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Antigenic determinants of *Trypanosoma cruzi* defined by cloning of parasite DNA

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A genomic DNA library from *Trypanosoma cruzi*, the agent of Chagas' disease, was constructed in the gt11 lambda vector and was screened with serum from a Chagasic patient. Out of 53 positive clones, 23 plaques were purified to homogeneity and 10 different groups were defined by cross-hybridization experiments and by reaction of antibodies selected with products from each recombinant clone. Native *T. cruzi* proteins of molecular mass ranging from 85 to larger than 205 kDa that share antigenic determinants with products of the recombinant clones were observed in Western blots of parasite extracts. Some of the native proteins were detected in the trypomastigote stage of the parasite, while others were present in epimastigotes as well. The latter result was confirmed for some recombinant clones by hybridization of the cloned DNA with Northern blots of parasite RNA. Clones from each group reacted differently with nine sera from rabbits infected with several *T. cruzi* strains as well as with eight sera from human patients. Clone 7 was detected by all rabbit sera but not by three human sera. Conversely, clones 1, 2 and 30 were detected by all human sera but failed to be detected by most rabbit sera. We conclude that several proteins from *T. cruzi* are antigenically active during infection and that some of them differ in their ability to generate antibodies in rabbit or human infections.

Key words: Antibody response; Chagas' disease; Gene cloning; *Trypanosoma cruzi* antigens

Introduction

Chagas' disease is one of the most important endemic problems in Central and South America, for which no definitive chemotherapeutic or immunological treatment is available. Even diagnosis is a practical problem to be worked out, since most of the current serological methods make use of non-standardized and heterogeneous populations of antigens [1]. Its etiological agent, *Trypanosoma cruzi*, has a complex life cycle which involves different forms of the parasite in the insect vector and the mammalian host [2]. There are two parasite stages in the vector: epimastigotes (in the midgut) and metacyclic trypomastigotes (in the rectum), whereas the stages present in the mammalian host are bloodstream trypomastigotes and intracellular amastigotes.

Recent studies provided important information on the host immune response in Chagas' disease (see ref. 3 for a recent review). Protective or lytic antibodies have been detected in the blood of chronically infected mice [4]; in mice immunized with irradiated *T. cruzi* trypomastigotes [5] and in mice immunized with killed trypomastigotes [6]. Interestingly, neither serum transfer of protective immunity nor lytic antibodies were given on immunization with epimastigote forms of the parasite [4–6]. It is not clear yet which *T. cruzi* protein(s) are involved in the production of lytic antibodies, since different research groups have identified proteins with different molecular weights related to the immunological response [5–7].

The surface (glyco)protein makeup of *T. cruzi* has also been analyzed by iodination or biosynthetic labelling of live parasites followed by im-

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Abbreviations: BSA, bovine serum albumin; IPTG, isopropyl-β-D-thiogalactopyranoside; SDS, sodium dodecyl sulfate; SSC, saline sodium citrate.

munoprecipitation with immune sera [8–14], lectin affinity chromatography [11,14,15] or reaction with monoclonal antibodies [16–20]. Despite early reports describing one major surface glycoprotein common to the three stages of the parasite [8], it is now evident that a number of surface (glyco)proteins are present in the *T. cruzi* membrane, some of which are stage-specific [5,6,10, 19]. Among them, an 85 kDa protein, present only in trypomastigotes, has been partially correlated with the process of parasite internalization in mammalian cells [21]; another protein of the same molecular weight has been characterized as a fibronectin receptor [22]. Recently, and for the first time, a gene for a *T. cruzi* surface protein was cloned and its product characterized as a 85 kDa protein [23].

Given the complex antigenic composition of *T. cruzi*, further work is required not only for a better understanding of the biology of the parasite but also to find molecules that could be applied to practical problems like diagnosis and vaccination. In this paper we address the question of the antigenic makeup of *T. cruzi* by asking which parasite molecules are detected by sera from Chagasic patients.

Materials and Methods

Parasites and sera. T. cruzi epimastigotes were grown in liquid medium [24]. Trypomastigotes from an infected Vero cell-culture were obtained as described by Zingales et al. [21]. The strains used in this study were the Miranda/76 clone [25] and the RA strain [26]. Human sera were obtained from patients diagnosed by serology in Buenos Aires (H1, H2, H3 and controls) or from Chaco province (Northern Argentina) (H8, H9, H11, H27, H33 and controls). Rabbit sera were from animals infected intraperitoneally with 2×10^6 live trypomastigotes. Strains used for the inoculation of the rabbits were as follows (numbers indicate that different antisera against the same strain were used), Tul-20 and -24, Y-22 and -27, AWP-12 and -25, CA1, RA and UP (see ref. 26 for further information on the above *T. cruzi* strains). Sera were obtained 30 days post-infection except in the case of Tul-20 and AWP-12 (15 days post-infection) and CA1 and Y-27 (60 days post-infection).

