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Surface antigens of stocks and clones of *Trypanosoma cruzi* isolated from humans

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Summary

The total surface polypeptide pattern was analyzed after radioiodination of 12 different stocks and clones of *Trypanosoma cruzi* belonging to four different zymodemes isolated from humans of the region of Bambuí (M.G., Brazil). Although some minor differences were encountered, it is concluded that this pattern cannot be used as a method for classification of strains. Sera obtained from chagasic patients harboring parasites typed according to each zymodeme and from rabbits immunized with either epimastigotes or trypomastigotes from the Y strain immunoprecipitated surface antigens of apparent M_r 55 kDa, 80 kDa and 95 kDa in all the stocks (epimastigotes) tested. These antigens thus appear to be conserved among stocks of *T. cruzi* and to be common to epimastigotes and trypomastigotes. Immunoprecipitation of antigens of surface radioiodinated trypomastigotes (Y strain) with 16 different chagasic sera indicates a remarkable identity among the observed patterns, suggesting that the antigenic characteristics of the surface of *T. cruzi* infective forms are highly conserved and insensitive to the zymodeme type.

Key words: *Trypanosoma cruzi;* isoenzymes (zymodemes); surface antigens.

Introduction

The species *Trypanosoma cruzi* encompasses a variety of populations differing from each other in the morphology of their evolutive stages, extent of

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differentiation when grown in axenic media, biological behavior in the vertebrate and invertebrate hosts, sensitivity to drugs, etc. (for reviews see Brener, 1973, 1980). These biological characteristics have been used as criteria for defining and discriminating *T. cruzi* populations.

Recently, two other criteria were introduced: (a) the analysis of the isoenzyme patterns, dividing the species in groups called zymodemes, and (b) the analysis of kDNA fragments generated by restriction endonucleases, cataloguing the species into groups called schizodemes (Morel et al., 1980).

On the basis of the first criterion, Miles et al. (1977, 1978) and Miles (1979) proposed the existence of three zymodemes from analyses of strains derived from humans and sylvatic animals. Romanha et al. (1979a, b), studying epimastigote strains isolated from human patients living in the region of Bambuí (Minas Gerais, Brazil), have found four basic zymodemes designated A, B, C, and D.

Our interest was directed toward determining whether these differences observed at the phenotypic and genotypic levels would also manifest themselves in the polypeptide composition of the plasma membrane. In this initial study, 12 stocks and clones of *T. cruzi* isolated from human chagasic patients from Bambuí have been analysed. The chagasic population of Bambuí has been extensively studied (Dias, 1979), with well-documented clinical histories available for each case.

The stocks selected in the present study include representatives of the four zymodemes described by Romanha et al. (1979a, b). We report the general cell surface polypeptide patterns of these stocks and clones, as well as their surface antigens recognized by sera from patients harboring these same stocks.

Material and Methods

Parasites. The *T. cruzi* stocks and clones used in this study were obtained by hemoculture from patients living in an endemic area of Chagas' disease (Bambuí, Minas Gerais, Brazil) (Chiari et al., 1979). Stabilates of positive hemocultures were maintained at −196° C until required. When required, the stabilates were thawed and the cells grown in LIT medium (Camargo, 1964; Castellani et al., 1967) to obtain a sufficient number of parasites for electrophoretic determination of the isoenzyme patterns. With the exception of stocks D143, D150 and D207, the other samples used throughout this work were all clones. The clones were obtained from culture forms on agar-LIT plates (Goldberg and Chiari, 1980) and were chosen by their isoenzymatic classification. The culture forms of *T. cruzi* were classified electrophoretically into zymodemes A, B, C, and D (Romanha et al., 1979a) by a combination of profiles generated on starch-gel by eight soluble enzymes. Epimastigotes from the Y strain of *T. cruzi* were grown in LIT medium. Cultures were kept at 28° C at exponential growth (98% epimastigotes). Trypomastigotes of the Y strain were obtained from cultures of LLC-MK_2 cells (rhesus monkey kidney epithelial cells) originally infected with bloodstream trypomastigotes. Tissue culture trypomastigotes were propagated as described (Andrews and Colli, 1982) by sequential reinfection of the monolayers. The parasites were freed from cell debris and transition forms by centrifugation of the culture medium, followed by incubation at 37° C for 60 min. The swimming trypomastigotes were recovered from the supernatant with less than 5% contamination by amastigotes.

Antisera. Human chagasic sera were obtained from chronic patients from Bambuí. Clinical histories for each human isolate used in this study are available. Antiserum against trypomastigotes was obtained from a rabbit infected with three doses of 10^3 live trypomastigotes at four week intervals. Anti-vesicles serum was prepared by immunizing rabbits with epimastigote plasma membrane vesicles (Zingales et al., 1982). Sera were titrated by indirect immunofluorescence (IIF) tests using fixed epimastigotes and were stored at –70° C.

