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ANTIGEN CHARACTERIZATION OF VECTOR-BORNE AND CULTURED METACYCLIC TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI

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SUMMARY

Metacyclic trypomastigotes of the CL strain of **Trypanosoma cruzi** obtained from triatomid vectors and from axenic cultures were comparatively analysed as to their antigen make-up and immunogenic characteristics. They were found to be similar by the various parameters examined. Thus, sera of mice immunized with either one of the two metacyclic types precipitated a 82Kd surface protein from ^{131}I-labeled culture metacyclics. Sera of mice protected against acute **T. cruzi** infection by immunization with killed culture metacyclics of a different strain (G) recognized, by immunoblotting, a 77Kd protein in both types of CL strain metacyclics. A monoclonal antibody raised against G strain metacyclics, and specific for metacyclic stages of this strain, reacted with both CL strain metacyclic types. Both metacyclic forms were similarly lysed by various anti-**T. cruzi** sera, in a complement-mediated reaction.

KEY-WORDS: Chagas'disease — vector borne — **Trypanosoma cruzi**, cultured metacyclic tripomastigotes.

INTRODUCTION

Natural infection by **Trypanosoma cruzi**, the protozoan parasite that causes Chagas' disease, is initiated when metacyclic trypomastigotes contained in the feces of triatomid vectors enter the vertebrate host. Of the various developmental stages of **T. cruzi**, trypomastigotes derived from insect vectors are the most poorly studied. Until recently, when the vector-borne metacyclics were shown to have antigens common to all the developmental forms of **T. cruzi** (VILLALTA & KIERSZENBAUM [15]) or to be susceptible to antibody-dependent, complement-mediated, destruction **in vitro** (KIERSZENBAUM & LIMA [6]), one of the few informations available on this insect stages was their high infective capacity. This scarcity of data on the insect trypomastigotes is greatly due to the difficulties in obtaing appreciable numbers of these forms from triatomid feces. Since metacyclics can be easily obtained in axenic cultures, these trypomastigotes have been generally used as equivalents of their insect counterparts (NOGUEIRA et al. [12]). However, up to the present, the actual similarities between metacyclic trypomastigotes from different origins have not been demonstrated.

In this study we attempted to determine whether metacyclic trypomastigotes from triatomids and from culture media are immunologically equivalent. For that purpose both types of metacyclics of the CL strain were examined as to their immunogenicity, antigen make-up and susceptibility to complement-mediated immunolysis.

MATERIAL AND METHODS

The CL strain of **T. cruzi**, isolated from **Triatoma infestans** (BRENER & CHIARI [1]),

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was used for antigen characterization. This strain has been maintained cyclically in mice and in either the insect vector **T. infestans** or liver infusion tryptose (LIT) medium (CAMARGO [2]). To immunize mice, in addition to the CL strain, we used the G strain, isolated from an opossum by Mena Barreto (YOSHIDA [17]).

Cultured metacyclics were purified through passage in DEAE-cellulose column, as previously described (YOSHIDA [17]). Insect-derived trypomastigotes were prepared as follows: **T. infestans** infected with **T. cruzi** were fed on mice and soon after the blood meal most insects eliminated clear transparent drops of liquid (urine) that were individually examined under phase contrast microscope to verify the presence of parasites. Samples containing trypomastigotes, either uncontaminated or mixed with a small number of epimastigotes, were pooled so that the final preparation contained more than 95% metacyclics. Urine from equivalent numbers of non-infected **T. infestans** was also collected to serve as controls.

Outbred albino male mice were immunized with metacyclic trypomastigotes inactivated by heating at 50°C for 10 min. All animals received 6 weekly doses by the intraperitoneal route. One group of mice received 5 x 10^4 killed CL strain metacyclics derived from **T. infestans.** Simultaneously another group of mice was immunized with 5 x 10^4 culture-derived trypomastigotes of the same strain. A third group of mice was immunized with 5 x 10^6 inactivated G strain metacyclics purified from LIT medium. Immunized mice were bled from the retro-orbital plexus one week after the last immunizing dose. Mice infected with the CL strain also served as source of immune sera. Sera from infected mice were collected 4 weeks and 7 weeks after inoculation of **T. cruzi.** All antisera were aliquoted and stored at-20°C until used.