Nucleic acids purification and DNA cloning. Total DNA was prepared from the parasites and the kinetoplast DNA was separated from nuclear DNA by centrifugation [27]. RNA was purified by the urea-LiCl method [28], electrophoresed in 1.2% agarose gels and blotted onto nitrocellulose filters using the method of Thomas [29]. The molecular weight marker for RNA gels was HindIII-digested glyoxal-treated lambda DNA. Hybridization of Southern and Northern blots with DNA probes was done as described [25]. Final washes of the filters were performed in 0.1 × SSC (1 × SSC = 150 mM NaCl/15 mM sodium citrate), plus 0.1% sodium dodecyl sulfate (SDS) at 65°C.

Nuclear DNA from epimastigotes of the *T. cruzi* Miranda/76 was cloned in λ gt11 by conventional methods [30]. Briefly, random fragments were generated by passing the DNA through a syringe, methylated with EcoRI methylase, their ends repaired with T4 DNA polymerase and ligated with EcoRI linkers. After digestion of the linkers with EcoRI, 1 to 8 kb fragments were purified by chromatography on a Sephacryl S-1000 column. This DNA was ligated with λ gt11 DNA treated with EcoRI and bacterial alkaline phosphatase, and the ligated DNA was packaged in vitro. *Escherichia coli* strains used were Y1088 for infection with the in vitro packaged virus and Y1090 for immunological screening [30]. Part of the library was directly used for screening without further amplification.

Immunological screening. The *T. cruzi* DNA library was screened with a human Chagasic serum (H2) which was cleaned of nonspecific antibodies by incubation with an *E. coli* Y1090 lysate plus pieces of nitrocellulose filter incubated on λ gt11 phage grown on a lawn of *E. coli* Y1090. Plaques of λ gt11 (300000; 90% containing inserts) were grown at 42°C on three dishes (30 cm in diameter) during 3 h. Duplicate filters containing 10 mM IPTG (isopropyl-β-D-thiogalactopyranoside) were used for induction, the first one during 2 h and the second one overnight at 37°C. Filters were successively incubated in TBS (50 mM Tris, pH 7.8, 150 mM NaCl) 10 min, TBS plus 3% BSA (bovine serum albumin, fraction V, Sigma) 30 min, 1:100 diluted H2 serum in TBS plus 3% BSA 120 min, TBS four times 10 min each, ^{125}I labelled

protein A 60 min (1×10^6 cpm ml^{-1}) in TBS plus 3% BSA and finally washed 5 times in TBS 10 min each, the third washing solution also containing 0.1% Nonidet P-40. The filters were exposed overnight with intensifying screens at −70°C. Plaques detected on both filters were rescreened three to four times until all plaques in the petri dish were positive.

Antibody selection and Western blotting. Total protein extracts from epimastigotes and trypomastigotes of the RA strain and of the non-pathogenic trypanosomatid *Crithidia fasciculata*, were prepared by resuspending parasite pellets in a solution containing dithiothreitol 0.77 g, SDS 1 g, 80 mM Tris pH 6.8, 5 ml glycerol, 2% bromophenol blue and water up to 50 ml. This extract was boiled 4 min, centrifuged 10 min at 13000 × *g* and the supernatant layered in 10% polyacrylamide gels containing 0.1% SDS (approximately 15 μg parasite protein per lane), electrophoresed in Tris-glycine buffer [31] and blotted onto nitrocellulose filters essentially as described by Burnette [32]. Molecular weight markers were: ovalbumin (45 kDa), BSA (66 kDa), phosphorylase B (97.4 kDa), β-galactosidase (116 kDa) and myosin (205 kDa).

Antibodies were selected [33] from the human serum H_2 diluted 1:50 in TBS plus 3% BSA. Filters soaked in 10 mM IPTG were layered on petri dishes (9 cm) containing recombinant λ gt11 clones, induced 3 h on one side and overnight on the other at 37°C. Filters were then processed essentially as described for the immunological screening up to the step of the incubation with the serum except for the blockage in TBS plus 3% BSA which was performed twice (60 and 30 min). Filters were then washed five times in TBS, 10 min each (the third wash also containing 0.05% of Nonidet P-40) and the selected antibodies eluted during 6 min in 8 ml of a solution containing 0.1 M glycine and 0.15 M NaCl, pH 2.6. After elution, the solution was adjusted to pH 8 with 1 M Tris-HCl pH 8, 3% BSA was then added and used for the reaction with Western blots. Western blots of parasite proteins were reacted with the selected antibodies following the same procedure used for the immunological screening of the DNA library, except for the final activity of the ^{125}I protein A in the solution which was 5×10^5 cpm ml^{-1}.