Cell surface iodination. Epimastigotes and trypomastigotes were washed three times with PBS and medium 199, respectively, by centrifugation at 800 × *g* for 10 min. Iodination was carried out as described before (Zingales et al., 1982). Briefly, 10^8 parasites in 1 ml of PBS were incubated with 150 μCi of $Na^{131}I$ (IPEN, São Paulo) in iodogen (1, 3, 4, 6-tetrachloro-3-6-diphenyl-glycoluril, Pierce) precoated tubes (20 μg iodogen/tube), during 10 min at 4° C, with occasional agitation. After iodination, the cells were washed twice and lysed in 0.5 ml of lysing buffer (10 mM Tris-HCl, pH 7.5, 1% Nonidet P-40, 1 mM N-α-tosyl-L-lysylchloromethyl ketone, 1 mM phenylmethylsulfonyl fluoride, 2.8 μg/ml aprotinin) for 10 min at 37° C. The lysates were centrifuged at 10.000 × *g* at 4° C for 30 min and the supernatants were used for total pattern analysis by SDS-polyacrylamide gel electrophoresis and for immunoprecipitation studies.

Immunoprecipitation. The procedure adopted (cf. Zingales et al., 1982) was as follows: iodinated extracts (equivalent to 3×10^7 cells) were incubated for 2 h at 4° C with the required amount of antisera. Controls were incubated with the same amount of a pool of normal human sera or with a preimmune rabbit serum. Subsequently, 100 μl of a 10% (w/v) suspension of formalin-fixed *Staphylococcus aureus* (Kessler, 1975) were added. After 30 min at room temperature, the samples were diluted with 1 ml of NET buffer (Kessler, 1975) and washed three times by centrifugation. A last washing was performed with 10 mM Tris-HCl (pH 8.7), 0.3 M NaCl, 0.1% sodium dodecyl sulfate and 0.05% Nonidet P-40. The proteins were eluted by boiling the immunoprecipitates for 3 min in 70 μl of electrophoresis sample buffer (Laemmli, 1970). The tubes were centrifuged in a Microfuge for 5 min. Aliquots of the supernatants were counted and loaded on SDS-polyacrylamide gels.

Polyacrylamide gel electrophoresis. Polyacrylamide slab gel electrophoresis in the presence of SDS was performed as described (Laemmli, 1970), using a gradient of acrylamide concentration from 7 to 14%. After fixing, staining and destaining, the gels were dried and exposed to X-ray films for different lengths of time. The relative molecular weights (M_r) of the radioactive protein bands were determined using Coomassie Blue stained standard proteins.

Results

The patterns of cell surface polypeptides from clones and stocks of *T. cruzi* isolated from human chagasic patients of Bambuí are shown in Fig. 1. Several clones from zymodemes A, B and C were selected, while in the case of zymodeme D three stocks directly isolated from independent patients were studied, since cloning of this particular zymodeme has not been achieved (cf. Romanha, 1982). The general pattern is complex, many polypeptides being represented in all clones and stocks analysed. A clear identity can be noted among clones of the same zymodeme, with, however, a somewhat higher variability among the three stocks of zymodeme D.

In order to assess whether these samples presented real differences at the antigenic level, surface antigens were analysed by immunoprecipitation with different sera from humans harboring *T. cruzi* typed according to a given zymodeme. In the case of zymodeme A, all sera precipitated three major antigens of apparent M_r 95, 80 and 55 kDa (Fig. 2). Antigens of apparent M_r 80 and 55 kDa

