EXPERIMENTAL PARASITOLOGY 77, 405–413 (1993)

Trypanosoma cruzi: Antibody Production and T Cell Response Induced by Stage-Specific Surface Glycoproteins Purified from Metacyclic Trypomastigotes

NOBUKO YOSHIDA, JORGE ENRIQUE ARAYA, JOSÉ FRANCO DA SILVEIRA, AND SELMA GIORGIO

Department of Microbiology, Immunology and Parasitology, Escola Paulista de Medicina, R. Botucatu, 862, São Paulo, Brazil

YOSHIDA, N., ARAYA, J. E., FRANCO DA SILVEIRA, J., AND GIORGIO, S. 1993. *Trypanosoma cruzi*: Antibody production and T cell response induced by stage-specific surface glycoproteins purified from metacyclic trypomastigotes. *Experimental Parasitology* 77, 405–413. The main surface glycoproteins of metacyclic trypomastigotes of *Trypanosoma cruzi*, gp90, gp82, and gp35/50, were purified and the immune response elicited by these antigens was analyzed. Balb/c mice immunized with antibody-affinity-purified gp82, plus alum as adjuvant, produced antibodies that recognized both the gp82 and the heterologous gp90 and gp35/50. On the other hand, antisera to gp90 reacted only with the homologous antigen, either by immunoprecipitation or by immunoblotting. Neither sera reacted with unrelated proteins in ELISA. Both antisera lysed 90–100% metacyclic forms in a complement-mediated reaction, a property associated with protection. However, in contrast to gp90, previously shown to induce protective immunity against acute *T. cruzi* infection, gp82 was not immunoprotective. Lymph node (LN) cells of mice primed with gp82 or gp90, which display 40% amino acid sequence identity at the carboxy terminal domain, were strongly stimulated *in vitro* by either one of these antigens. Proliferation, inhibitable by anti-CD4 but not by anti-CD8 antibodies, was *T. cruzi*-specific, no activation being observed with irrelevant antigens. LN cells of mice immunized with unrelated proteins did not proliferate *in vitro* in the presence of gp82 or gp90. The 35/50-kDa glycoconjugate, which was phenol-extracted, did not elicit any detectable antibody or T cell response. © 1993 Academic Press, Inc.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Trypanosoma cruzi;* Metacyclic trypomastigotes; Surface glycoproteins; T and B cell responses; Indirect immunofluorescence assay (IFA), Freund's complete adjuvant (FCA); Stimulation index (SI); Lymph node (LN); Keyhole lympet hemocyanin (KLH); Ovalbumin (OVA); Bovine serum albumin (BSA); Enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Infection by *Trypanosoma cruzi*, the protozoan parasite that causes Chagas' disease, stimulates both humoral and cellular immune responses (Brener 1980) but the outcome is not necessarily beneficial to the host. While antibodies, particularly those with trypanolytic activity, and $CD8^+$ cells have been reported to confer resistance to *T. cruzi* (Kierszenbaum 1980; Takehara *et al.* 1981; Krettli and Brener 1982; Araguth *et al.* 1988; Tarleton *et al.* 1992), helper T cells have been implicated in pathogenesis in the chronic infection (Said *et al.* 1985; Russo *et al.* 1988; Ribeiro dos Santos *et al.* 1992). In this context, the identification of parasite antigens that induce protective immune response, or trigger pathogenic mechanisms that lead to tissue lesions, is of crucial importance.

In the past several years, we have attempted to determine the immunoprophylactic potential of surface antigens of *T. cruzi* metacyclic forms, the insect stages infective to the mammalian host. Metacyclic trypomastigotes express on the surface a set of antigens, of 90, 82, and 35/50 kDa, referred to as gp90, gp82, and gp35/50, with no apparent counterpart in the blood trypomastigotes (Teixeira and Yoshida 1986;

0014-4894/93 $5.00
Copyright © 1993 by Academic Press, Inc.
All rights of reproduction in any form reserved.

Yoshida *et al.* 1989). These glycoproteins constitute the main surface molecules of metacyclic forms in different *T. cruzi* strains (Teixeira and Yoshida 1986; Mortara *et al.* 1988, 1992) and appear to be highly immunogenic. Mice immunized with heat-killed metacyclic trypomastigotes produce antibodies which are directed predominantly to these antigens (Teixeira and Yoshida 1986; Yoshida *et al.* 1989) and lyse metacyclic forms in a complement-dependent reaction (Yoshida, 1986; Yoshida *et al.* 1986), a property associated with protection (Krettli and Brener, 1982). In accord with this, immunized mice have been found to resist acute infection upon challenge with vector-derived metacyclic forms (Yoshida *et al.* 1984; Yoshida 1986).