Results

Identification of recombinant clone groups. Screening of 300000 recombinant λ gt11 clones with sera from a Chagasic patient (H_2) resulted in 53 positive clones on both duplicated filters. Twenty-three clones were purified to single positive plaques. Cross-hybridization experiments (not shown) allowed us to define 10 groups of

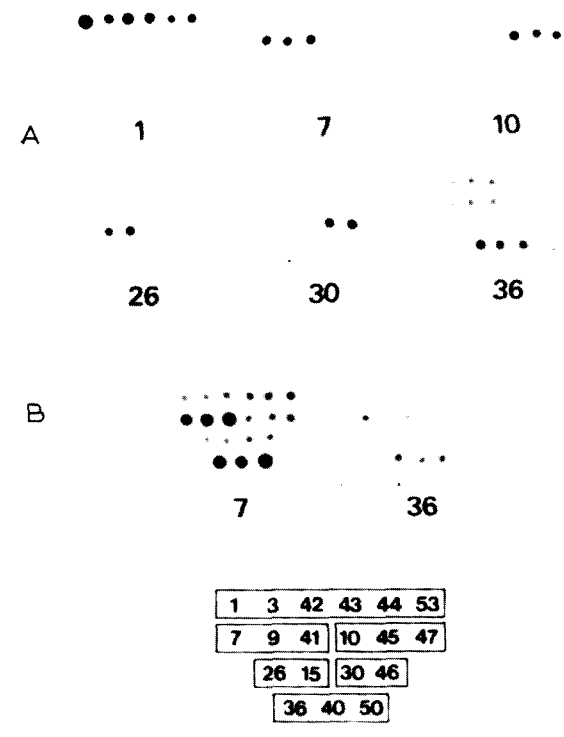

Fig. 1. Identification of recombinant clones with selected antibodies. Ten groups of clones were identified by cross-hybridization experiments (see text). Six of the ten groups contained two or more clones which are indicated by the numbers in the rectangles at the bottom of the figure. The other four groups have a single clone whose numbers are: 2, 13, 22 and 54. Filters containing phage products distributed as indicated in the bottom of the figure were reacted with antibodies selected from the human serum H_2 with the recombinant clone indicated under each photograph (A). In panel B, antibodies selected from a rabbit serum against *T. cruzi* AWP strain (AWP-12) with clones 7 or 36 as indicated were reacted with duplicated filters containing recombinant clone products as in panel A.

clones without sequence homology. For this purpose, isolated inserts from the recombinant clones were hybridized with Southern blots of EcoRI digested DNA from the 23 positive phages. Six of the ten groups have two or more clones. Further confirmation of the homology among clones of the same group was obtained by antibody reaction (Fig. 1A). In all cases, antibodies from the H_2 human serum selected with each clone reacted with proteins produced by all clones of the same group, but not with the products of the others. When two rabbit sera were used for antibody selection with clones 7 and 36, a different result was obtained (Fig. 1B). Thus, clone 36 was able to select antibodies from rabbit sera against *T. cruzi* strain AWP (AWP-12, Fig. 1B, and -25, not shown) that cross-react with the products from clones 7, 9 and 41, that is, with the three members of another group. Furthermore, antibodies selected with clone 7 from rabbit serum against *T. cruzi* AWP-12 also cross-reacted with clones 36, 40 and 50 (Fig. 1B). These results were not due to the fact that sera from different infected species (human and rabbit) were used since antibodies against *T. cruzi* CA1 selected from a third rabbit with clone 36, only reacted with members of the same clone group but not with the products from clones 7, 9 and 41 (not shown).

Native proteins and RNA species identified with the recombinant clones. To identify the native proteins that share antigenic determinants with the products of each recombinant clone, we used the antibody selection method followed by reaction of the selected antibodies with Western blots of protein extracts from epimastigotes and trypomastigotes of the RA *T. cruzi* strain (Fig. 2). Negative control experiments included reaction of Western blots of *Crithidia fasciculata* protein extracts with antibodies selected with each of the recombinant clones, as well as reaction of Western blots of *T. cruzi* protein extracts with antibodies selected with λ gt11 wild type (not shown). Detection of RNA species homologous to the recombinant clones was performed by hybridization of Northern blots of total RNA from epimastigotes and trypomastigotes of the RA *T. cruzi* strain with DNA inserts from each recombinant clone (Fig. 3). Clone 22 was not further analyzed since its product did not react with any of the