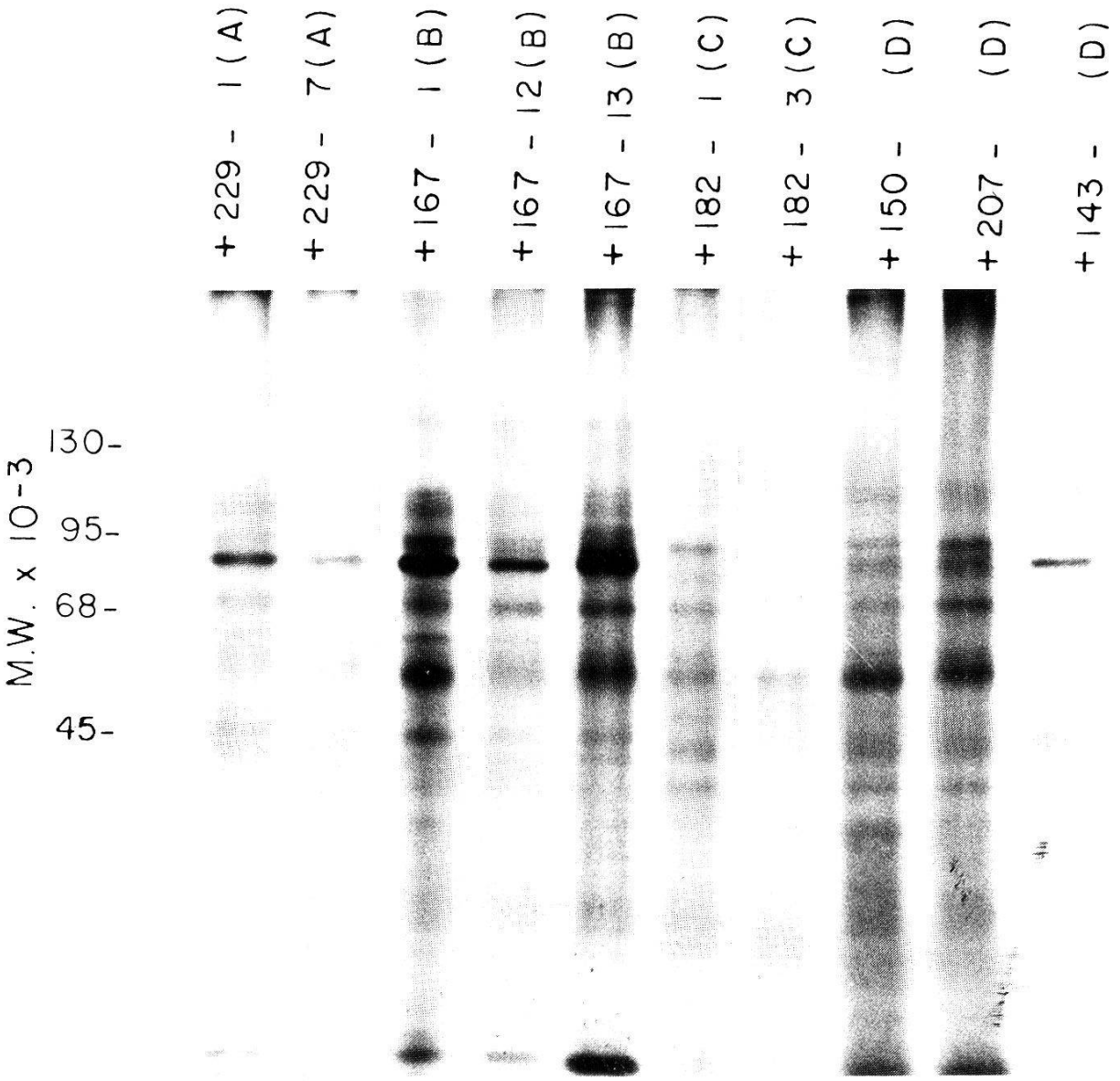

Fig. 1. Autoradiograph of iodinated cell surface proteins of epimastigotes from different clones and stocks of *T. cruzi* analysed by SDS-polyacrylamide gel electrophoresis. Numbers refer to different isolates. The zymodeme types are given in parentheses.

were also represented on the surface of clones from zymodeme B (Fig. 3). However, a somewhat greater complexity was observed in the gel region of apparent M_r between 80 and 110 kDa. In this region at least three antigens were immunoprecipitated by all sera screened, among them the 95 kDa glycoprotein. Patterns very similar to that of A were encountered for clones of zymodeme C (Fig. 4) and stocks of zymodeme D (not shown). Both 100 kDa and 80 kDa antigens were recognized by human sera. We believe that the slight difference in M_r between the 100 kDa antigen and the 95 kDa antigen observed in zymodemes A and B is not significant (vide infra).

In summary, the antigens of apparent M_r 55, 80 and 95 (100) kDa were detected on the surface of all clones and stocks screened, these antigens having

