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IMMUNE RESPONSE IN MICE IMMUNIZED WITH ACIDIC ANTIGENIC FRACTIONS FROM Trypanosoma cruzi CYTOSOL.

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SUMMARY

The humoral and cellular immune responses as well as the resistance to infection with bloodstream forms of **T. cruzi** were studied in mice immunized with acidic antigenic fractions from parasite cytosol, F III and F IV, plus **Bordetella pertussis** as adjuvant. The immunization with F III induced positive ITH and DTH responses to homologous antigens. In mice immunized with F IV, the ITH was negative and four out of six animals presented positive DTH reactions. In both groups of mice the analysis of IgG aginst **T. cruzi** showed that the major isotype elicited was IgG1. Specific IgE was also detected in sera from F III immunized mice, thus confirming the presence of homocytothropic antibodies. The parasitemias reached by F III and F IV immunized mice after challenge were lower than those of the controls showing in this way a partial protection against the acute infection. The histological studies of heart and skeletal muscle performed two months after the infection revealed variable mononuclear infiltration in all infected mice despite immunization.

KEY WORDS: Trypanosoma cruzi; Immune response; Acidic antigens.

INTRODUCTION

Several evidences indicate that the immune response plays an important role in protection against infection with **Trypanosoma cruzi** in mice[1,12,17-19]. It is therefore important to define the relative contributions of humoral and cellular immunity to either protective or pathogenic mechanisms involved in **T. cruzi** infection.

Previously, it was demonstrated that an heterogeneous humoral immune response in chagasic patients occurs[5, 24] and that sera from chagasic patients have highly reactive antibodies with acidic antigenic fractions from **T. cruzi** cytosol, F III (pI 5-6) and F IV (pI 4-5)[6] and from parasite exoantigens[8]. So, we think that these antigens are interesting for immune protection studies.

The purpose of the present work was to study the immune response in mice immunized with F III and F IV in order to look into the role of this response on the protection and/or pathogenesis during infection with **T.cruzi**. Our data showed that mice inoculated either with F III or with F IV yielded IgG antibodies being IgG1 the predominant isotype. Immediate and delayed type hipersensitivity responses in mice immunized with F III were also observed. In both groups of animals a significant degree of protection against the infection with **T.cruzi** was obtained as observed from a reduction in the parasitemia levels.

MATERIAL AND METHODS

Parasites: Tulahuén strain of **T.cruzi** was used. Bloodstream forms were obtained from 4-6 week-old BALB/c mice one week after infection by intraperitoneal subinoculations. Epimastigote forms were cultured and collected as described elsewhere[2].

Antigens: F III and F IV antigens were obtained from epimastigotes (EPI) as previously described[6]. Briefly, the EPI homogenate centrifuged at 105.000 g yielded the supernatant fluid named F 105. The F III and F IV fractions were obtained by isoeletric focusing on agarose gels of F 105 at pH gradient 3 to 10. The F III zone (pI 5-6) and the F IV zone (pI 4-5) were cut, homogenized and used for the immunizations.

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The components of F III and F IV eluted from agarose gels with phosphate buffer saline (PBS) pH 7.2 were used for swelling footpad tests.

Immunization: Male BALB/c albino mice two month old were immunized intradermally with three weekly doses of F III or F IV antigens. Each inoculation dose contained 70 µg of protein. Inactivated **Bordetella pertussis**, strain 10536 (Instituto Nacional de Microbiología "Carlos Malbrán", Buenos Aires) was used as adjuvant in a concentration of 1.25 x 10^9 U per mouse. Control animals were injected with **Bordetella pertussis**.

Skin test: It was performed 10 days after the last antigen injection by a challenge with 25 µl of PBS containing 40 µg of F III or F IV into a hind footpad. The thickness of both the antigen injected and the contralateral footpad injected with 25 µl of PBS was measured with a dial micrometer 20 min and 48 hr after the inoculation. The results are expressed as the difference in swelling between the thickness of antigens inoculated footpad and the thickness of PBS inoculated footpad.

Antibodies assay: ELISA was done as previously described[23]. Briefly, **T. cruzi** F 105 at concentration 100 µg/ml in carbonate - bicarbonate buffer pH 9.6 was adsorbed to polystyrene plates overnight at 4°C. After washing with PBS containing 0.05% Tween 20 (PBS-Tween), the plates were blocked with PBS containing 5% human serum albumin (PBS-HSA) for 30 min. at 37°C. The diluted test sera in PBS-5% HSA were added. After 30 min of incubation at 37°C the plates were washed with PBS Tween, and the peroxidase labeled goat anti mouse IgG diluted 1/100 in PBS-HSA was added and incubated for 30 min at 37°C followed by a washing and the addition of the substrate H_2O_2 and the cromogen o-phenylendiamine. For the analysis of IgG isotypes, goat anti mouse isotypes (IgG 1, IgG 2 and IgG 3) (Sigma) were added after incubation of the mouse sera. After washing, the peroxidase labeled rabbit anti-goat IgG diluted 1/500 (Sigma) was added. The mean absorbancy of duplicates was used for statistical analysis.