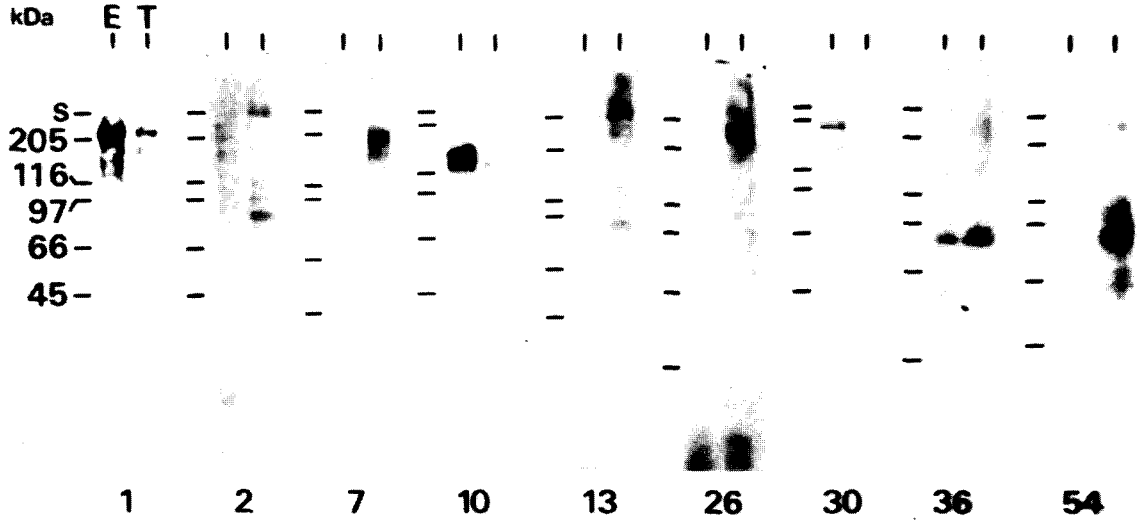

Fig. 2. Detection of native *T. cruzi* proteins with selected antibodies. Protein extracts from epimastigotes (E) and trypomastigotes (T) of *T. cruzi*, RA strain, were run on 10% polyacrylamide gels, blotted onto nitrocellulose filters and reacted with antibodies selected from the human serum H_2 with the indicated recombinant clones. 7.5% polyacrylamide gel was used for the reaction with antibodies selected with clone 1 since the band detected did not enter into 10% gels. Molecular weight markers are those described under Materials and Methods. S indicates the separation between stacking and 10% polyacrylamide gels.

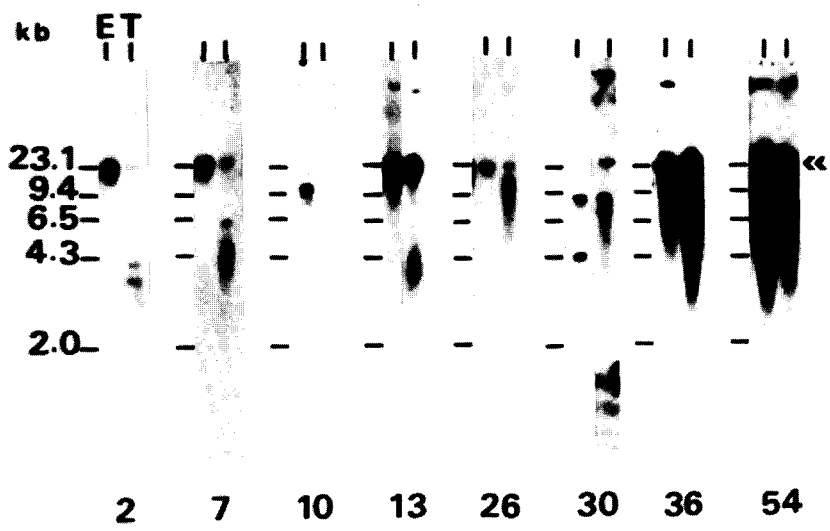

Fig. 3. Detection of RNA species homologous to the recombinant cloned DNAs. Total RNA from epimastigotes (E) and trypomastigotes (T) of *T. cruzi*, RA strain, were electrophoresed on 1.2% agarose gels, blotted onto nitrocellulose filters, and hybridized with the insert of each recombinant cloned DNA as indicated. Arrows on the right side of the figure (<<) indicate contaminant DNA band. Molecular weight markers are those described under Materials and Methods.

other sera used (see below). The results of Western and Northern blot experiments are summarized in Table I, which also includes the sizes of the inserts of the recombinant clones studied.

Antibodies selected with clone 1 detect a protein larger than 205 kDa in epimastigote and trypomastigote extracts. No RNA species could be detected with this clone, and this may be due to the possible large size of the corresponding RNA. Antibodies to clone 2 recognize a protein of 85 kDa and clone 2 DNA detected RNA species of 4.1 and 3.5 kb, in both cases only in the trypomastigote stage of the parasite. Five proteins (from 205 to 165 kDa) and four RNA species (from 5.8 to 4 kb) were observed only in trypomastigotes with clone 7. Detection of more than one protein and RNAs with this clone, as well as with others (see below) may have several explanations (epitopes shared by different proteins, presence of precursor or breakdown products),

TABLE I

Native proteins and RNA species detected by the recombinant clones

λ gt11 clones	Insert size (kb)	Native protein size (kDa)	E or T	RNA size (kb)	E or T
1	1.3	>205	E-T	N.D.	N.D.
2	2.5	85	T	4.1/3.5	T
7	2.5	205/200/190/175/165	T	5.8/4.8/4.3/4	T
10	3.0	150/140/125	E-T	10	E
13	2.2	85	T	3.8	T
26	2.3	>205	T	10	T
30	0.65	205/195/160	E	8.4(E-T)/6.8(T)/4.3(E)	E-T
36	0.70	85	E-T	N.D.	N.D.
54	1.5	90	T	N.D.	N.D.

