobulin from a rabbit immunized with a synthetic peptide, DSSAHSTPSTPADSSAH, based on the repeats of the carboxyl terminus of TS (anti-CTR) (6). For the inhibition assays, 5 to 10 μl of the indicated total sera, defatted serum, or the immunoglobulins purified by protein A-Sepharose was preincubated with an enzyme source for 10 to 30 min and the substrates were added to complete 50 μl. The results are expressed as counts per minute of [^{14}C]sialyllactose that is formed or as the percentage of inhibition relative to the activity obtained without sera or purified antibodies. Neuraminidase activity was measured by the removal of sialic acid from human erythrocytes by TS of culture supernatants followed by agglutination with peanut agglutinin (PNA) as described previously (11). Briefly, 2% human erythrocytes in PBS were incubated in 96-well plates with culture supernatants of trypomastigotes released from infected LLCMK$_2$ cells. After 4 h, an equal volume of serially diluted PNA was added, and the agglutination was detected after 30 min of incubation. For the inhibition assays, the serum was preincubated with the enzyme source for 30 min. The maximal activity, or no inhibition, was determined by the minimal agglutinating concentration of PNA. When agglutination was completely inhibited at 500 μg of PNA per ml, the sera were diluted before incubation with the enzyme source. For each antiserum dilution, the PNA concentration increased inversely. The maximal inhibition was obtained at serum dilutions of 1/64. It was extrapolated that at a one-half serum dilution the minimal agglutinating concentration of PNA was 32,000 μg/ml. The percentages of inhibition were obtained by dividing the log of the PNA concentration in micrograms per milliliter by the log of 32,000, which was considered the maximal inhibition. Some inhibition was consistently observed by the addition of nonimmune sera. Such inhibition could be due to the presence of cross-reacting antibodies in normal and nonimmune sera since it is also observed for *Vibrio cholerae* neuraminidase (not shown). Alternatively, it could be related to the presence of sialic acid donors and acceptors in sera and immunoglobulins, which interfere with the enzymatic assays.

Inhibition of *T. cruzi* sialylation. Trypomastigotes released from LLCMK$_2$ cells in Dulbecco's modified Eagle's medium containing 0.2% bovine serum albumin (BSA) (Ultrapure;

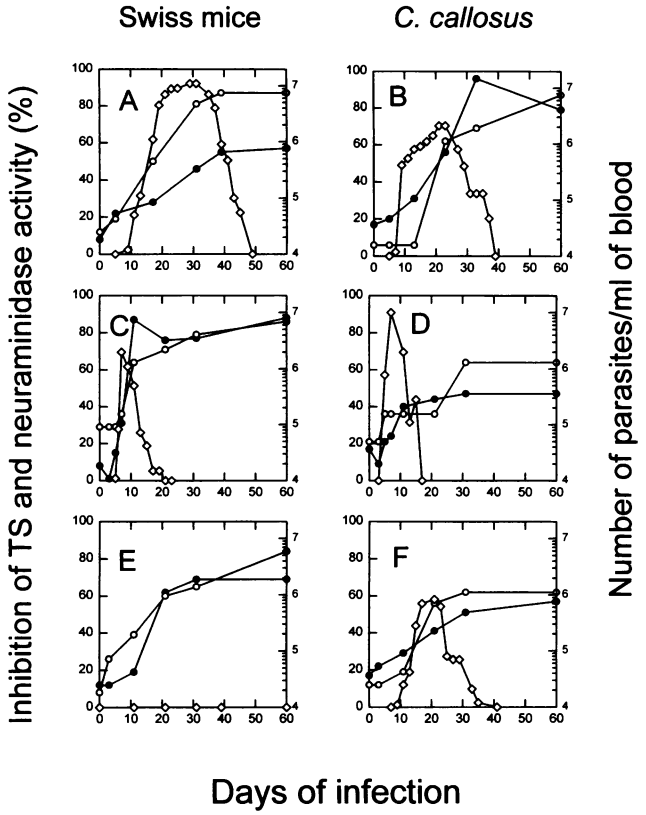

FIG. 1. Inhibition of TS and neuraminidase activity by sera of Swiss mice and *C. callosus* animals infected with *T. cruzi*. The values correspond to the experiment whose results are shown in Table 1, and the percentage of inhibition of TS and neuraminidase was calculated as described in Materials and Methods. At day 0, the animals were infected with *T. cruzi* F (A and B), Y (C and D), and Costalimai (E and F), and the number of parasites per milliliter of blood (◇) and the inhibition of neuraminidase activity (●) and TS activity (○) were determined with defatted and total sera, respectively, pooled from five animals. The inhibition of activity was measured in duplicate and is expressed as a percentage of the activity in PBS as explained in Table 1 and Materials and Methods.