Parasitemia: Three weeks after last antigens dose, immunized and controls mice were challenged intraperitoneally with 10^3 bloodstream forms of **T. cruzi**. The parasitemia was determined every 7 days, starting on day 10 post infection. Ten microliters of blood samples were taken from the tails of infected mice, mixed with 90 µl 0.85% ammonium chloride to lyse red cells and then the parasites counted in a Neubauer chamber.

Histology: Infected immunized and infected normal **mice** were killed two months after the infection. The heart and skeletal muscle were fixed in 10% buffered formalin for at least 24 hr, then processed and embedded in paraffin. After cutting the sections were stained with hematoxilin-eosin.

Statistical analysis: Student's t test was used for the statistical analysis of the skin tests, antibody assays and parasitemias results.

RESULTS

In order to study the immune response induced by **T. cruzi** cytosol acidic antigens, mice were immunized with F III or F IV antigens. F III and F IV were obtained by isoelectric focusing of **T. cruzi** epimastigote cytosol antigens at pH gradient 3-10 as described in Material and Methods. The F III (pI 5-6) and F IV (pI 4-5) antigens were obtained as show in the Fig.1.

Skin tests

When the skin test was performed ten days after the last antigen injection, mice immunized with F III showed a significantly higher reaction to homologous antigen at 20 min (ITH) and 48 hr (DTH) than control animals ($p<0.001$) indicating that reaginic antibodies and cellular immunity oc-

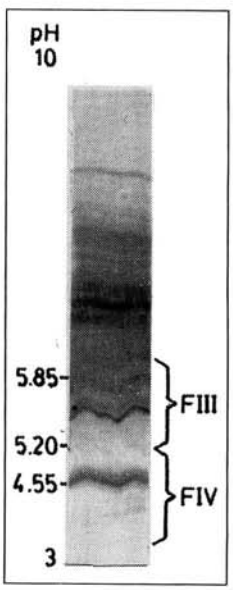

Fig. 1. IEF of F105 on agarose gel. Samples from F105 (200µg) were focused on agarose, pH gradient 3-10, as described in Material and Methods. The gel was fixed and stained with Coomassie brilliant blue R-250. The pH of isoelectric point markers are also indicated.

curred. The skin reactivity induced by F IV was not detected at 20 min and only 4 out of 6 animals showed DTH reactivity (Fig.2).

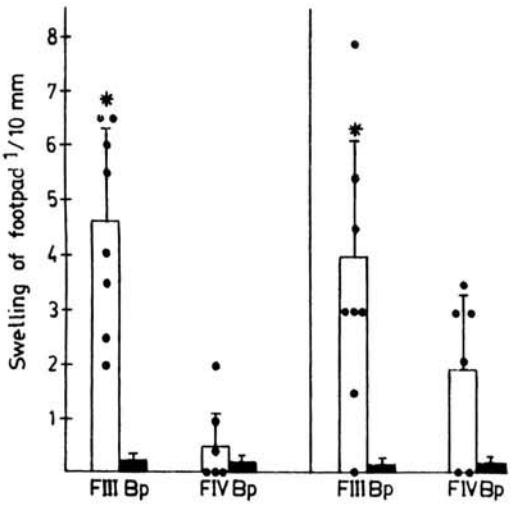

Fig. 2. Immediate (ITH) and delayed (DTH) skin test in mice immunized with F III (n=8), F IV (n=6) and with **B. pertussis**, Bp, (n=6). The skin test was performed using the homologous antigen. The bars represent the mean of the footpad swelling ± SD. The circles represent the individual values. Significant differences with the control group are shown as *p<0.001.