The relative molecular mass in kilodaltons (kDa) of native proteins and the molecular weight of RNA species in kilobases (kb) identified with each recombinant clone are shown. The column E or T indicates whether proteins or RNAs have been identified in epimastigotes, trypomastigotes or in both stages. (E) or (T) in brackets are included after some RNA species, to indicate whether they were present in one of the parasite forms or in both. N.D. (non-detected) indicates that identification of the RNA species was not possible (see text).

which cannot be proved, nor refuted, at present.

Antibodies selected with clone 10 detected three proteins (150, 140 and 125 kDa) present in much larger amount in epimastigotes than in trypomastigotes. However, RNA (10 kb) could only be detected in epimastigotes. Thus with clone 10 it was possible to detect proteins but not RNA species in the trypomastigote form of *T. cruzi*. This result might be due to the presence of a very small amount of this RNA species, in the trypomastigote stage, as suggested by the much smaller amount of proteins.

Antibodies selected with clones 13 and 26 allowed detection of proteins of 85 and 205 kDa, respectively, only in the trypomastigote stage of the parasite. Accordingly, RNA species were detected in the same parasite stage with both recombinant clones (Fig. 3 and Table I). In the case of clone 30, a different result at the protein and RNA level was observed. Proteins were detected only in epimastigotes (205, 195 and 160 kDa); however, RNA species that differed in size between epimastigotes and trypomastigotes, are present in both *T. cruzi* stages (Table I). Finally, antibodies selected with clone 36 mainly detected one 85 kDa protein in both parasite stages, while antibodies to clone 54 recognized a protein band of 90 kDa only in the trypomastigote protein extract. Both recombinant clone inserts (36 and 54) seem to have a repetitive sequence also present in different RNA species (Fig. 3), that precludes the identification of discrete RNA bands. The same result was obtained when poly(A)$^+$ RNA was used instead of total RNA for the Northern blots (not shown).

Reactivity of human and rabbit sera with the products of the recombinant clones. In order to assess how representative of the parasite antigens were the products of the isolated clones, each clone product was reacted with sera obtained from 9 rabbits infected with different *T. cruzi* strains and from 8 chronically infected humans (Fig. 4). Clones reacting very strongly (++) or stronger (+) than the background observed with λ gt11 were tabulated (Table II). Some of the clones were detected by all sera from either rabbits or humans, while others were only detected by some of them or, in the case of clone 22, by none but for the serum used for the initial screening (H_2), which gave a very faint reaction. Clone 7 reacted with antibodies from all rabbit sera, but did not react with three out of eight human sera. Conversely, clones 1, 2 and 30 were detected by all human sera tested, but by few of the rabbit sera. Interestingly, rabbit serum against CA1 *T. cruzi* strain reacted in a similar way as human sera since it was the only one that detected clones 1 and 2. Two rabbit and two human control sera gave negative results with all recombinant clones (not shown).

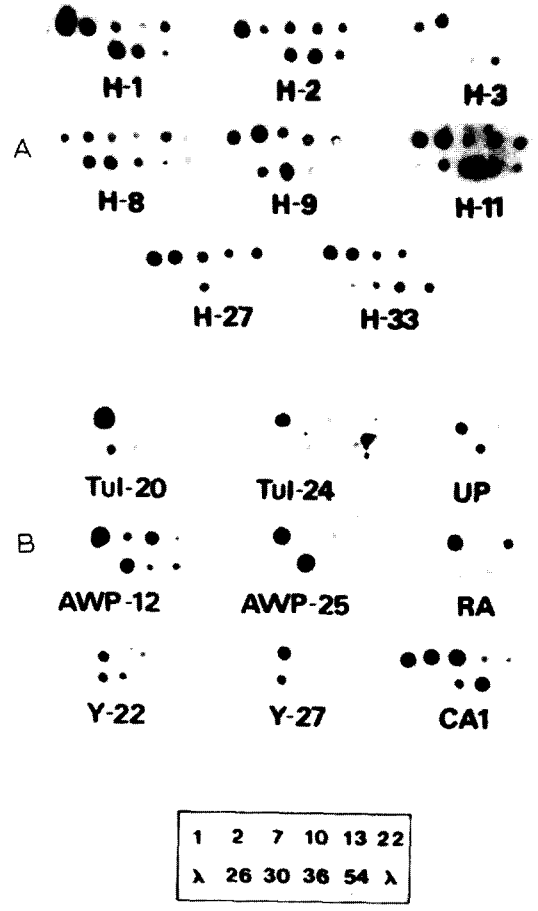

Fig. 4. Reaction of human and rabbit sera with recombinant DNA products. Nitrocellulose filters containing recombinant products distributed as shown on the bottom of the figure, were reacted with each of the indicated human sera from chronic Chagasic patients (A) or with rabbit sera against each of the *T. cruzi* strains stated (B). λ, λ gt11 phage.

