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DIFFERENCES IN THE ANTIGENIC PROFILE OF BLOODSTREAM AND CELL CULTURE DERIVED TRYPOMASTIGOTES OF Trypanosoma cruzi.

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SUMMARY

A comparative study of the antigenic profile of bloodstream and cell culture derived trypomastigotes showed many differences in their components. Using mouse anti-**T. cruzi** antibodies the differences were located mostly in the 120 kDa band, whereas using chagasic patient sera the differences were located in the 85 and 52 kDa bands. These findings might explain known physiological differences between trypomatigotes obtained from cell culture and from infected blood. A brief report of this work has already been published[9].

KEY WORDS: Trypanosoma cruzi; T. cruzi antigens; Trypomastigotes.

INTRODUCTION

Trypanosoma cruzi, the protozoan responsible for Chagas' disease has a life cycle involving both mammalian and insect host. In the mammalian host, **T. cruzi** proliferates as the intracellular amastigote form which differentiates to trypomastigote. Trypomastigotes will enter other cells or reach the bloodstream from where they can be sucked by the insect vector or spread to other tissues. The importance of the mammalian trypomastigotes in the life cycle of **T. cruzi** justifies the great number of reports available on the antigenic composition of this evolutive form of the parasite. However, due to the difficulties in isolating bloodstream trypomastigotes (BT), most studies have been performed with cell culture derived trypomastigotes (CCDT). Although BT and CCDT are very similar in many aspects, several reports indicate differences in their infectivity to fibroblast cells (L-cells[3]) or macrophages[7] and in their reactivity with anti-**T. cruzi** antibodies[4].

These observations prompted us to make a comparative study of BT and CCDT antigens which are recognized by antibodies present in sera from mice and humans infected with **T. cruzi.**

MATERIAL AND METHODS

Parasites: The Y strain of **T. cruzi**[10] was used in all experiments. The BT were obtained from the blood of A/Sn mice 7 days after infection with 10^5 parasites/animal. The parasites were isolated from the blood components according to the technique described by SILVA et al.[8] which concisely consists in separating the parasites from blood components by differencial centrifugation followed by hypotonic shock in order

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to eliminate contaminant red cells and passage through a CM cellulose column for removal of leucocytes and platelets.

CCDT were obtained from LLC-MK2 cells infected 7 days previously and maintained in Eagle's medium containing 10% FSC (fetal calf serum) at 37° under 5% CO_2. After isolation BT and CCDT were washed 3 times with PBSG (phosphate buffered saline plus 1% glucose) containing PMSF (phenylmethylsulfonyl fluoride), TLCK (tosyl-lysine chloro-methyl ketone) and EDTA (ethylenediaminetetraacetic acid) at the concentration of 1 mM. The parasite containing pellet was stored at -20° until use.

Immune sera: groups of 20 A/Sn mice, 5 weeks old, were injected intraperitoneally (i.p.) with 15 blood forms of the parasite. Mice were treated with nifurtimox in drinking water (0.5 mg/ml) until day 21 post infection (p.i.), in order to avoid mortality due to acute infection. The immune sera were obtained by bleeding the animals by the axial plexus under ether anaesthesia on day 15 and 28 p.i. The sera collected were pooled separately and kept frozen (-20°) until use; to obtain hyperimmune sera the animals received 3 additional doses of 100 BT on day 49, 56 and 63 p.i. and were bled on day 70 p.i. Sera were also collected from uninfected mice.

Sera from Chagas' disease patients: sera were obtained from patients with the chronic phase of Chagas' disease with positive specific serological tests (complement fixation, haemagglutination and immunofluorescence test) and stored at -20°.

Detection of IgG anti-T. cruzi antibodies: titrations of IgG anti-**T. cruzi** antibodies in sera were performed by ELISA using as antigen lysates of epimastigotes grown in LIT (Liver Infusion Tryptose) medium[2]. Anti-mouse IgG and anti-human IgG labeled with peroxidase were used as conjugates and OPD (ortophenilenediamine) /H_2O_2 as the enzyme substrate.