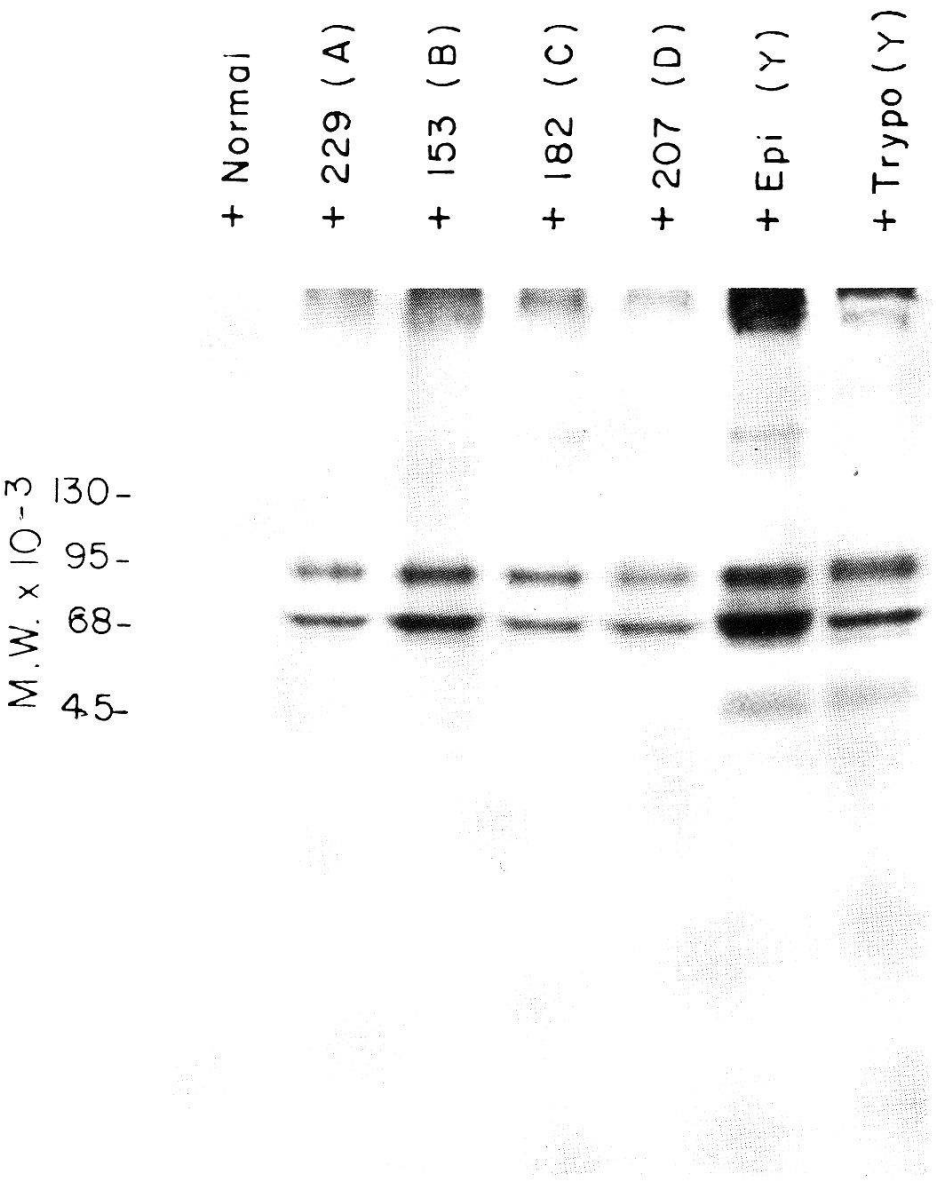

Fig. 2. Labeled cell surface antigens (^{131}I) of an epimastigote clone (229-10) belonging to zymodeme A, immunoprecipitated with sera from chagasic patients of Bambuí (carrying zymodemes A to D) and with sera from rabbits immunized with either epimastigote plasma membrane vesicles [Epi (Y)] or tissue culture trypomastigotes [Trypo (Y)] from Y strain. A pool of normal human sera was used as control.

the same apparent M_r as those previously detected on the membrane of epimastigotes from the Y strain (Zingales et al., 1982). This observation was further confirmed by incubating epimastigote lysates from the four zymodemes with rabbit sera obtained against trypomastigotes or epimastigote plasma membrane vesicles from the Y strain (Fig. 2–4). The results strongly suggest that the 95 kDa and 80 kDa antigens are conserved among clones and stocks of *T. cruzi* and further support the contention that these polypeptides are common to epimastigote and trypomastigote forms (Zingales et al., 1982).

Parallel experiments were conducted with epimastigotes and trypomastigotes from the Y strain. The lysates of radioiodinated cells were incubated with