TABLE II

Reactivity of rabbit and human sera with products from each recombinant clone

λ gt11 clones	Sera																
	Rabbit									Human							
	Tul-20	Tul-24	UP	AWP-12	AWP-25	RA	Y-22	Y-27	CA1	H1	H2	H3	H8	H9	H11	H27	H33
1									++	++	++	+	+	++	++	++	++
2									++	++	++	++	++	++	++	++	++
7	++	++	++	++	++	++	++	++	++	+	++			+		++	+
10									+		++			+	+	++	+
13	+		+	++		+	+		+	++	++		+	+	+	++	
22											+						
26											+		++	+	+		+
30	+						++	+	+	++	++	+	++	++	++	++	+
36	+		++	++	++		++		++	++	++	+	+		+		++
54										+	++						++

The results shown in Fig. 4 were tabulated. Only signals much stronger (++) or stronger (+) than the background observed with λ gt11 wild type, were included.

Discussion

We have analyzed the antigenic determinants of *T. cruzi*, searching for those genes whose products can be detected with serum from a chronic Chagasic patient. In four of the ten groups defined, a single clone was detected, while in other groups, two, three and up to six homologous clones were isolated. The differences in the number of recombinants isolated per group may indicate the presence of repeated genes, as already seen in *T. cruzi* for those of an unknown protein [34] and heat shock genes [35]. In any case, these results showed that at least ten different protein epitopes are antigenically active in *T. cruzi* infections. Five of them are expressed only in trypomastigotes, while three other proteins were present in epimastigotes as well. The product of the recombinant clone 30 was only detected in epimastigotes, but this could not be confirmed after RNA analysis (Table I). In most cases, antigenically active epitopes were located in proteins of different molecular weights. However, antibodies to four apparently unrelated clone products 2, 13, 36 and 54, detected native proteins of similar sizes (85–90 kDa), three of which, 2, 13 and 54, seem to be specific of trypomastigotes. We do not know yet whether the latter three recombinant clones contained different DNA sequences from the same gene. In this context, several research groups have reported the presence of proteins of around 85 kDa in *T. cruzi* related to either cell penetration [21], to the binding of fibronectin [22] and also a heat-shock protein [35]. Recently, a gene for one 85 kDa protein was cloned [23]. None of our clones seem to be related to the latter since synthetic oligonucleotides obtained according to the published sequence [23] failed to detect any of them under conditions that allowed detection of bands in *T. cruzi* nuclear DNA digests (unpublished observations).

The above mentioned results provide new information on the antigenic makeup of *T. cruzi*. Preliminary immunofluorescence studies (not shown) using living parasites and selected antibodies suggest that the native proteins homologous to the products of clones 2, 10, 13, 36 and 54 are located on the surface of *T. cruzi*. However, these results need to be confirmed using antibodies to each of the fusion proteins raised in rabbits. Cloned DNA products gave, in addition, some insight into the immune response against each of the several protein antigens of *T. cruzi*. It has been demonstrated that several *T. cruzi* strains differed in their antigenic composition [9,12,17,20], which may explain the variable reactivity of some clone products with different sera (Table II). However, epitopes contained in clone 7 product, were antigenically active in all infected rabbits tested, but not in three of the eight infected humans, while antibodies against clone 1, 2 and 30 products were detected in all human sera, but only in few rabbit sera. This difference between rabbit and human response, does not seem to be related to differences in the antigenic composition of *T. cruzi* strains since, firstly, six *T. cruzi* strains were used to obtain rabbit sera and all of them strongly reacted with the product of clone 7 and, but for one serum, none reacted with the products of clones 1 and 2, and secondly, even though we do not know the *T. cruzi* strains that infected the humans from which the sera used were derived, we consider unlikely that they might be identical, specially because of the different reaction observed among human sera with some of the cloned gene products (see Table II). The hypothesis we consider more likely is related to the possible change with time of the antigens presented to the immune system and to difference between hosts. Thus, rabbit sera were obtained from 15 to 60 days post-infection, whereas the human sera were from chronic infections in which the parasites may have been present for years. In this context, it is worthwhile noticing that differences in the kinetics of appearance of circulating antibodies in the mouse as measured by complement mediated lysis or conventional serology have been reported [36]. These observations may have practical implications in the serological diagnosis of Chagas' disease, since different antibody types may be present in the course of an infection. Thus, the product of the clone 7 may be useful to follow infections in rabbit models, while clones 1, 2 and 30 seem useful for diagnosis in humans. Antibodies to clones 7 and 36 were specific for each clone product if selected from one (human) serum but