To produce hybridoma, Balb/c mice were immunized intravenously with 3 doses of 10^7 heat-inactivated metacyclic trypomastigotes of **T. cruzi** G strain purified from LIT medium. Four days after the last immunizing dose, when anti-**T. cruzi** antibodies were detected in the serum of immunized mice, spleen cells from a mouse were recovered and fused with P3U1 plasmacytoma cells (KOHLER & MILSTEIN [7], POTOCNJAK et al. [13]). The hybridoma supernatants were screened by indirect immunofluorescence (IF) test using formaldehyde-fixed G strain metacyclic trypomastigotes as antigens.

For lysis assay, metacyclic trypomastigotes of the CL strain were incubated at room temperature for 15 min with 50 μl of either normal or immune mouse serum previously heated at 56°C for 30 min. Thereafter 50 μl of normal serum was added as source of complement and the parasites were incubated at 37°C for 1 hour. To determine the percentage of trypomastigote lysis, samples were examined under phase contrast microscope and the number of motile intact metacyclics were counted against the number of lysed parasites.

Trypomastigotes were labeled with ^{131}I by the Iodo-Gen method (MARKWELL & FOX [11]) as described by CAMARGO et al. [3]. Preparation of parasite extract, immunoprecipitation by KESSLER's method [5], sodium dodecyl sulphate polyacrylamide gel electrophoresis (LAEMMLI [10]) using 7% slab gels, and radioautography were performed as already described (YOSHIDA [17], YOSHIDA et al. [18]).

For metacyclic antigen determination we also used Western blot analysis ,TOWBIN et al. [14]). Live parasites were placed in reducing buffer containing 2% SDS, 10% glycerol, 10% β-mercaptoethanol and 6M urea in the presence of protease inhibitors. The extracts were subjected to SDS-PAGE, using 7% slab gels for separation and 5% gels for stacking, and then electroblotted overnight at 4°C to a nitrocellulose membrane. The membrane was incubated at room temperature with phosphate buffered saline (PBS) containing 5% defatted milk and then incubated for 1 hr with anti-**T.cruzi** mouse serum at a final dilution of 1:50 in PBS-milk. Negative controls were incubated under the same conditions with normal mouse serum. All preparations were washed 3 times with PBS containing 1% milk and thereafter incubated for 1 hr with affinity-purified goat antimouse ^{125}I-labeled immunoglobulin (10^6 cpm/ml). After 4 washings with PBS containing 0.05% Tween 20, the membrane was dried and exposed to an X-ray film at -70°C.

RESULTS

Sera of mice immunized with inactivated CL strain metacyclics, derived from either triatomids of LIT medium, immunoprecipitated a

major 82Kd surface protein upon reaction with ^{131}I-labeled culture CL strain trypomastigote extracts (Fig. 1, lanes a and b). We were not able to determine the insect-derived metacyclic antigens, in spite of repeated attempts, due to smaal number of parasites obtained from **T. infestans**, on the order of 10^6, i.e., about 100 times lower than the number of culture metacyclics used for labeling.

The antigen make-up of CL strain metacyclics from **T. infestans** and from LIT medium was compared by immunoblotting technique, using sera of mice immunized with killed G strain culture metacyclics. This anti-G serum was selected among others because it reacted with polypeptides of CL strain culture metacyclics, with apparent molecular weights between 70Kd and 100Kd, (Fig. 1, lane c), previously shown to be the main surface antigens of CL strain culture trypomastigotes (YOSHIDA [16]). As shown in Fig. 2 (lanes a and b) a band of 77Kd was detected in both culture and vector-borne CL strain metacyclics. The fact that only one of the metacyclic antigens is identified by immunoblotting procedure may be due to the small number of parasites used (10^6). Another possibility is that the 77Kd protein is more efficiently transferred to the nitrocellulose membrane and, therefore, more easily detected.