In view of these observations, we have focused on the determination of the immunologic properties of purified metacyclic surface antigens. The 90-kDa glycoprotein, for instance, has been shown to induce specific antibody and T-cell responses in mice (Araguth *et al.* 1988) and to confer protection against acute *T. cruzi* infection when given with alum as adjuvant (Gonzalez *et al.* 1991).

To further proceed in this series of studies, in the present paper we analyzed the immune response elicited in mice by purified gp82 and gp35/50, compared to that elicited by gp90.

Materials and Methods

T. cruzi G strain (Yoshida 1983) was used throughout this study. Parasites were maintained cyclically in mice and in liver infusion tryptose medium (Camargo 1964). Metacyclic trypomastigotes were purified through passage in DEAE–cellulose column as previously described (Yoshida 1986).

Immunoblotting (Towbin *et al.* 1979) and the standard procedures for cell surface iodination (Markwell and Fox 1978), preparation of parasite extracts, immunoprecipitation (Kessler 1975), and SDS–PAGE (Laemmli 1970) have been detailed elsewhere (Teixeira and Yoshida 1986; Yoshida *et al.* 1989).

To purify gp82, the following procedure was used. Antibody affinity column was prepared by coupling monoclonal antibody 3F6 to CNBr-activated Sepharose 4B (Pharmacia LKB, Uppsala, Sweden) according to the manufacturer's instructions. Parasites were lysed with 0.5% Nonidet P-40 in PBS containing protease inhibitors phenylmethylsulfonyl fluoride (1 m*M*), iodoacetamide (1 m*M*), antipain (25 μg/ml), and leupeptin (25 μg/ml). After centrifugation at 12,000*g* for 5 min, the supernatant was collected and mixed with 3F6-Sepharose for 2 hr at 4°C under constant shaking. The resin was then packed at 5-cm height in a 10-ml plastic syringe, washed several times with PBS, followed by three washings with distilled water. The bound antigen was eluted with 50 m*M* triethylamine, pH 11.6, and dried in a speed vacuum concentrator. The amount of protein in the antigen preparation was determined in 96-well microtiter plates (Falcon–Becton Dickinson & Co., NJ) by reaction with 0.01% (w/v) Coomassie brilliant blue G solution containing 5% ethanol and 10% (v/v) phosphoric acid, followed by reading of optical density in Multiscan MCC/340 P, at 620 nm.

The 90-kDa glycoprotein was obtained as described by Araguth *et al.* (1988), by using 1G7-antibody affinity chromatography. To determine the amount of the purified preparation we followed the procedure used for gp82. Purification of gp35/50 by antibody affinity column was not possible because the monoclonal antibody 10D8 precipitates at 4°C even at relatively low concentrations. Therefore, an alternative method of phenol extraction (Previato *et al.* 1985, Ruiz *et al.* 1993) was used. The amount of antigen was estimated by colorimetric method for sugar and related substances (Dubois *et al.* 1956) in 96-well microtiter plates.

For immunization experiments, 6- to 8-week-old Balb/c mice from Escola Paulista de Medicina were used. To elicit antibody response, mice were inoculated intraperitoneally with 10 μg of purified *T. cruzi* antigen absorbed into aluminium hydroxide, an adjuvant devoid of side effects and shown to be effective in immunization with purified *T. cruzi* antigen (Gonzalez *et al.* 1991). Starting on Day 10 upon priming, each mouse received three weekly doses of 5 μg of antigen plus 0.5 mg of alum. One week after the last immunizing dose, the sera were assayed for *T. cruzi*-specific antibodies.

To study the proliferative response of lymph node (LN) cells, mice were immunized in the hind footpads with 40 μg of purified antigen emulsified in Freund's complete adjuvant in a total volume of 0.1 ml per mouse. The draining lymph nodes were excised 10 days after priming and cell suspensions were obtained by homogenization in RPMI 1640 with a loose tissue grinder. Cells were washed twice with RPMI 1640 and adjusted to a concentration of 4×10^6 cells/ml, in RPMI 1640 supplemented with 10 m*M* Hepes, 2 m*M* L-glutamine, 1% minimum essential medium containing nonessential amino acids, 1 m*M* sodium pyruvate,

5×10^{-5} *M* 2-mercaptoethanol, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 0.5% normal syngeneic mouse serum. Aliquots of 0.1 ml of the cell suspension were deposited onto 96-well plates (Costar, Cambridge, MA) to which varying concentrations of parasite antigen were added, in triplicates. The cultures were incubated at 37°C in 5% CO_2 for 4 days. After this period, cells were pulsed with 1 μCi of [^{3}H]thymidine per mmol) per well for 18 hr. At the end of the incubation period, lymphocytes were harvested onto glass fiber paper, dried, and counted by beta emission spectroscopy. The results are presented as the mean of triplicates and the standard deviation of the experimental mean, which was never higher than 20% of the mean control values, was omitted. Lymphocyte proliferation was analyzed by Student's *t* test ($P < 0.02$). Proliferation assay was also performed in the presence of anti-CD4 monoclonal antibody GK1.5 (Wilde *et al.* 1983), a gift from Dr. Olga Martinez, Instituto Butantan, São Paulo, Brazil, or anti-CD8 monoclonal antibody H35-89.9 (Pierres *et al.* 1982), kindly provided by Dr. Momtchilo Russo, Instituto de Ciências Biomédicas, USP, São Paulo, Brazil.