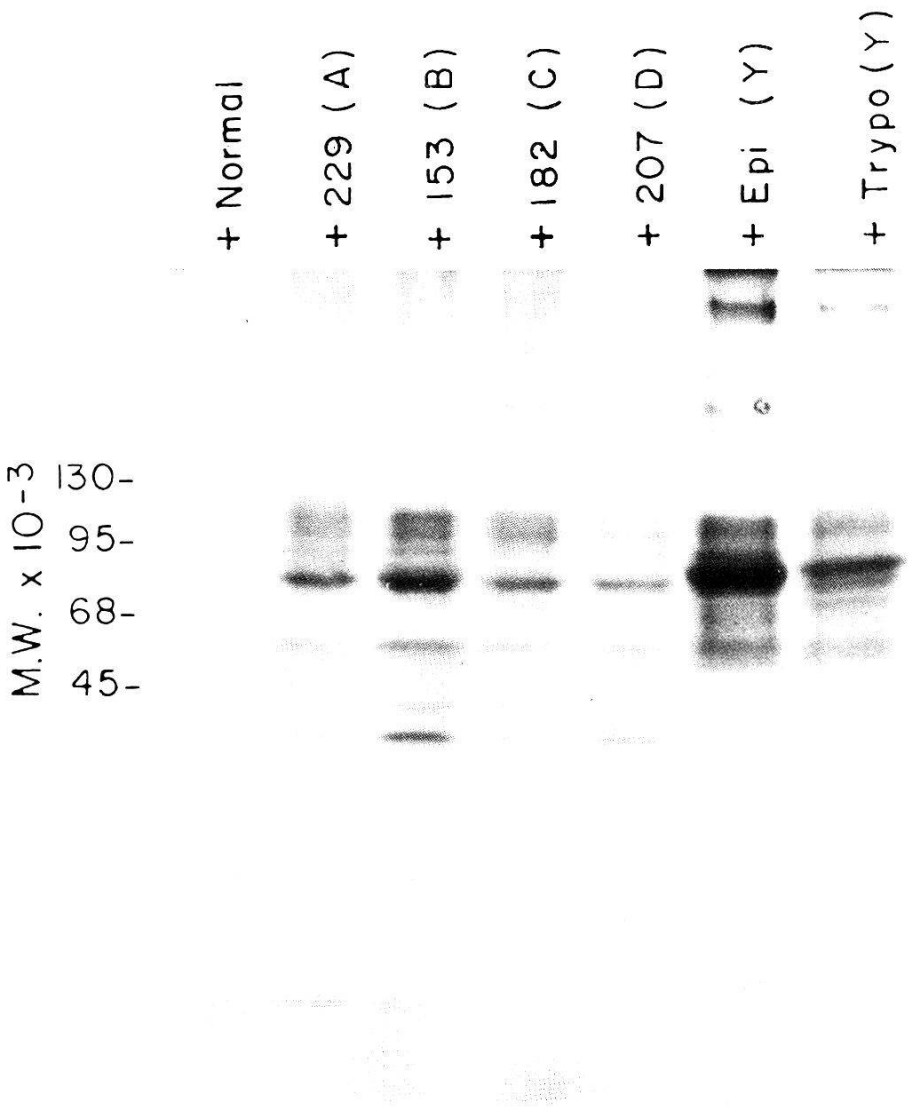

Fig. 3. Labeled cell surface antigens (^{131}I) of an epimastigote clone (167-13) belonging to zymodeme B, immunoprecipitated as described in the legend of Fig. 2.

sera from 16 patients from Bambuí, classified according to each zymodeme. Fig. 5 exemplifies some of the results obtained for the epimastigote surface antigens. Independent of the zymodeme harbored by the donor patient, all sera tested recognized the 95 and 80 kDa antigens, the latter being more intense on the autoradiographs. Fig. 6 shows that additional antigens of apparent M_r higher than 95 kDa were detected on the surface of the trypomastigotes by sera from patients harboring zymodemes A, B, C and D. The pattern is also highly conserved and identical to that obtained by incubation with rabbit anti-trypomastigote serum, as previously described (Zingales et al., 1982).