SDS-PAGE and Western Blotting: CCDT and BT pellets were solubilized in Tris buffer pH 6.8 containing 2.5% SDS (sodium dodecyl sulfate), 2.5% 2-mercaptoethanol, 10% glicerol, 0.001% bromophenolblue, 1 mM PMSF, 1 mM TLCK and 1 mM EDTA, at concentrations of 4×10^8 cells/ml. The suspensions were boiled for 3 min and 0.5 ml of the mixtures were applied to a 10 cm polyacrylamide gel 10% or 7.5%. The electrophoresis was performed as described by LAEMMLI[5] and the separated antigens transferred to nitrocellulose membranes according to TOWBIN et al[11]. In order to analyse BT and CCDT antigens, the nitrocellulose membranes were treated with sera of different groups of mice or sera from chagasic patients, followed by addition of anti-mouse IgG or anti-human IgG labeled with peroxidase and H_2O_2 plus 4-chloro-1-naphtol as the enzyme substrate.

RESULTS

Pattern of antigens recognized by antibodies obtained during the course of murine infection: in order to compare the antigenic profile of BT and CCDT, we analysed by Western blotting the parasite antigens recognized by sera of infected mice collected on day 15 p.i., when parasitaemia reachs its peak, day 28 p.i., when it reachs its lowest level and day 70 p.i., in the chronic phase of the infection when the animals are fully resistant to a lethal dose of the parasite. The ELISA titres of the anti-**T. cruzi** antibodies in these sera were 20, 640 and 1280 respectively.

In spite of the very low antibody titre detected in sera from day 15 p.i., these antibodies already detected faint bands in the blots of proteins having about 100 to 150 kDa, present on both BT and CCDT (Figure 1). Hyperimmune sera and sera from the 28th day p.i., detected almost the same antigens. These sera showed an expected homology between the BT and CCDT antigenic profiles, major homology antigens being located on the 72 and 76 kDa regions. In addition, these 2 sera detected also differences between BT and CCDT antigens. As shown in Figure 1, the 120 kDa band was more intensely stained in CCDT than in BT, and the 40 kDa band was present only in BT.

BT and CCDT antigens recognized by antibodies present in sera of chronic chagasic patients: the antigenic profiles of BT and CCDT revealed by 9 human chagasic sera were also studied.

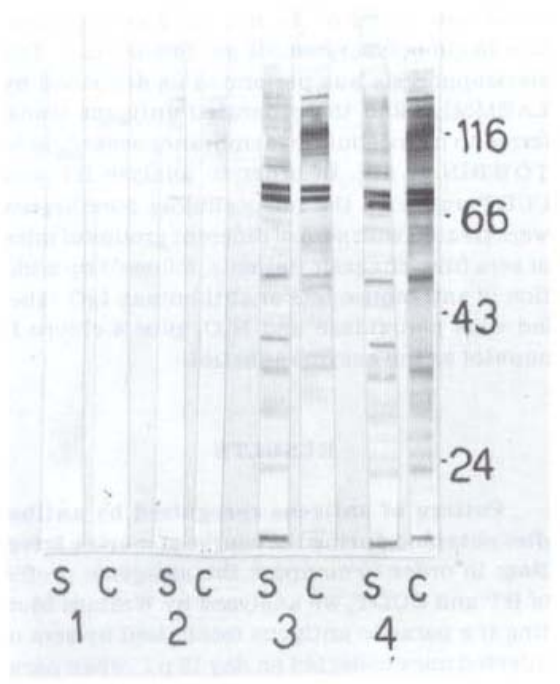

Fig. 1 — Antigens of bloodstream (S) and cell culture derived (C) trypomastigote forms of **T. cruzi** fraccionated by SDS-PAGE (10%) and recognized by normal mouse serum (1), serum of mice on the 15th day p.i. (2), serum of mice on the 28th day p.i. (3) and hyperimmune serum (4) by Western blotting. Mr values are shown on the right.

It is interesting to observe that the antigenic profile revealed by human sera (Figure 2) is different of that obtained by mouse sera (Figure 1). The recognition pattern of trypomastigote antigens detected by these different human sera was also variable. Some of chagasic patient sera (serum number 2, 3, 4, 5 and 6) detected only a few differences between BT and CCDT antigens related to band mobility and intensity of staining, whereas others (serum number 7, 8 and 9) detected more differences in distinct regions of the blots. These differences consisted either in the presence of bands in only one of the antigens or in bands more intensively stained in one antigen than in the other.