Indirect immunofluorescence assay (IFA) was carried out using formaldehyde-fixed metacyclic forms. Assay for complement-dependent lysis (Krettli and Brener 1982) was performed essentially as described by Mortara *et al.* (1992).

Amino acid sequences of gp82 and gp90 were deduced from nucleotide sequences of cDNA clones isolated from a *T. cruzi* expression library constructed in phage λ gt11 (Franco *et al.* 1993; Araya *et al.*, in preparation).

RESULTS

We used several criteria to ascertain the purity of metacyclic trypomastigote glicoproteins. The purity of gp90 was checked by Coomassie blue and silver staining of SDS–PAGE gels, and also by autoradiography of ^{125}I-labeled preparation, as previously described (Gonzalez *et al.* 1991). In addition to Coomassie blue and silver staining, the purity of gp82 preparation was determined by probing the glycoprotein blotted on nitrocellulose membranes with ^{125}I-labeled monoclonal antibodies 3F6 and 1G7. The monoclonal antibody 3F6 recognizes an 82-kDa antigen in several *T. cruzi* strains (Y, Cl, and Tulahuen) and a doublet band of 75/82 kDa in G strain metacyclic trypomastigotes (Teixeira and Yoshida 1986), whereas the monoclonal antibody 1G7 reacts with a single band of 90 kDa in metacyclic forms of different strains (Mortara *et al.* 1988). Figure 1 shows that the gp82 contains 3F6-reactive but not 1G7-reactive antigens. In the case of phenol-extracted parasite preparation, the silver staining and Schiff's reagent has consistently revealed the 35/50-kDa bands as the sole components, as previously reported by Schenkman *et al.* (1993).

To determine the antibody response induced by purified *T. cruzi* antigens, mice were immunized according to the schedule described under Materials and Methods and their sera were examined for reactivity with metacyclic trypomastigote antigens. By immunoprecipitation of ^{125}I-labeled parasite preparations, sera of mice immunized with gp82 revealed, in addition to the homologous antigen, the heterologous gp90 (Fig. 2A, lane 3). The converse, i.e., recognition of gp82 by sera of mice immunized with purified gp90, was not observed (Fig. 2A, lane 2). By immunoblotting, both sera were found to react essentially with the homologous antigen (Fig. 2B). Our interpretation for these findings is that gp90 carries

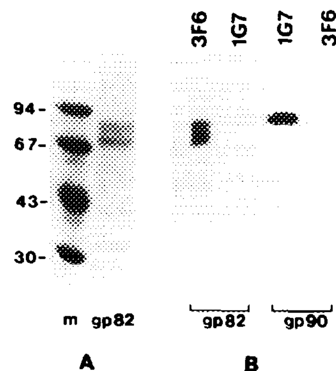

FIG. 1. Analysis of the purified gp82. The antigen purified by 3F6 antibody affinity column was subjected to SDS–PAGE and the gel stained with Coomassie blue (A) or blotted onto nitrocellulose membrane and probed with ^{125}I-labeled monoclonal antibodies 3F6 and 1G7 (B). As a control, nitrocellulose strips containing purified gp90 were probed with the same labeled antibodies. Molecular size markers (m) are shown on the left.

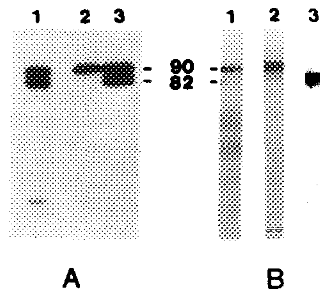

FIG. 2. Reactivity of *T. cruzi* metacyclic trypomastigote antigens with different immune sera. (A) Metacyclic forms were surface labeled with ^{125}I and the detergent-lysed cell extracts were precipitated with sera of mice immunized with heat-killed metacyclic forms (lane 1), purified gp90 (lane 2), or gp82 (lane 3), and analyzed by SDS–PAGE. (B) Immunoblot analysis of metacyclic trypomastigote extracts upon reaction with the same sera used in experiment A (lanes 1–3), followed by sequential incubation with anti-mouse IgG conjugated to horseradish peroxidase and the substrate diaminobenzidine. The numbers between A and B correspond to the molecular mass of parasite antigens.