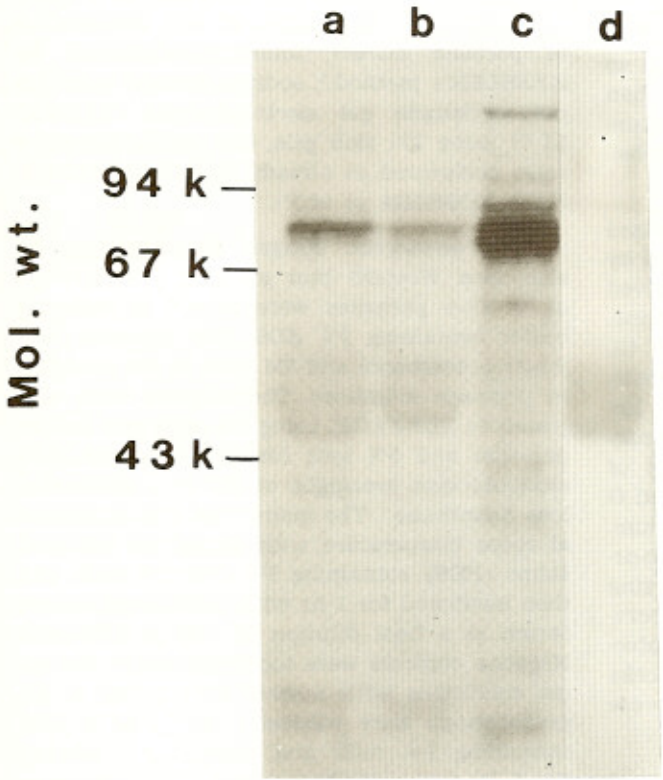

Fig. 1 — Surface antigens of metacyclic trypomastigotes of **T. cruzi** CL strain purified from LIT medium. ^{131}I-labeled parasite extracts were immunoprecipitated with: sera of mice immunized with heat-inactivated CL strain metacyclics derived from **T. infestans** (lane a) or purified from culture medium (lane b); sera of mice immunized with heat-killed G strain metacyclics (lane c). Precipitation patterns with normal mouse serum are shown in lane d.

Both insect-derived and cultured CL strain trypomastigotes were found by IF test to react with a monoclonal antibody that did not recognize epimastigotes or blood trypomastigotes. This monoclonal antibody immunoprecipitated a surface protein with an apparent molecular weight of 90Kd from ^{131}I-labeled extracts of CL strain metacyclics purified from LIT medium (Fig. 3, lane a). The presence of this 90Kd antigen could not be determined in the insect-borne metacyclics due to the already mentioned limitations.

The CL strain metacyclic trypomastigotes from different origins displayed similar susceptibility to complement-mediated immunolysis **in vitro** (Fig. 4). They were both extensively lysed by sera of mice immunized with killed G strain metacyclics as well as by sera of chroni-

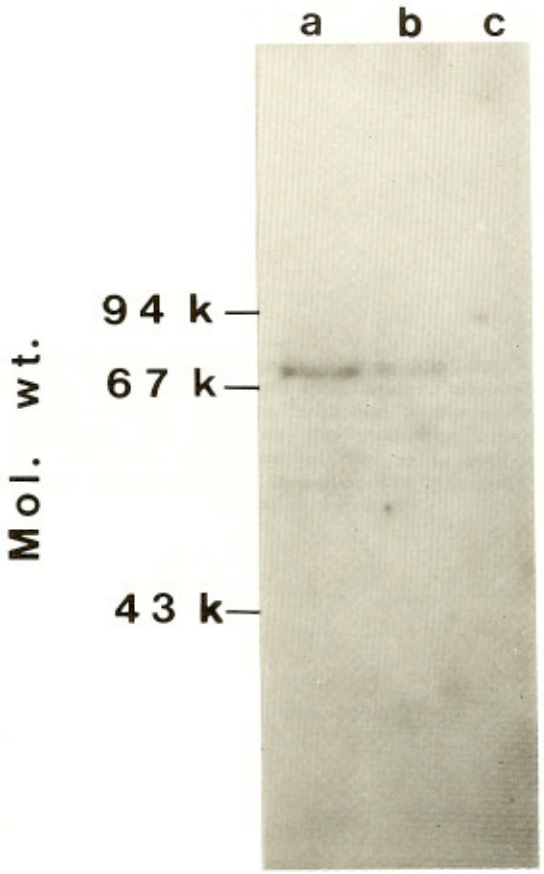

Fig. 2 — Western immunblot analysis of extracts of 10^6 metacyclic trypomastigotes of **T. cruzi** CL strain. Sera of mice immunized with heat-killed G strain metacyclics identified a protein with an apparent molecular weigth of 77 Kd in both culture (lane a) and triatomid (lane b) derived metacyclics. This polypeptide was not detected in the preparation obtained from non-infected **T. infestans** (lane c).

cally infected mice (7 week-old infection). Sera of acutely infected mice (4 week-old infection) showed low lytic activity while the monoclonal antibody reacting with both metacyclic forms had no trypanolytic effect.

DISCUSSION

The present findings indicate that metacyclic trypomastigote stage of **T. cruzi**, derived from triatomid vectors and from culture media, are immunogenically and antigenically similar. Thus, sera of mice immunized with either one of the two CL strain metacyclic forms precipitated from ^{131}I-labeled culture metacyclics of the CL strain a surface protein of 82Kd. On the other hand, a 77Kd polypeptide was detected in both metacyclic trypomastigotes by the immunoblot technique using sera of mice immunized with killed G strain metacyclics.