cross-reacted with each other if selected from other (rabbit) serum. One likely explanation may be the existence of common epitopes in part of the proteins coded by both clones, despite the difference in the relative molecular mass of the native proteins detected with each clone (Table I). Antibodies against common epitopes may be present in some sera but not in others. Epitopes not shared by both proteins allowed selection of antibodies specific for each clone product.

In summary, we have isolated several genes for *T. cruzi* antigenic determinants, and identified their products in the epimastigote and trypomastigote stages of the parasite life cycle. The results obtained suggest that using cloned gene products it will be possible to dissect the immune response, which in organisms with a complex life cycle, like *T. cruzi*, may provide new clues on the interesting and delicate equilibrium of the host–parasite relationship.

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References

1 Krettli, A.U. (1982) Antibodies to *Trypanosoma cruzi* in experimental and human infections. Afr. J. Clin. Exp. Immunol. 3, 327–345.
2 Brener, Z. (1973) Biology of *Trypanosoma cruzi*. Annu. Rev. Microbiol. 27, 347–383.
3 Brener, Z. (1986) Why vaccines do not work in Chagas disease. Parasitol. Today 2, 196–197.
4 Krettli, A.U. and Brener, Z. (1982) Resistance against *Trypanosoma cruzi* associated to anti-living trypomastigote antibodies. J. Immunol. 128, 2009–2012.
5 Zweerink, H.J., Weston, H.D., Andersen, O.F., Garber, S.S. and Hayes, E.C. (1984) Immunity against infection with *Trypanosoma cruzi* in mice correlates with presence of antibodies against three trypomastigotes polypeptides. Infect. Immun. 46, 826–830.
6 Yoshida, N. (1986) *Trypanosoma cruzi*: recognition of trypomastigote surface antigens by lytic antisera from mice resistant to acute infection. Exp. Parasitol. 61, 184–191.
7 Martins, M.S., Hudson, L., Krettli, A.U., Cancado, J.R. and Brener, Z. (1985) Human and mouse sera recognize the same polypeptide associated with immunological resistance to *Trypanosoma cruzi* infection. Clin. Exp. Immunol. 61, 343–350.
8 Snary, D. and Hudson, L. (1979) *Trypanosoma cruzi* cell surface proteins: identification of one major glycoprotein. FEBS Lett. 100, 166–170.
9 Araujo, F.G. and Remington, J.S. (1981) Characterization of stages and strains of *Trypanosoma cruzi* by analysis of cell membrane components. J. Immunol. 127, 855–859.
10 Nogueira, N., Unkeless, J. and Cohn, Z. (1982) Specific glycoprotein antigens on the surface of insect and mammalian stages of *Trypanosoma cruzi*. Proc. Natl. Acad. Sci. USA 79, 1259–1263.
11 Andrews, N.W., Katzin, A.M. and Colli, W. (1984) Mapping of surface proteins and antigens of *Trypanosoma cruzi* by two-dimensional electrophoresis: a correlation with the cell invasion capacity. Eur. J. Biochem. 140, 599–604.
12 Gonzalez, N.S., Sanchez, D.O., Frasch, A.C.C. and Algranati, I.D. (1984) Surface proteins in different isolates of *Trypanosoma cruzi* epimastigotes. Mol. Cell. Biochem. 63, 157–164.
13 Contreras, V.T., Morel, C.M. and Goldemberg, S. (1985) Stage specific gene expression preceeds morphological changes during *Trypanosoma cruzi* metacyclogenesis. Mol. Biochem. Parasitol. 14, 83–96.
14 Dragon, E.A., Brothers, V.M., Wrightsman, R.A. and Manning, J. (1985) A M_r 90 000 surface polypeptide of *Trypanosoma cruzi* as a candidate for a Chagas' disease diagnostic antigen. Mol. Biochem. Parasitol. 16, 213–229.
15 Katzin, A.M. and Colli, W. (1983) Lectin receptors in *Trypanosoma cruzi*: an *N*-acetyl-D-glucosamine-containing surface glycoprotein specific for the trypomastigote stage. Biochim. Biophys. Acta 727, 403–411.
16 Snary, D., Ferguson, M.A.J., Scott, M.T. and Allen, A.K. (1981) Cell surface antigens of *Trypanosoma cruzi*: use of monoclonal antibodies to identify and isolate an epimastigote specific glycoprotein. Mol. Biochem. Parasitol. 3, 343–356.