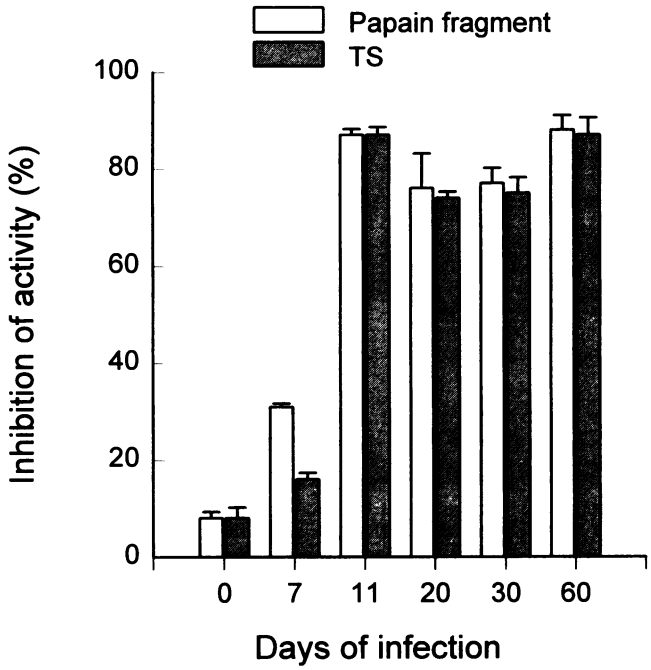

FIG. 2. Antibodies inhibit the activity of TS and the papain fragment of TS. Swiss mice were infected with the Y strain of *T. cruzi*, and sera collected at indicated times were used to inhibit activity of TS and the papain fragment. The results are expressed as means ± the standard deviations of triplicate determinations. The 0% value of inhibition was 3,000 cpm for TS-pap and 3,400 cpm for TS (0.1 and 0.11 U, respectively, as defined in reference 6).

Boehringer, Mannheim, Germany) (19) were centrifuged, washed in Hanks' balanced saline, and resuspended to 10^8 parasites per ml of Hanks' balanced saline. Ten microliters of the parasite suspension was added to immunofluorescence slides, and after 10 min, the excess of parasites was removed. The live and adsorbed parasites were washed with Hanks' balanced saline and incubated with antisera diluted one-half in Dulbecco's modified Eagle's medium with 0.2% BSA. After 15 min, the parasites were washed, fixed for 1 h with 4% paraformaldehyde, washed three times with Hanks' balanced saline, resuspended in Dulbecco's modified Eagle's medium with 0.2% BSA, and incubated for 30 min with 50 μg of purified immunoglobulins of the monoclonal antibody 3C9 per ml, specific to the sialylated antigen of trypomastigotes (19). Bound antibodies were detected with a 1/200 dilution of anti-mouse immunoglobulins conjugated with fluorescein isothiocyanate (Boehringer). Controls were run in parallel without monoclonal antibody 3C9, and no fluorescence whatever was detected.

Immunoblot. TS and TS-pap were boiled in a sample buffer containing 2% sodium dodecyl sulfate (SDS), 50 mM Tris-HCl, 5% 2-mercaptoethanol, 10% glycerol, and 0.01% bromophenol blue, and run in 7.5% polyacrylamide-SDS gels. The gels were transferred to nitrocellulose membranes and probed with Chagasic sera, or with an antiserum to TS-pap diluted 100 times with 1% BSA in 0.15 M NaCl–10 mM Tris-HCl, pH 7.4. This antiserum was obtained from rabbits immunized with TS-pap in complete Freund's adjuvant, followed by four subcutaneous boosters, monthly spaced, in incomplete Freund's adjuvant (anti-papain fragment). Bound antibodies were detected with protein A labeled with ^{125}I and autoradiography.