Our results also show that the metacyclic trypomastigotes harvested from distinct milieu have specific antigens not shared by other developmental stages of **T. cruzi**. A monoclonal antibody that failed to recognize epimastigotes or blood trypomastigotes reacted with both insect and culture metacyclics of the CL strain. A 90Kd polypeptide was detected by this monoclonal antibody on the surface of culture metacyclics.

Complement-mediated trypanolytic activit of immune sera **in vitro** has been associated with the presence of protective antibodies (KRETTLI & BRENER [9]). Of relevance is, therefore, the finding that both insect and culture CL strain metacyclics were almost completely lysed by sera of mice immunized with killed G strain metacyclics, and previously shown to be resistant to **T. cruzi** infection (YOSHIDA et al.[19]), as well as by sera of chronically infected mice, which have been demonstrated to have protective effect (HANSON [4], KRETTLI & BRENER [8]).

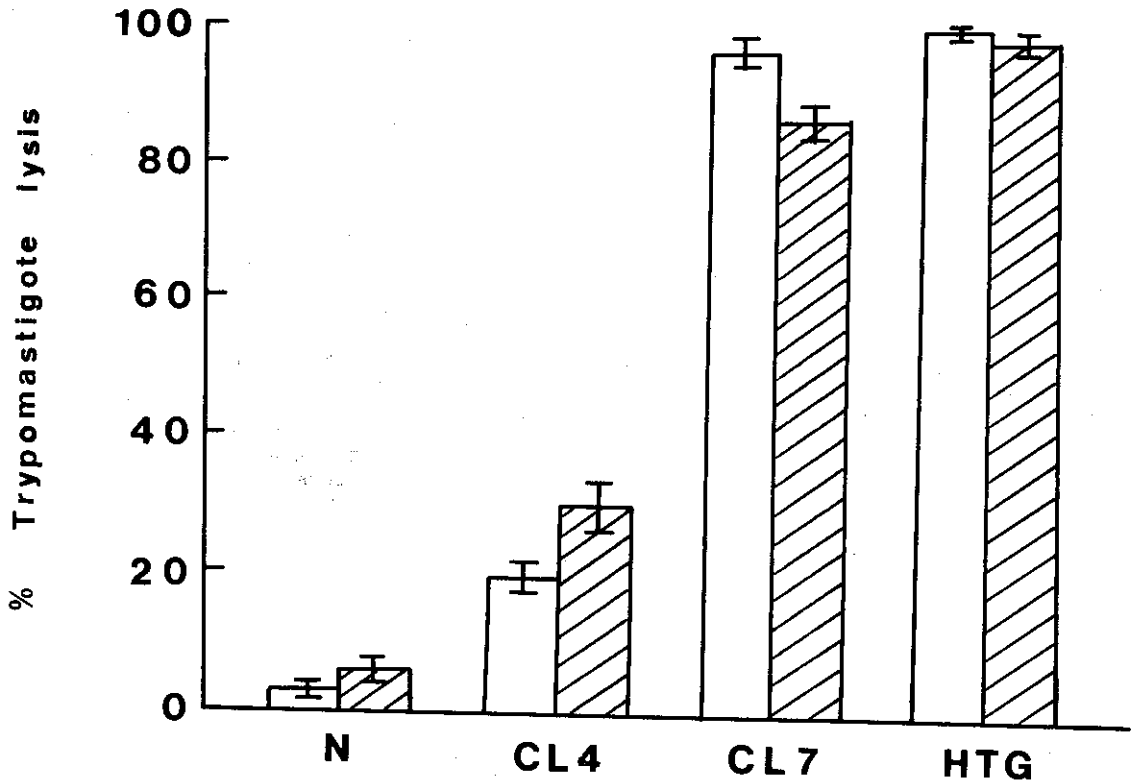

Fig. 4 — Susceptibily it of metacyclic trypomastigotes of **T. cruzi** CL strain to complement-mediated immunolysis. Metacyclics purified from LIT medium (□) or obtained from **T. infestans** (▨) were incubated with: normal mouse serum (N), sera from mice with 4 week-old (CL4) or 7 week-old (CL7) infection by the CL strain, and sera from mice immunized with heat-kiilled metacyclics of the G strain (HTG), in the presence of normal human serum as source of complement.

tripomastigotas metacíclicos, reagiu com formas metacíclicas da cepa CL tanto do vetor quanto de cultura. As duas formas metacíclicas mostraram suscetibilidade semelhante à lise por vários soros anti-**T. cruzi**, em uma reação mediada por complemento.

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