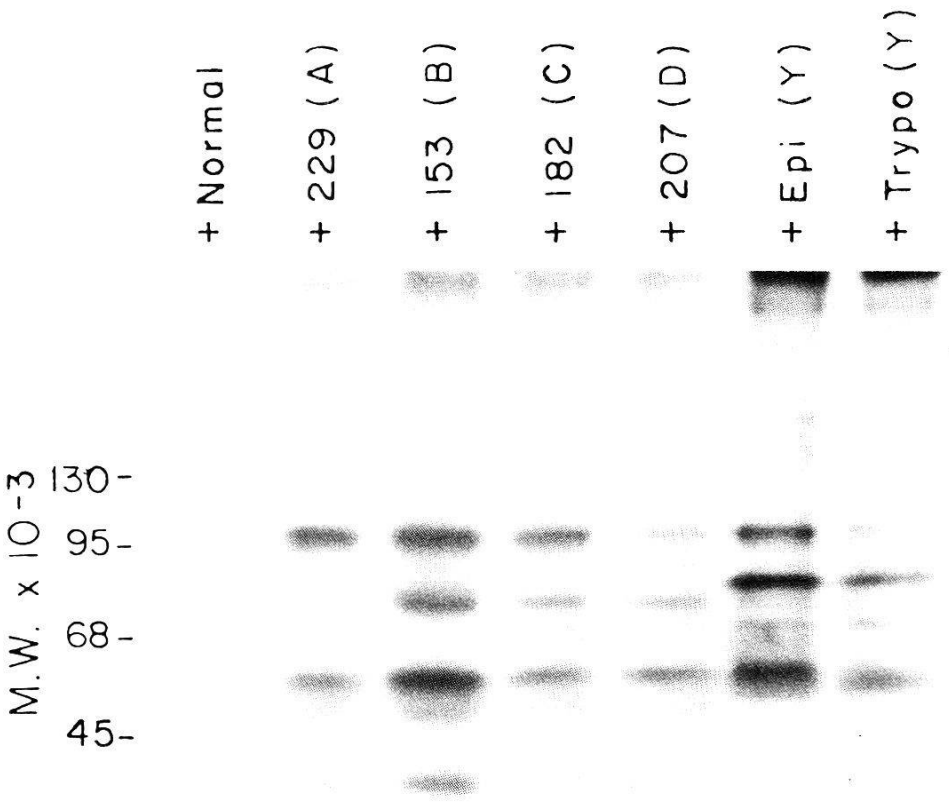

Fig. 4. Labeled cell surface antigens (^{131}I) of an epimastigote clone (182-1) belonging to zymodeme C, immunoprecipitated as described in the legend of Fig. 2.

Discussion

The cell surface polypeptide patterns from different clones and stocks of *T. cruzi* are very complex. Many components are common to all samples analysed, although there are some differences with respect to the minor proteins. Moreover, greater homogeneity is observed among clones belonging to the same zymodeme, at least when analysed by one-dimensional electrophoresis. Nonetheless, unlike analysis of isoenzymes (Miles et al., 1977, 1978; Romanha et al., 1979a, b) and endonuclease-generated fragments of kDNA (Morel et al., 1980), our results suggest that analysis of cell surface patterns cannot be used as a reliable method for classification of *T. cruzi* strains. Recently, Camargo et al. (1982) detected variation in the cell surface proteins of different species of lower