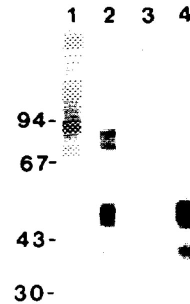

FIG. 3. Immunoblot analysis of *T. cruzi* metacyclic trypomastigote antigens. Nitrocellulose strips containing parasite extracts were probed with sera of mice immunized with gp90 (lane 1), gp82 (lane 2), or gp35/50 (lane 3). Lane 4 shows the strip containing gp35/50 purified by phenol extraction probed with antiserum to gp82. ^{125}I-labeled protein A was used for detection of bands. Molecular sizes, in kilodaltons, are shown on the left.

immunodominant epitopes not shared by gp82 and that antibodies elicited by immunization with gp90 are most, if not exclusively, directed to these epitopes. On the other hand, the epitopes on gp90 shared by gp82 would be minor epitopes and apparently conformational, their reactivity with anti-gp82 sera being abolished when the antigen is immobilized on nitrocellulose filters after denaturation. Unlike gp90, the gp82 presumably bears several nondominant epitopes, which would induce antibodies of different specificities, including those directed to conformational epitopes shared by gp90.

In addition to gp90, in some preparations gp35/50 could also be visualized by antisera to gp82. This finding was confirmed by using ^{125}I-labeled protein A, instead of peroxidase-conjugated anti-mouse Ig, to reveal the immunocomplexes (Fig. 3, lane 2). Since gp35/50 is a mucin-like glycoprotein containing at least 60% carbohydrate (Schenkman *et al.* 1993), the epitopes recognized by anti-gp82 sera could be carbohydrates. To test this possibility, we treated nitrocellulose strips containing metacyclic trypomastigote extract with sodium periodate for 1 hr, followed by reduction with sodium borohydride, as described by Woodward *et al.* (1985). This treatment, which destroys carbohydrate epitopes, has been used to reveal cross-reactive carbohydrate determinants of *T. cruzi* antigens (Xu and Powell 1991). As shown in Fig. 4, the reactivity of anti-gp82 sera with gp35/50 was completely abolished, and with gp82 decreased, upon periodate treatment. Of note is the finding that the gp35/50 purified by phenol extraction failed to induce antibody response. Sera of mice immunized with gp35/50 were negative by IFA (Table I) and were unable to reveal any parasite antigen on immunoblots (Fig. 3, lane 3). However, the antigenic properties of gp35/50 appear to be preserved since it is recognized by anti-gp82 sera (Fig. 3, lane 4).

Immune sera against gp82 and gp90 displayed comparable IFA titers and extensively lysed metacyclic trypomastigotes in the presence of complement (Table 1). Lytic antibodies have been associated with protection (Krettli and Brener 1982) and accordingly, mice immunized with gp90 plus

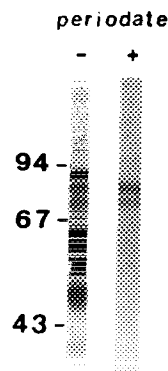

FIG. 4. Effect of periodate treatment on the reactivity of gp35/50 with anti-gp82 sera. G strain metacyclic trypomastigote extract was subjected to SDS–PAGE and blotted onto nitrocellulose membrane. One of the strips was treated with sodium periodate before reaction with anti-gp82 sera and ^{125}I-labeled protein A. Note that reactivity of gp82 sera with gp35/50 was completely abolished upon periodate treatment.

alum as adjuvant, have been shown to resist acute infection when challenged with metacyclic forms of virulent *T. cruzi* strains (Gonzalez *et al.* 1991). However, immunization with gp82, following the same protocol, did not induce protective immunity, with immunized mice developing parasitemia levels comparable to those of nonimmunized controls upon challenge.

TABLE I
IFA Titer and Complement-Mediated Trypanolytic Activity of Mouse Antisera to *T. cruzi* Metacyclic Antigens

Antiserum[a] to	IFA titer[b]		Lysis[c] (%)	
	Expt I[d]	Expt II[d]	Expt I	Expt II
gp82	1600	1600	100	90.8
gp90	1600	3200	100	100
gp35/50	—	—	2	9
NMS[e]	—	—	8	5

[a] Antisera were obtained by immunization of Balb/c mice with purified parasite antigen, according to the schedule described under Materials and Methods.

[b] IFA titer is expressed as the reciprocal values of the highest serum dilution.

[c] Percentage of lysis was determined by counting under a phase-contrast microscope the relative numbers of lysed parasites versus live motile metacyclic trypomastigotes.

[d] Sera from two different groups of immunized mice were used in experiments I and II.

[e] Normal mouse serum.