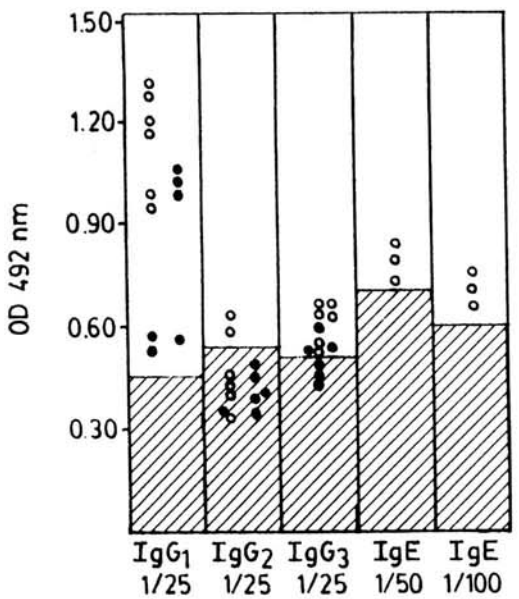

Fig. 3. IgG isotype profiles in sera of mice immunized with F III (○), F IV (●). The numbers indicated below show the serum dilution used in the test. The shaded area represents the mean + 2 SD obtained with the control sera. The results of specific IgE obtained in three sera anti F III assayed are also plotted.

Antibody assays

On day 15 post the last immunization, IgG antibodies against **T.cruzi** F 105 antigens were detected in all animals by ELISA. Figure 3 shows the IgG isotype of anti F III and F IV sera from immunized mice. The predominant isotype was IgG1, with low level of IgG3 and very low level of IgG2. Specific IgE was also revealed in the three sera assayed anti F III at dilution 1/50 and 1/100.

The IgE against F IV was not assayed in the F IV immunized mice because this group of animals showed negative ITH reaction.

Parasitemia

The levels of parasitemia reached after a challenge of immunized animals with 10^3 blood forms of **T. cruzi** performed three weeks after the last injection were significantly diminished as compared with the control group. Figure 4 shows the parasite

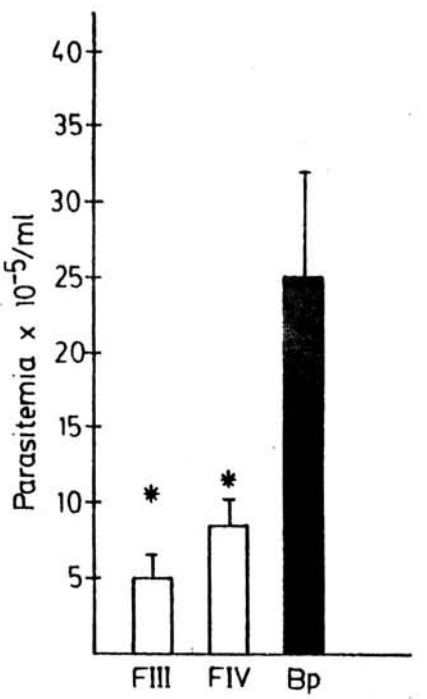

Fig. 4. Parasitemia of mice challenged with 10^3 **T. cruzi** after immunization with F III (n=6), F IV (n=6) and with **B. pertussis** (Bp) (n=6). Data at the peak of parasitemia are presented as mean ± SD day 17 post infection. The cumulative mortality percentage on day 60 post infection was for **B. pertussis** control 100%, F III group 25% and F IV group 30%. Significant differences with the control group are shown as * p< 0.01, t'test.

numbers detected in circulation of immunized mice and controls. Hence, the animals immunized with F III and F IV had a mean of $5 \pm 1.6 \times 10^5$ and $8.4 \pm 1.8 \times 10^5$ parasites/ml respectively. The value obtained with the controls was $25 \pm 7 \times 10^5$ parasites /ml ($p < 0.01$) immunized vs controls.

Histology

The histological modifications in heart from immunized and control mice, two months after the infection, were similar. They were characterized by a focal and light mononuclear infiltration. Amastigote nests were found in one of two controls and were not observed in the immunized animals. In the skeletal muscle the infiltration was focal and the intensity light in the controls, focal or diffuse and light to moderate in the F IV immunized mice and diffuse and heavy in the F III immunized mice (Fig. 5). Owing to the low animal number the statistical significance of these results is unknown.

DISCUSSION

One of the major activities in immunoparasitology is the identification of parasite antigens that have different immunological effects under circumstances of infection of vaccination such as host-protective antigens or immunopathologic antigens.

During the infection with **T. cruzi**, inflammatory lesions in the heart, skeletal muscles and other tissues are probably due to the immune response induced by the parasite [7, 10, 11, 15, 16].