The most interesting observation in this study was that with all the 9 human sera analysed the bands located in the 85 and 52 kDa regions were more intensively stained in CCDT antigens, whereas the region of the high molecular weight antigens showed a larger number of more strongly stained bands in BT antigens (Figure 2).

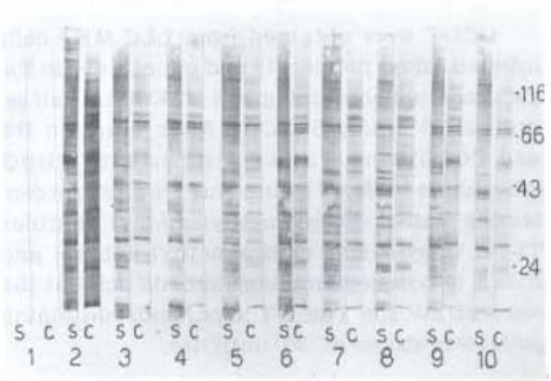

Fig. 2 — Antigens of bloodstream (S) and cell culture derived (C) trypomastigote forms of **T. cruzi** fraccionated by SDS-PAGE (10%) and recognized by normal human serum (1), or chronic patients sera (2-10) by Western blotting. Mr values are shown on the right.

DISCUSSION

Comparison between BT and CCDT antigens recognized by antibodies present on different sera showed differences among some of their components detected either singly by some of the sera or by all of them. These differences are probably not due to antigen loss during the isolation of the BT since the CM cellulose method used for their isolation does not seem to alter the antigenic profile of the parasites[8].

The antigenic pattern of both BT and CCDT was very much dependent on the serum used to detect the antigens. Thus, antigens revealed by mouse sera always showed a simpler pattern than those detected by chagasic patient sera. Even within the small number of human sera tested — 9 — we detected considerable differences in the antigens recognized by their antibodies. On this regard, it must be pointed out that in contrast with infection in humans, experimental mice infection is usually performed by inoculation of a fixed number of one specific strain of the parasite in a specific strain of inbred mice. This probably explains why several pools of hyperimmunized mice sera detected almost the same trypomastigote antigens. Conversely, natural infection of humans differs according to several unknown variables such as the dose of the

inoculum, strain of the infectant parasite and the genetic background of each infected host. Thus, the variation in the antigenic composition of the parasite plus the uniqueness of the immune response result in activation of different clones of lymphocytes and in the production of antibodies with different specificities. On the other hand, differences in the reactivity of each antiserum with BT or CCDT antigens may also be due to either qualitative differences in the epitopes of their antigens or to quantitative differences in the expression of the whole antigen. In order to define this matter, experiments must be done with the antigens fractionated by 2D-electrophoresis and/or monoclonal antibodies against each of the peptides where the differences are detected.

The observation that the 85 kDa band is more intensely stained in CCDT than in BT may be related to the function that this antigen may play in the infectivity of trypomastigotes to cells[1]. If that is true, it might offer an explanation for previous studies showing that CCDT are more infective to cells "in vitro" than BT[3, 7].

LANAR & MANNING[6] have also made a comparative study of CCDT and BT antigens using the Western blotting technique but they did not detect any difference between these antigens. However, the sera samples they used detected only the major antigens of the parasite whereas in our experiments, differences between BT and CCDT were not related to the major antigens. This fact probably explains the discrepance between both results.

Concluding, in spite of the great homology observed between the antigenic profile of BT and CCDT, there are also important differences among some of their antigenic components that might offer an explanation to some discrepant results found in the literature where BT and CCDT were used assuming that they have the same antigenic composition.

RESUMO

Diferenças no perfil antigênico de tripomastigotas sangüícolas e de cultura celular do Trypanosoma cruzi.

O estudo comparativo do perfil antigênico de tripomastigotas sangüícolas e tripomatigotas obtidos por cultura celular do **Trypanosoma cruzi** revelou que estas formas apresentam diferenças em alguns de seus componentes. Utilizando-se anticorpos provenientes da infecção murina, as diferenças foram detectadas na região de 120 kDa enquanto soros de pacientes chagásicos detectaram diferenças nas regiões de 85 e 52 kDa. Estas observações podem oferecer uma explicação a diferenças fisiológicas que ocorrem entre os tripomastigotas sangüícolas e os obtidos de cultura celular.

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