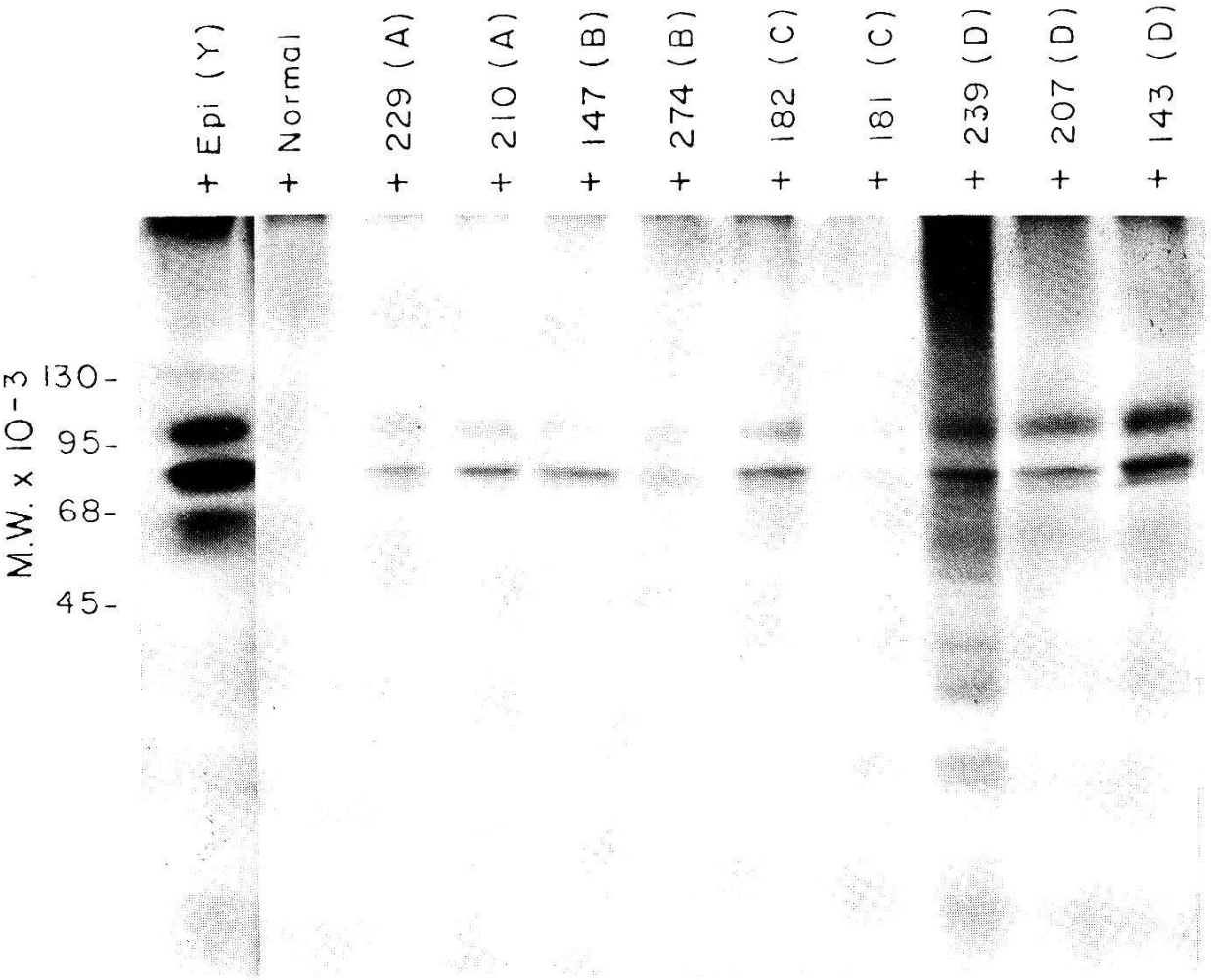

Fig. 5. Radioiodinated surface antigens of epimastigotes from the Y strain immunoprecipitated with rabbit anti-epimastigote plasma membrane vesicles serum [Epi (Y)] and with different human chagasic sera. The sera were classified according to the zymodeme type (in parentheses) of the parasite stock harbored by each patient. A pool of normal human sera was used as control.

trypanosomatids. However, intraspecies variation could not be detected by the methodology employed.

Despite the complexity, relatively few antigens are expressed in the membrane of the epimastigotes of the different clones and stocks studied. For zymodemes A, C and D, polypeptides of apparent M_r 55, 80 and 95 kDa (100 kDa) were immunoprecipitated by sera from chronic chagasic patients and sera from rabbits immunized with either epimastigotes or trypomastigotes from the Y strain of *T. cruzi*. This suggests that these antigens are common to all stocks analysed and are either identical or share common antigenic determinants recognized by these sera. The present immunoprecipitation data do not, however, permit conclusions as to the quantitative expression of the antigens on the cell surface of the stocks. In the case of zymodeme B, two other polypeptides (M_r 90 kDa and 110 kDa) could also be detected. These components are possibly related to the 95 kDa and/or 80 kDa antigens since they are immunoprecipitated by sera from patients carrying zymodemes A, C or D. Different degrees of