RESULTS AND DISCUSSION

We studied the immune response against TS in Swiss mice, which have been used extensively in experimental Chagas' disease, and *C. callosus*, a wild rodent, which is a natural *T. cruzi* reservoir. *C. callosus* is well adapted to laboratory breeding and offers an alternative model to study the immune mechanisms during the course of Chagas' disease. Therefore, mice and *C. callosus* animals were inoculated with three different *T. cruzi* strains and the inhibitory immune responses against the parasite neuraminidase-TS were determined during the course of infection (Table 1 and Fig. 1). Strain F produces a persistent parasitemia with a maximal number of parasites in blood on day 28 in Swiss mice, and on days 21 to 22 in *C. callosus*. After the 10th day of infection, when the parasites appear in the blood, both species develop antibodies that inhibit the removals of sialic acid from human erythrocytes (neuraminidase activity) (Fig. 1A) and the transference of sialic acid from sialyllactose to [^{14}C]lactose (TS activity) (Fig. 1B) by the soluble enzyme. The inhibition reaches maximal levels with the decrease of parasitemia after 40 days of

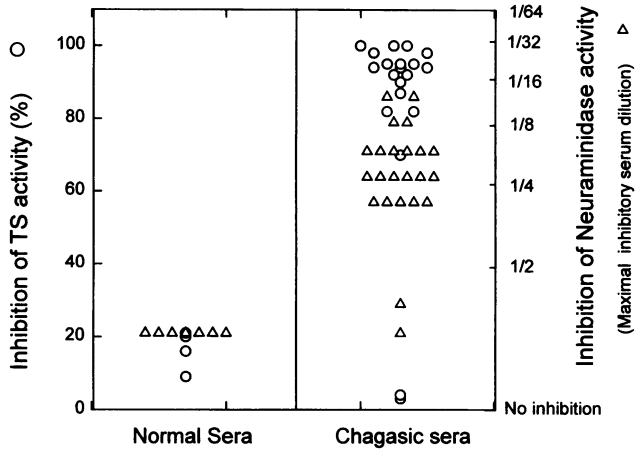

FIG. 3. Inhibition of enzymatic activity by sera from Chagasic patients. Defatted sera from human Chagasic patients were used to inhibit the neuraminidase activity, as measured by the PNA agglutination assay (triangles), or TS activity, by using TS-pap (circles). The figures are means of duplicates of each individual serum. The values are expressed as percentages of inhibition relative to TS without serum, which produces 1,200 cpm (0.13 U) in the case of the TS assay. For the neuraminidase assay is shown the maximal serum dilution that causes agglutination in the presence of 500 μg of PNA per ml, which roughly corresponds to the percentage of inhibition, as described in Materials and Methods. The sera used for the inhibition of neuraminidase and TS activities were different.

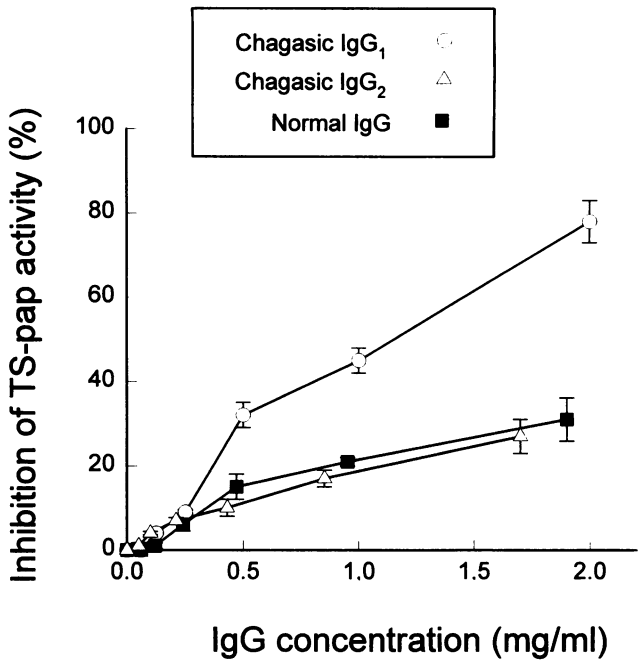

FIG. 4. Effect of immunoglobulin concentration on the TS activity. The papain fragment of TS was preincubated for 10 min with the purified immunoglobulins from an inhibitory Chagasic serum (○), a noninhibitory Chagasic serum (△), or from serum from a noninfected individual (■), and the substrates for the standard TS reaction were added. The antibody concentrations are final values after addition of substrates (40 μl), and the results are means ± standard deviations relative to controls without antibodies of triplicate measurements. The 0% value was 4,300 cpm, or 0.14 TS U.

infection. Similarly, inhibitory antibodies to both activities are detected in the infection with Y strain after the onset of parasitemia, at the sixth day, and maximal inhibition after the decline of parasitemia is at 25 days (Fig. 1C and D). The Costalimai strain, which causes patent parasitemia only in *C. callosus*, also induces inhibitory responses to neuraminidase and TS in both rodents (Fig. 1E and F). In all strains and animals, identical inhibitory responses are obtained with total serum, defatted serum, or purified immunoglobulins (not shown).