We also determined the T cell response induced by purified metacyclic trypomastigote antigens. LN cells from individual mice primed with gp82 were stimulated *in vitro* in the presence of varying concentrations of purified antigen. As shown in Fig. 5, LN cells proliferated toward gp82 in a dose-dependent manner, maximum stimulation being reached at a concentration as low as 2.5 μg/ml. No proliferation was observed with a nonrelated antigen from *Leishmania*. The proliferative response was abolished by anti-CD4 monoclonal antibody, but not by anti-CD8 antibodies, at 5 μg/ml. Stimulation of LN cells from mice that received sham emulsion was negligible (Fig. 5).

We also examined the proliferative response of gp82-primed LN cells toward other *T. cruzi* surface antigens as well as irrelevant antigens. Proliferation was observed in the presence of gp90 but not of KLH or OVA (Table II). Similar results were obtained with LN cells primed with gp90 that were strongly stimulated by both gp90 and gp82. This cross-reactivity is not surprising considering that the known carboxy terminal domain of gp90 displays 40% amino acid sequence identity with the corresponding domain of gp82 (Fig. 6).

Experiments of T cell proliferation with gp35/50 consistently gave negative results. LN cells primed with gp35/50 did not proliferate even toward the homologous antigen (Table II), up to 40 μg/ml.

DISCUSSION

In this study we have analyzed the immune response elicited in mice by purified *T. cruzi* metacyclic trypomastigote antigens. Immunization of Balb/c mice with gp82 or gp90 induced parasite-specific antibody as well as T cell responses, whereas gp35/50 failed to stimulate either T or B cell response. This lack of response does not

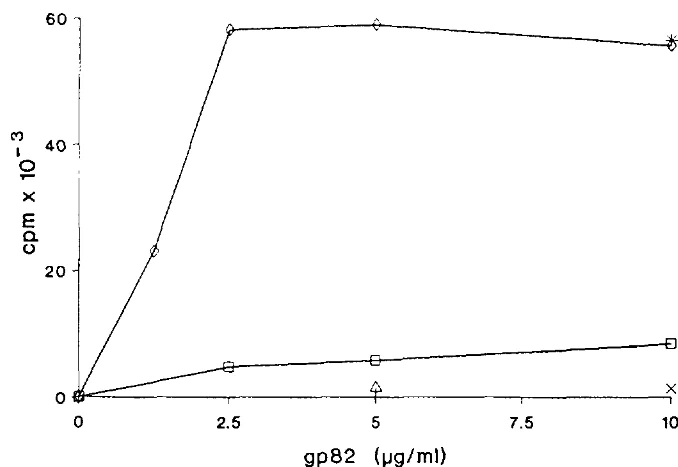

FIG. 5. Proliferative response of lymph node cells to gp82. Cells obtained from Balb/c mice 10 days after priming with purified gp82 (◇) or with FCA alone (□) were stimulated *in vitro* with the indicated doses of antigen in the absence or in the presence of anti-CD4$^+$ (X) or anti-CD8$^+$ (*) antibodies. Note that there was no proliferation with *Leishmania* antigen (△). Shown are the results of one of four comparable experiments, expressed as means of triplicates. Values of samples without antigen have been subtracted.

necessarily mean that gp35/50 is intrinsically devoid of immunogenic properties. Since this glycoprotein was prepared by phenol extraction, it is possible that the immunogenicity of the antigen has been impaired by this purification procedure.

We have found that gp82 shares T cell epitopes with gp90. *In vitro* proliferation experiments showed that gp82 and gp90 stimulate CD4$^+$ cells from mice primed with either one of these antigens. Since the amino acid sequence of the carboxy terminal domain of gp90, deduced from a recombinant cDNA clone (Franco *et al.* 1993), bears considerable sequence similarity with the corresponding domain of gp82 (Fig. 6), this cross-reactivity was not unexpected. Actually, preliminary experiments with recombinant antigens containing the referred sequences of gp82 and gp90 indicate that common T cell epitopes are contained within this domain.

TABLE II
Proliferative Response of Lymph Node Cells of Mice Immunized with Purified *T. cruzi* antigens

	SI[b] in response to				
In vivo	*T. cruzi* antigen[c]			Unrelated antigen[c]	
priming[a]	gp82	gp90	gp35/50	KLH	OVA
gp82	7.2	5.6	1.5	1.1	1.5
gp90	7.2	8.2	1.0	ND	ND
gp35/50	1.1	0.9	1.2	1.4	1.5
KLH	1.6	1.7	ND	5.3	1.7
OVA	1.6	1.4	ND	0.9	6.5

Note. ND, not determined.

[a] Mice were primed with 40–50 μg of purified *T. cruzi* antigens or irrelevant proteins.

[b] Stimulation index (SI) = cpm of culture containing antigen/cpm of culture without antigen.