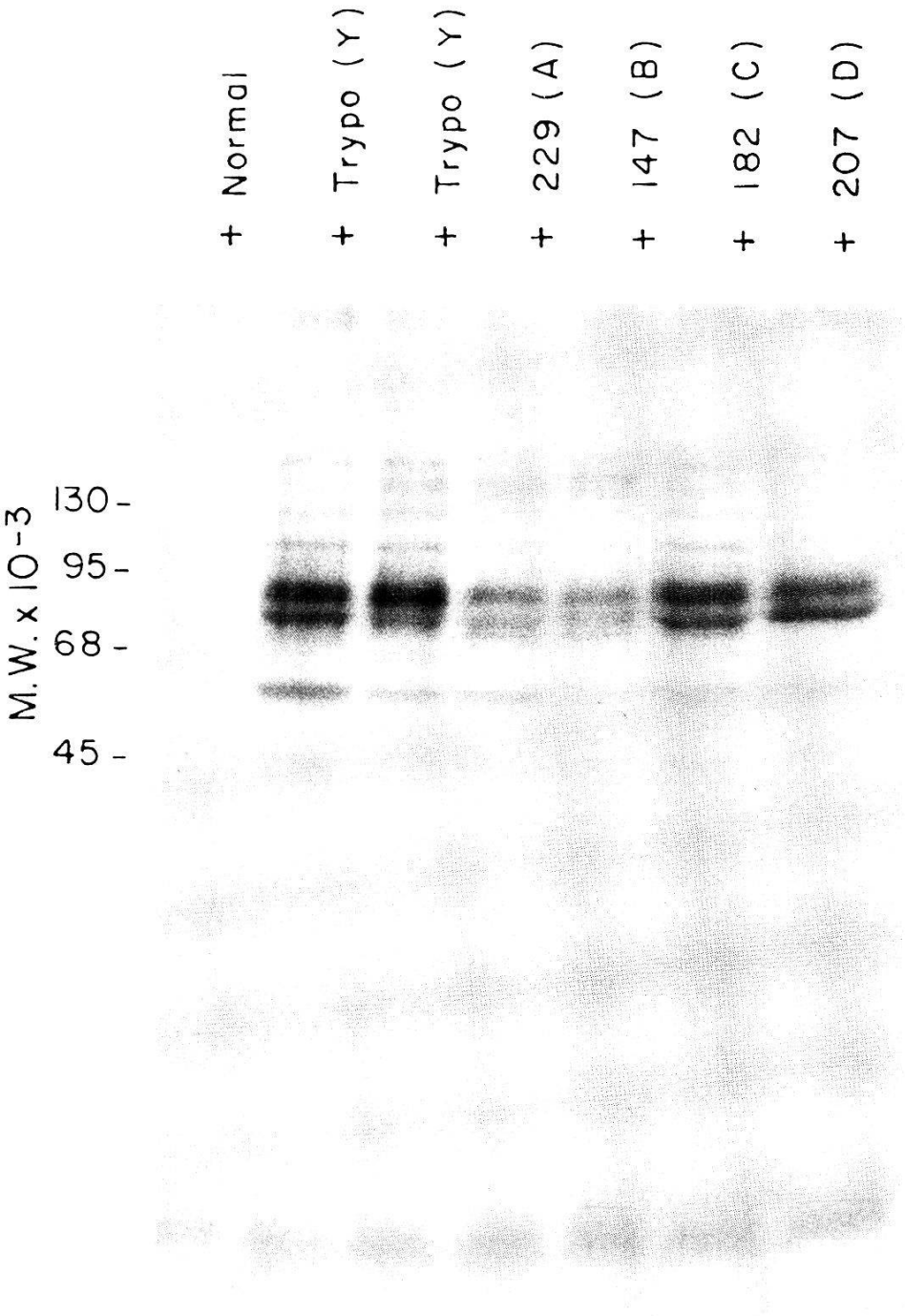

Fig. 6. Radioiodinated surface antigens of tissue culture trypomastigotes from the Y strain immunoprecipitated with two rabbit anti-trypomastigote sera [Trypo (Y)] and different human chagasic sera. These sera were classified according to the zymodeme type (in parentheses) of the parasite stock harbored by each patient. A pool of normal human sera was used as control.

glycosylation would explain the slight variability in their migration on SDS-polyacrylamide gels. An analogous situation has been found for the VSG of African trypanosomes in two-dimensional gel electrophoresis (Pearson et al., 1981). Alternatively, these two extra antigens observed in zymodeme B may also be present in the other zymodemes, but in amounts too small to be detected by radioiodination, yet still sufficient to induce antibody formation. A third

possibility, implied by the work of Gonçalves et al. (1982) is that mixed infections might exist among our patients from the region of Bambuí. Indeed, more than one population has been detected in the circulation of the same patient (Engel et al., 1982). Thus, despite the fact that we employed clones isolated from these patients in order to guarantee the genetic homogeneity of our samples, their sera might still contain antibodies against other zymodemes capable of expressing these extra antigens during the time course of the infection.

We believe that the 80 kDa and 95 kDa polypeptides are glycoproteins since their M_r coincide with the M_r of two main glycoproteins isolated by affinity chromatography from epimastigotes of the Y strain (Zingales et al., 1982). Furthermore, sera developed against plasma membrane vesicles isolated from epimastigotes of the Y strain immunoprecipitate the same antigens from the stocks studied.

Our results confirm previous contentions (Zingales et al., 1982; Colli et al., 1984) that the 80 kDa and 95 kDa antigens are common to epimastigote and trypomastigote forms since they are immunoprecipitated from cloned epimastigotes by both rabbit anti-trypomastigote (Y strain) and human chagasic sera.

As discussed elsewhere (Zingales et al., 1982), the 95 kDa antigen corresponds to the 90 kDa glycoprotein described by Snary and Hudson (1979). Using a somewhat different approach, Snary (1980) concluded that this glycoprotein is common to epimastigotes and trypomastigotes derived from different clones or geographic areas. In the present study, we confirm and extend these observations. The 80 kDa glycoprotein found in the clones and stocks examined most probably corresponds to the 75 kDa glycoprotein present on the surface of both epimastigotes and metacyclic trypomastigotes (Nogueira et al., 1982). We have found that this antigen is intensely precipitated by all chagasic sera screened, whereas the 95 kDa glycoprotein is less noticeably precipitated by sera with lower IIF titers. This observation could explain why some authors have not been able to identify this antigen in their studies of the epimastigote stage (cf. Nogueira et al., 1982).

Using trypomastigotes from the Y strain, we observed that several antigens are recognized by human chagasic sera from Bambuí, in particular those with apparent M_r above 95 kDa, confirming previous data from our laboratory (Zingales et al., 1982). It is remarkable that the immunoprecipitation patterns are essentially the same, independent of the zymodeme type of the serum used suggesting that the antigenic characteristics of the plasma membrane of *T. cruzi* infective forms are essentially constant. Recently, Nogueira et al. (1982) detected conserved antigens on the surface of six different strains of trypomastigotes from different regions of South America. Furthermore, common antigens were also detected on the surface of amastigotes from four distinct strains, using monoclonal antibodies (Araujo et al., 1982).

Our data not only confirm the existence of specific high molecular weight surface antigens on the trypomastigote form, but also indicate that the majority

of the antigens in both stages of the *T. cruzi* are highly conserved in distinct populations of this parasite.

Acknowledgments

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