Therefore, the immune response that is inhibitory to neuraminidase-TS is mounted by animals after the contact with *T. cruzi*, independently of the appearance of the parasite in the blood. The outcome of infection varied with the strain and host. Whereas all Swiss mice die when inoculated with the Y strain, and about 50% die with F strain, *C. callosus* animals evolve to the chronic phase, with regression of most histopathologic lesions (2). In both hosts, the inhibitory antibodies appear in equal numbers when the number of parasites rises in the bloodstream. The case of Swiss mice infected with strain Costalimai is noteworthy. Although bloodstream trypomastigotes are not detected, myocardial cells contain extensive lesions with intracellular amastigotes, while *C. callosus* parasitemia is patent with low tissular aggression (2). We point out that TS is synthesized by intracellular trypomastigotes and accumulates in the cytoplasm of the infected cells (8, 15). This intracellular TS may be the source of antigenic stimulation in Swiss mice infected with the Costalimai strain. Whether the anti-TS antibodies contribute to the clearance of the parasites from the blood during the acute stages is not known.

Recently, we found that digestion of TS with papain generates a 70-kDa fragment (TS-pap) with full enzymatic activity (17). This papain fragment contains the amino-terminal domain, lacks the CTRs, and when used to immunize animals induces the formation of antibodies that inhibit TS activity. As the carboxyl-terminal repeats of TS are highly immunogenic (5), we tested whether the antibodies present in sera from infected animals also inhibit the activity of TS-pap. As shown in Fig. 2, sera of Swiss mice infected with the Y strain of *T. cruzi* inhibit the activity of entire TS or TS-pap. Similar results are obtained in mice infected with the other parasite strains.

Next, we examined the presence of inhibitory antibodies in sera from chronic Chagasic patients infected with *T. cruzi*. Out of 45 sera, only 4 did not inhibit neuraminidase or TS activities (Fig. 3). The inhibitory activity is present in whole serum, defatted serum, or purified immunoglobulins (not shown). Lipoproteins obtained from normal or Chagasic patients were not inhibitory by the TS assay and inhibit about 20% of the neuraminidase activity as measured by the PNA lectin assay. Figure 4 shows that the inhibitory effect of Chagasic sera is conserved in purified immunoglobulins. Immunoglobulins isolated either from a normal individual or from the Chagasic patients without inhibitory activity in whole sera do not inhibit TS-pap significantly, even at 2 mg/ml. In contrast, immunoglobulins isolated from most Chagasic patients inhibit TS-pap activity at concentrations as high as 0.5 mg/ml.

We have previously shown that antibodies directed to the carboxy-terminal repeats do inhibit the transfer of soluble or membrane-bound substrates by TS (17), whereas others have found inhibition of sialic acid removal from erythrocytes by the same type of antibodies (14). One possible reason for this discrepancy is that the antibodies directed to the repeats may aggregate and precipitate TS. To exclude this possibility, we repeated the assays with immobilized TS. The group of inhibitory sera decreased the activity of TS immobilized by the