[c] The concentration of *T. cruzi* antigen in culture was 10 μg/ml and that of unrelated protein was 40 μg/ml.

In addition to T cell epitopes, gp82 and gp90 also share B cell epitopes. One intriguing aspect of these cross-reactive epitopes is that immunization of mice with gp82, but not with gp90, elicit antibodies to these epitopes. As a consequence, sera of mice immunized with gp82 immunoprecipitate both gp82 and gp90, but anti-gp90 sera only recognize the homologous antigen (Fig. 2A). We presume that gp90 bears immunodominant epitopes not present in gp82 so that antibodies generated by immunization

```
gp-82   PVNNRESNGPH-------TFVITFTLCDVIVHKVPKNSTTLLGAVLAEPISTLFIGLSYGTDGTWE
gp-90   PVNTRVNNVRHVSLSHNFTLVASVTI-----EEAPSADAPLMGAMLGDTNSQYTMGVLYTADKEWV
        *** *  *  *       * * . *.        *    .*.**.* .  *   .*. * .*  *

gp-82   TVFNGETTTSGSTWMPGKEYQVALMLQDGNKGSVYVDGMSVGSLATLPTPEVRGAEIADFYFVGGE
gp-90   TIFNGKKTTESGTWEPGKEYQKALMLQ-GNKSSVYVDGKSLG------------------------
        *.***  **   ** ****** ***** *** ****** *.*

gp-82   DEEDK---------------------KSSSVTVKNVFLYNRPLGADELRMVKKIDGSMHGGVSR
gp-90   -EEELPLLQSERPLEYLSFCFGGCGIKNFPVTVKNVFLYNRPLNPTEMTAIK--DRKPKDEKGR
         **.                      *   *************   *.  .*  *        *
```

FIG. 6. Comparison of sequences of the carboxy terminal domain of gp82 and gp90. The deduced partial amino acid sequences of gp90 (Franco *et al.* 1993) and gp82 (Araya *et al.*, in preparation) are shown. Asterisks and points indicate identical and similar residues, respectively.

with gp90 are mostly directed to these epitopes. In fact, Gonzalez *et al.* (1991) have shown that most antibodies induced by purified gp90 recognize the same or topographically related sites as the monoclonal antibody 1G7, which reacts exclusively with gp90 and is known to reduce infectivity of metacyclic forms *in vitro* and *in vivo* (Araguth *et al.* 1988; Yoshida *et al.* 1990). On the other hand, immunization with gp82 generates a repertoire of antibodies directed to different epitopes, including the gp82-specific epitopes, the conformational epitopes shared by gp90, and the periodate-sensitive carbohydrate epitopes.

These differences between gp82 and gp90 may explain why immunization of mice with gp90 confers resistance to acute *T. cruzi* infection (Gonzalez *et al.* 1991) whereas no such effect is observed in mice immunized with gp82. The indications are that antibodies to gp90, induced by immunodominant epitopes not shared by other metacyclic antigens, play a role in protective immunity. In contrast, although gp82 induces antibodies of different specificities, it is possible that none of these antibodies are protective. Alternatively, the correct antibodies may be produced but the concentration appropriate to confer resistance is not achieved.

Another aspect of immunization with purified *T. cruzi* antigens that has been addressed in preliminary experiments is whether it engenders nonspecific polyclonal cell activity, such as described by Pestel *et al.* (1992). These authors have reported that an octadecapeptide derived from an 85-kDa surface protein of *T. cruzi*, coupled to a carrier protein, can stimulate lymph node cells from mice primed with FCA, KLH, or tetanus toxoid and, in addition, antibodies elicited by immunization with the coupled peptide react in ELISA with irrelevant antigens, such as KLH, OVA, BSA, and fibronectin. Since nonspecific polyclonal lymphocyte activation may play a role in the autoimmunity in chronic *T. cruzi* infection (Minoprio *et al.* 1986), it has been proposed that the *T. cruzi* octadecapeptide could be one of the molecules that, through activation of polyclonal response, leads to the appearance of autoimmune reaction (Pestel *et al.* 1992). In our study, by testing antisera to gp80 and gp90 in ELISA, we did not detect any reactivity with the nonparasite proteins used by Pestel *et al.* (1992) (data not shown). Neither have we seen nonspecifically primed T cells respond to gp82 or gp90.

All these data indicate that, if immune response can be biased toward the protective epitopes by specific *T. cruzi* antigens following particular immunization procedures, control of infection without damaging effects to the host may not be out of reach.

ACKNOWLEDGMENTS

This work was supported by the United Nations Development Program/World Health Organization Special Programme for Research and Training in Tropical

Disease, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), PADCT-Conselho Nacional de Desenvolvimento Científico e Tecnológico (PADCT-CNPq). We thank Dr. Marcello A. Barcinski for critical reading of this manuscript.