In this work the immunization of mice with partially purified acidic antigens from **T. cruzi** cytosol, F III and F IV, induced a partly protective immune response evidenced by the fact that immunized mice presented levels of parasitemia significantly lower than the controls indicating that the immune mice were more efficient to eliminate the parasites. The analysis of the humoral immune response revealed that both antigenic fractions F III and F IV were able to induce specific antibodies mainly of IgG1 isotype. The effectiveness of humoral immunity against parasites depends on the presence of antigen epitopes which bind antibody isotypes capable of mediating the killing of parasites[22]. It can be suggested that in our experimental model the IgG1 isotype could have a protective effect through their binding to the Fc receptors present in macrophages, natural killer cells, eosinophils and other cells and thus be envolved in the death of the parasite by antibody - dependent cellular cytotoxicity or phagocytosis[3, 4, 13, 21]. Moreover, the IgG1 antibodies that bind to mast cells can elicit anaphylatic reactions[19] atract eosinophils and cause the death of parasites[4, 19]. The results obtained with the cutaneous reactions showed specific cellular immunity in all animals immunized with F III and in four out of six mice immunized with F IV. In mice immunized with F III was also

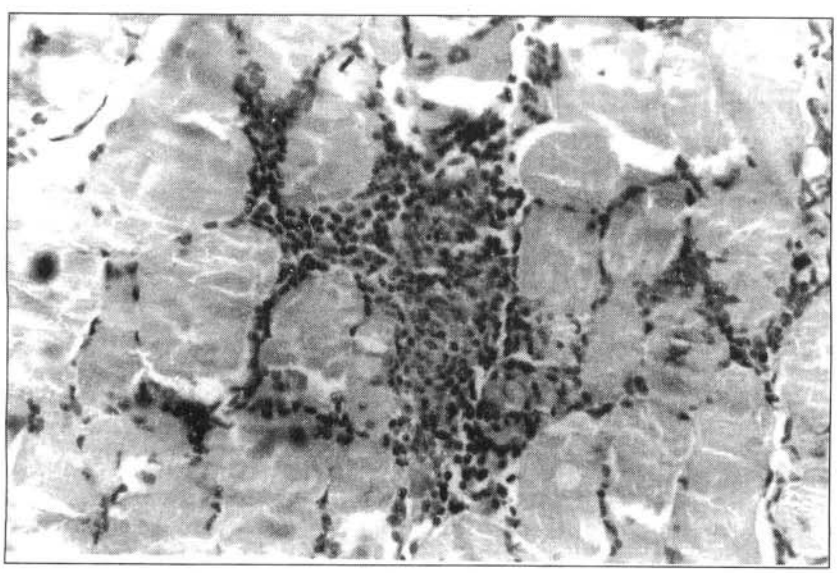

Fig.5. Section of skeletal muscle from FIII immunized and infected mice, obtained two months after the infection. Heavy mononuclear cells infiltration is observed. (40 x).

detected a significant reactivity of ITH and the presence of specific IgE.

The histological modifications in heart from mice immunized and controls, two months after the infection with trypomastigotes, were similar but a mononuclear infiltration in the skeletal muscle was higher in the immunized animals mainly those immunized with F III, suggesting a possible role of the memory cells in the tissue damage when the chronic phase disease occur. It has been demonstrated that during the chronic phase of mice **T. cruzi** infection the cellular immunity can be involved in the tissue damage [10, 15, 16].

It is possible that the preferential induction of different subsets of T-helper cells by specific antigens of an infectious agent[14] or the host genetic background[20] may be crucial in the immunoregulation through production of different lymphokynes, with important consequences for both protective and immunopathogenic responses.

RESUMO

Resposta imune em camundongos imunizados com a fração ácida antigênica do Citosol do *Trypanosoma cruzi.*

As respostas humorais e celulares, assim como a resistência à infecção a formas sanguícolas do **T. cruzi** foram estudadas em camundongos imunizados com as frações antigênicas ácidas do Citosol parasitário, F III e F IV com **Bordetella pertussis** como adjuvante. A imunização com F III induziu respostas positivas ITH e DTH aos antígenos homólogos. Em camundongos imunizados com F IV o ITH foi negativo e quatro dos seis animais apresentaram reações DTH positivas. Em ambos os grupos de camundongos a análise de IgG contra **T. cruzi** mostrou que o principal isotipo produzido foi IgG1. IgE específico foi também detectado em soros de camundongos imunizados com F III, portanto confirmando a presença de anticorpos homocitotrópicos. As parasitemias atingidas pelos camundongos imunizados por F III e F IV após desafio foram mais baixas do que aquelas dos controles mostrando proteção parcial contra a infecção aguda. Os estudos histológicos do coração e do músculo esquelético realizados dois meses depois da infecção revelaram infiltrado mononuclear de intensidade variável em todos os camundongos infectados a despeito da imunização.

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