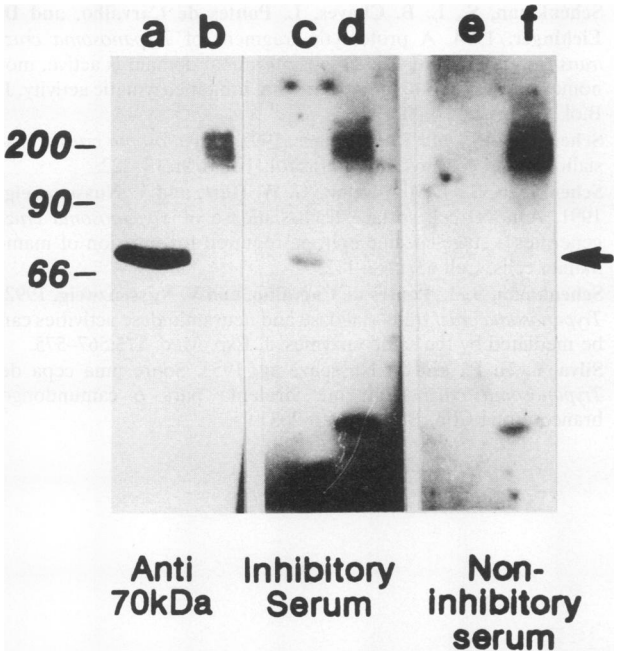

FIG. 5. Only inhibitory immunoglobulins recognize the papain fragment of TS in immunoblot. TS-pap (lanes a, c, and e) and purified TS (lanes b, d, and f) were run in SDS–7.5% polyacrylamide gels for electrophoresis, transferred to nitrocellulose membranes, and probed with the antiserum to the papain fragment (lanes a and b), an inhibitory Chagasic serum (lanes c and d), and a noninhibitory Chagasic serum (lanes e and f). The numbers at the left are molecular mass standards in kilodaltons, and the arrow on the right shows the position of migration of the papain fragment.

antirepeat antibodies preadsorbed to protein A-Sepharose by (50 ± 5)% and of the activity of TS immobilized on nitrocellulose membranes by (81 ± 8)%. Immunoglobulins isolated from the group of noninhibitory sera or from normal individuals did not decrease at all the activity of immobilized TS.

To clarify the mechanism of inhibition of TS activity, we did immunoblots with entire TS and TS-pap as antigens, probed with inhibitory and noninhibitory immunoglobulins from Chagasic sera. As shown in Fig. 5, only the immunoglobulins isolated from inhibitory sera recognize TS-pap, whereas both antibodies reacted with the same intensity to the entire TS.

Antibodies of Chagasic sera also totally inhibit the sialylation of tissue culture-derived trypomastigotes grown in the absence of serum (not shown). This was assayed by indirect immunofluorescence with the monoclonal antibody 3C9, which recognizes a sialic acid-dependent epitope (19). Unexpectedly, however, the noninhibitory Chagasic serum also inhibits sialylation, although to a lesser extent. The reasons for this are unknown and might be related to the aggregation of TS in the parasite surface, or to the presence of antibodies to the sialic acid acceptors or even unrelated antigens. In any case, the present results show that, in the blood of chronic patients and of infected animals, the sialylation of the parasite is inhibited. Since *T. cruzi* sialylation has been implicated in cell invasion, resistance to complement, and escape from the vacuole (18), the in vivo inhibition of TS may have profound consequences.

We cannot define the mechanism of inhibition or the binding sites recognized by the inhibitory antibodies. The fact that inhibition can be obtained with immobilized TS suggests that it is not due to enzyme aggregation or precipitation. It could be explained by (a) the direct covering of the catalytic or substrate binding sites, since it was obtained with soluble substrates, or (b) inactivation of TS upon antibody binding. We have no evidence to exclude each of these possibilities, and only the use of more-specific antibodies could distinguish among them. Development of such antibodies could help to delineate strategies to control trypanosome-borne diseases and provide tools to understand the TS role for these parasites.

On the other hand, the reasons for the lack of inhibition by the sera of a few patients are also unknown. It is possible that the inhibitory antibodies recognize limited epitopes in the TS molecule and that the immunogenicity of such epitopes is genetically restricted. We are currently investigating whether this restriction is related to differences in the major histocompatibility complex, and whether it reflects differential disease evolution.