REFERENCES

ARAGUTH, M. F., RODRIGUES, M. M., AND YOSHIDA, N. 1988. *Trypanosoma cruzi* metacyclic trypomastigotes: Neutralization by the stage-specific monoclonal antibody 1G7 and immunogenicity of 90 kD surface antigen. *Parasite Immunology* **10**, 707–712.

BRENER, Z. 1980. Immunity to *Trypanosoma cruzi*. *Advances in Parasitology* **18**, 247–292.

CAMARGO, E. P. 1964. Growth and differentiation in *Trypanosoma cruzi*. I. Origin of metacyclic trypanosomes in liquid media. *Revista do Instituto de Medicina Tropical de São Paulo* **6**, 93–100.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., AND SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350–356.

FRANCO, F. R. S., PARANHOS-BACALLA, G. S., YAMAUCHI, L. M., YOSHIDA, N., AND FRANCO DA SILVEIRA, J. 1993. Characterization of a cDNA clone encoding the carboxy-terminal domain of a 90-kilodalton surface antigen of *Trypanosoma cruzi* metacyclic trypomastigotes. *Infection and Immunity* **61**, in press.

GONZALEZ, J., ARAGUTH, M. F., AND YOSHIDA, N. 1991. Resistance to acute *Trypanosoma cruzi* infection resulting from immunization of mice with a 90-kilodalton antigen from metacyclic trypomastigotes. *Infection and Immunity* **59**, 863–867.

KESSLER, S. W. 1975. Rapid isolation of antigens from cells with staphylococcal protein A antibody absorbent: Parameters of the interaction of antibody–antigen complexes with protein A. *Journal of Immunology* **115**, 1617–1624.

KIERSZENBAUM, F. 1980. Protection of congenitally athymic mice against *Trypanosoma cruzi* infection by passive antibody transfer. *Journal of Parasitology* **66**, 673–675.

KRETTLI, A. U., AND BRENER, Z. 1982. Resistance against *Trypanosoma cruzi* associated to anti-living trypomastigote antibodies. *Journal of Immunology* **128**, 2009–2012.

LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**, 680–685.

MARKWELL, M. A. K., AND FOX, C. F. 1978. Surface-specific iodination of membrane proteins of viruses and eukariotic cells using 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril. *Biochemistry* **17**, 4807–4817.

MINOPRIO, P. M., EISEN, H., FORNI, L., D'IMPERIO LIMA, M. R., JOSKOWICZ, AND COUTINHO, A. 1986. Polyclonal lymphocyte responses to murine *Trypanosoma cruzi* infection. *Scandinavian Journal of Immunology* **24**, 661–668.

MORTARA, R. A., ARAGUTH, M. F., AND YOSHIDA, N. 1988. Reactivity of stage-specific monoclonal antibody 1G7 with metacyclic trypomastigotes of *Trypanosoma cruzi* strains: Lytic property and 90000 mol. wt surface antigen polymorphism. *Parasite Immunology* **10**, 369–378.

MORTARA, R. A., SILVA, S., ARAGUTH, M. F., BLANCO, S. A., AND YOSHIDA, N. 1992. Polymorphism of the 35- and 50-kilodalton surface glycoconjugates of *Trypanosoma cruzi* metacyclic trypomastigotes. *Infection and Immunity* **60**, 4673–4678.

PESTEL, J., DEFOORT, J., GRASS-MASSE, H., AFCHAIN, D., CAPRON, A., TARTAR, A., AND OUAISSI, A. 1992. Polyclonal cell activity of a repeated peptide derived from the sequence of an 85-kilodalton surface protein of *Trypanosoma cruzi* trypomastigotes. *Infection and Immunity* **60**, 715–719.

PIERRES, M., GORIDIS, C., AND GOLSTEIN, P. 1982. Inhibition of murine T cell-mediated cytolysis and T cell proliferation by a rat monoclonal antibody immunoprecipitating two lymphoid cell surface polypeptides of 94,000 and 180,000 molecular weight. *European Journal of Immunology* **12**, 60–69.

PREVIATO, J. O., ANDRADE, A. F., PESSOLANI, M. C., AND MENDONÇA-PREVIATO, L. 1985. Incorporation of sialic acid into *Trypanosoma cruzi* macromolecules: A proposal for a new metabolic route. *Molecular and Biochemical Parasitology* **16**, 85–96.

RIBEIRO DOS SANTOS, R., ROSSI, M. A., LAUS, J. L., SILVA, J. S., SAVINO, W., AND MENGEL, J. 1992. Anti-CD4 abrogates rejection and reestablishes long-term tolerance to syngeneic newborn hearts grafted in mice chronically infected with *Trypanosoma cruzi*. *Journal of Experimental Medicine* **175**, 29–39.