17 Kirchhoff, L.V., Engel, J.C., Dvorak, J.A. and Sher, A. (1984) Strains and clones of *Trypanosoma cruzi* differ in their expression of a surface antigen identified by a monoclonal antibody. Mol. Biochem. Parasitol. 11, 81–89.
18 Orozco, O., Afchain, D., Dissous, C., Rodriguez, C., Ovlaque, G., Lemesre, J.L., Loyens, M. and Capron, A. (1984) Different monoclonal antibodies against the component 5 specific for *Trypanosoma cruzi*. Am. J. Trop. Med. Hyg. 33, 560–568.
19 Teixeira, M.M.G. and Yoshida, N. (1986) Stage-specific surface antigens of metacyclic trypomastigotes of *Trypanosoma cruzi* identified by monoclonal antibodies. Mol. Biochem. Parasitol. 18, 271–282.
20 Wrightsman, R., Leon, W. and Manning, J. (1986) Variation in antigenic determinants specific to the infective stage of *Trypanosoma cruzi*. Infect. Immun. 53, 235–239.
21 Zingales, B., Katzin, A.M., Arruda, M.V. and Colli, W. (1985) Correlation of tunicamycin-sensitive surface glycoproteins from *Trypanosoma cruzi* with parasite interiorization into mammalian cells. Mol. Biochem. Parasitol. 16, 21–34.
22 Ali Ouaissi, M., Cornette, J. and Capron, A. (1986) Identification and isolation of *Trypanosoma cruzi* trypomastigote cell surface protein with properties expected of a fibronectin receptor. Mol. Biochem. Parasitol. 19, 201–211.
23 Peterson, D.S., Wrightsman, R.A. and Manning, J.E. (1986) Cloning of a major surface-antigen gene of *Trypanosoma cruzi* and identification of a nonapeptide repeat. Nature 322, 566–568.
24 Warren, L. (1960) Metabolism of *Schizotrypanum cruzi*. Chagas I: effect of culture age and substrate concentration on respirative rate. J. Parasitol. 46, 529–538.
25 Macina, R.O., Sanchez, D.O., Affranchino, J.L., Engel, J.C. and Frasch, A.C.C. (1985) Polymorphisms within minicircle sequence classes in the kinetoplast DNA of *Trypanosoma cruzi* clones. Mol. Biochem. Parasitol. 16, 61–74.
26 Gonzalez Capa, S.M., Katzin, A.M., Anasco, N. and Lajmanovich, S. (1981) Comparative studies on infectivity and surface carbohydrates of several strains of *Trypanosoma cruzi*. Medicina (Buenos Aires) 41, 549–555.
27 Borst, P., Fase-Fowler, F., Frasch, A.C.C., Hoeijmakers, J.H.J. and Weijers, P.J. (1980) Characterization of DNA from *Trypanosoma brucei* and related trypanosomes by restriction endonuclease digestion. Mol. Biochem. Parasitol. 1, 221–246.
28 Auffray, C. and Rougeon, F. (1980) Purification of mouse immunoglobulin heavychain messenger RNAs from total myeloma tumor RNA. Eur. J. Biochem. 107, 303–314.
29 Thomas, P.S. (1980) Hybridization of denatured RNA and small DNA fragments to nitrocellulose filters. Proc. Natl. Acad. Sci. USA 77, 5201–5205.
30 Young, R.A., Mehra, V., Sweetser, D., Buchanan, T., Clark-Curtiss, J., Davis, R.W. and Bloom, B.R. (1985) Genes for the major protein antigens of the leprosy parasite *Mycobacterium leprae*. Nature 316, 450–452.
31 Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–682.
32 Burnette, W.N. (1981) 'Western blotting': electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. 112, 195–203.
33 Saint, R.B., Beall, J.A., Grumont, R.J., Mitchell, G.F. and Garcia, E.G. (1986) Expression of *Schistosoma japonicum* antigens in *Escherichia coli*. Mol. Biochem. Parasitol. 18, 333–341.
34 Gonzalez, A., Lerner, T.J., Huecas, M., Pineda, B.S., Nogueira, N. and Lizardi, P.M. (1985) Apparent generation of a segmented mRNA from two separate tandem gene families in *Trypanosoma cruzi*. Nucleic Acids Res. 13, 5789–5804.
35 Dragon, E.A., Sias, S.R., Kato, E.A. and Gabe, J.D. (1987) The genome of *Trypanosoma cruzi* contains a constitutively expressed, tandemly arranged multicopy gene homologous to a major heat shock protein. Mol. Cell. Biol. 7, 1271–1275.
36 Krettli, A.U. and Brener, Z. (1982) Protective antibodies in *Trypanosoma cruzi* infections: detection, functional activity and possible mechanisms of trypomastigote killing in vivo and in vitro. J. Immunol. 128, 2009–2012.