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REFERENCES

1. **Affranchino, J. L., C. F. Ibanez, A. O. Luquetti, A. Rassi, M. B. Reyes, R. A. Macina, L. Aslund, U. Pettersson, and A. C. C. Frasch.** 1989. Identification of a *Trypanosoma cruzi* antigen that is shed during the acute phase of Chagas' disease. Mol. Biochem. Parasitol. **34**:221–228.
2. **Borges, M. M., S. G. de Andrade, C. G. Pilatti, J. C. do Prado, Jr., and J. K. Kloetzel.** 1992. Macrophage activation and histopathological findings in *Calomys callosus* and Swiss mice infected with several strains of *Trypanosoma cruzi*. Mem. Inst. Oswaldo Cruz Rio J. **87**:493–502.
3. **Brener, Z.** 1962. Therapeutic activity and criterion of cure in mice experimentally infected with *Trypanosoma cruzi*. Rev. Inst. Med. Trop. São Paulo **4**:389–396.
4. **Campetella, O., D. Sanchez, J. J. Cazzulo, and A. C. C. Frasch.** 1992. A superfamily of *Trypanosoma cruzi* surface antigens. Parasitol. Today **8**:378–381.
5. **Cazzulo, J. J., and A. C. C. Frasch.** 1992. SAPA/*trans*-sialidase and cruzipain: two antigens from *Trypanosoma cruzi* contain immunodominant but enzymatically inactive domains. FASEB J. **6**:3259–3264.
6. **Chaves, L. B., M. R. S. Briones, and S. Schenkman.** 1993. Trans-sialidase from *Trypanosoma cruzi* epimastigotes is expressed at the stationary phase and is different from the enzyme expressed in trypomastigotes. Mol. Biochem. Parasitol. **61**:97–106.
7. **Deane, M. P., and J. K. Kloetzel.** 1974. Lack of protection against *Trypanosoma cruzi* by multiple doses of *T. lewisi* culture forms. A discussion on some strains of "lewisi." Exp. Parasitol. **35**:406–410.
8. **Frevert, U., S. Schenkman, and V. Nussenzweig.** 1992. Stage-specific expression and intracellular shedding of the cell surface *trans*-sialidase of *Trypanosoma cruzi*. Infect. Immun. **60**:2349–2360.
9. **Mello, D. A., and M. M. Borges.** 1981. Primeiro encontro do *Triatoma costalimai* naturalmente infectado pelo *Trypanosoma cruzi*: estudos de aspectos biológicos da amostra isolada. Mem. Inst. Oswaldo Cruz Rio J. **76**:61–69.
10. **Parodi, A. J., G. D. Pollevick, M. Mautner, A. Buschiazzo, D. O. Sanchez, and A. C. C. Frasch.** 1992. Identification of the gene(s) coding for the trans-sialidase of *Trypanosoma cruzi*. EMBO J. **11**:1705–1710.
11. **Pereira, M. E.** 1983. A rapid and sensitive assay for neuraminidase using peanut lectin hemagglutination: application to *Vibrio cholerae* and *Trypanosoma cruzi*. J. Immunol. Methods **63**:25–34.
12. **Pereira, M. E. A., J. S. Mejia, E. Ortega-Barria, D. Matzilevich,**

and R. P. Prioli. 1991. The *Trypanosoma cruzi* neuraminidase contains sequences similar to bacterial neuraminidases, YWTD repeats of the low density lipoprotein receptor, and type III modules of fibronectin. J. Exp. Med. **174:**179–191.

13. **Previato, J. O., A. F. Andrade, M. C. Pessolani, and L. Mendonça-Previato.** 1985. Incorporation of sialic acid into *Trypanosoma cruzi* macromolecules. A proposal for a new metabolic route. Mol. Biochem. Parasitol. **16:**85–96.
14. **Prioli, R. P., E. Ortega-Barria, J. S. Mejia, and M. E. A. Pereira.** 1992. Mapping of a B-cell epitope present in the neuraminidase of *Trypanosoma cruzi*. Mol. Biochem. Parasitol. **52:**85–96.
15. **Rosenberg, I. A., R. P. Prioli, J. S. Mejia, and M. E. A. Pereira.** 1991. Differential expression of *Trypanosoma cruzi* neuraminidase in intra- and extracellular trypomastigotes. Infect. Immun. **59:**464–466.
16. **Schauer, R., G. Reuter, H. Muhlpfordt, A. F. Andrade, and M. E. Pereira.** 1983. The occurrence of N-acetyl- and N-glycoloylneuraminic acid in *Trypanosoma cruzi*. Hoppe-Seyler's Z. Physiol. Chem. **364:**1053–1057.
17. **Schenkman, S., L. B. Chaves, L. Pontes de Carvalho, and D. Eichinger.** 1994. A proteolytic fragment of *Trypanosoma cruzi trans*-sialidase lacking the carboxy-terminal domain is active, monomeric and generates antibodies that inhibit enzymatic activity. J. Biol. Chem. **269:**7970–7975.
18. **Schenkman, S., and D. Eichinger.** 1993. *Trypanosoma cruzi* trans-sialidase and cell invasion. Parasitol. Today **9:**218–222.
19. **Schenkman, S., J. Man-Shiow, G. W. Hart, and V. Nussenzweig.** 1991. A novel cell surface trans-sialidase of *Trypanosoma cruzi* generates a stage-specific epitope required for invasion of mammalian cells. Cell **65:**1117–1125.
20. **Schenkman, S., L. Pontes de Carvalho, and V. Nussenzweig.** 1992. *Trypanosoma cruzi* trans-sialidase and neuraminidase activities can be mediated by the same enzymes. J. Exp. Med. **175:**567–575.
21. **Silva, L. H. P., and V. Nussenzweig.** 1953. Sobre uma cepa de *Trypanosoma cruzi* altamente virulenta para o camundongo branco. Folia Clin. Biol. **20:**191–203.