RUIZ, R. C., RIGONI, V. L., GONZALEZ, J., AND YOSHIDA, N. 1993. The 35/50 kDa surface antigen of *Trypanosoma cruzi* metacyclic trypomastigotes, an adhesion molecule involved in host cell invasion. *Parasite Immunology* **15**, 121–125.

RUSSO, M., STAROBINAS, N., MINOPRIO, P., COUTINHO, A., AND HONTEBEYRIE-JOSKOWICZ, M. 1988. Parasitic load increases and myocardial inflammation decreases in *Trypanosoma cruzi*-infected mice after inactivation of helper T cells. *Annales de L'Institut Pasteur: Immunology* **139**, 225–236.

SAID, G., JOKOWICZ, M., BARREIRA, A. A., AND EISEN, H. 1985. Neuropathy associated with experimental Chagas' disease. *Annals of Neurology* **18**, 676–683.

SCHENKMAN, S., FERGUSON, M. A. J., HEISE, N., CARDOSO DE ALMEIDA, M. L., MORTARA, R., AND

Yoshida, N. 1993. Mucin-like glycoproteins linked to the membrane by glycosylphosphatidylinositol anchor are the major acceptors of sialic acid in a reaction catalysed by trans-sialidase in metacyclic forms of *Trypanosoma cruzi*. *Molecular and Biochemical Parasitology* 59, 293–304.

Takehara, H. A., Perini, A., Silva, M. H., and Mota, I. 1981. *Trypanosoma cruzi:* Role of different antibody classes in protection against infection in the mouse. *Experimental Parasitology* 52, 137–146.

Tarleton, R. L., Koller, B. H., Latour, A., and Postan, M. 1992. Susceptibility of β_2-microglobulin-deficient mice to *Trypanosoma cruzi* infection. *Nature* 356, 338–340.

Teixeira, M. M. G., and Yoshida, N. 1986. Stage-specific surface antigens of metacyclic trypomastigotes of *Trypanosoma cruzi* identified by monoclonal antibodies. *Molecular and Biochemical Parasitology* 18, 271–282.

Towbin, H., Staehelin, T., and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Science USA* 76, 4350–4354.

Wilde, D. B., Marrack, P., Kappler, J., Dialynas, D. P., and Fitch, F. W. 1983. Evidence implicating L3T4 in class II MHC antigen reactivity: Monoclonal antibody GK1.5 (anti-L3T4a) blocks class II MHC antigen-specific proliferation, release of lymphokines, and binding by cloned murine helper T lymphocyte lines. *Journal of Immunology* 131, 2178–2183.

Woodward, M. P., Young, W. W., and Bloodgood. 1985. Detection of monoclonal antibodies specific for carbohydrate epitopes using periodate oxidation. *Journal of Immunological Methods* 78, 143–153.

Xu, B., and Powell, M. R. 1991. Carbohydrate epitopes are responsible for antibody cross-reactivity in *Trypanosoma cruzi*-infected mice. *Journal of Parasitology* 77, 808–810.

Yoshida, N. 1983. Surface antigens of metacyclic trypomastigotes of *Trypanosoma cruzi*. *Infection and Immunity* 40, 836–839.

Yoshida, N., Teixeira, M. G., Christo, C. B., and Sbravate, C. A. 1984. Resistance of mice immunized with killed culture trypomastigotes against infection by insect-derived trypomastigotes of *Trypanosoma cruzi*. *Revista do Instituto de Medicina Tropical de São Paulo* 26, 329–333.

Yoshida, N. 1986. *Trypanosoma cruzi:* Recognition of trypomastigote surface antigens by lytic antisera from mice resistant to acute infection. *Experimental Parasitology* 61, 184–191.

Yoshida, N., Teixeira, M. M. G., and Sbravate, C. A. 1986. Antigen characterization of vector-borne and cultured metacyclic trypomastigotes of *Trypanosoma cruzi*. *Revista do Instituto de Medicina Tropical de São Paulo* 28, 80–86.

Yoshida, N., Mortara, R. A., Araguth, M. F., Gonzalez, J. C., and Russo, M. 1989. Metacyclic neutralizing effect of monoclonal antibody 10D8 directed to the 35 and 50 kilodalton surface glycoconjugates of *Trypanosoma cruzi*. *Infection and Immunity* 57, 1663–1667.

Yoshida, N., Blanco, S. A., Araguth, M. F., Russo, M., and Gonzalez, J. 1990. The stage-specific 90-kilodalton surface antigen of metacyclic trypomastigotes of *Trypanosoma cruzi*. *Molecular and Biochemical Parasitology* 39, 39–46.

Received 2 April 1993; accepted with revision 25 August 1993