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CELLULAR IMMUNITY TO *TRYPANOSOMA CRUZI* INFECTION IN MICE

Ataualpa P. REIS, Clea A. CHIARI, Ronan TANUS and Italia M. ANDRADE

SUMMARY

The *in vitro* response of spleen cells from mice experimentally infected with different strains of *Trypanosoma cruzi* in the presence of specific antigen was investigated by the leukocyte inhibition migration technique. With the minor technical modifications mentioned, the Rosemberg & David test was demonstrated to be an appropriate method for the study of cellular immunity in *T. cruzi* infections. In mice inoculated with strains showing a predominance of stout forms (CL and PNM strains) there was significantly more leukocyte inhibition migration than in mice inoculated with strains showing a predominance of slender forms (Y and Berenice strains).

INTRODUCTION

The migration inhibitory factor is now well established as a specific and sensitive indicator of delayed hypersensitivity states in man (SOBORG & BENDIXEN [24], SOBORG [25], EDDLESTON et al. [12], ANDERSEN et al. [3]), in guinea-pig (DAVID et al. [10], BENNETT & BLOOM [4], BLOOM et al. [5], DEKARIS et al. [11], AMOS & LACHMAN [2]) in rats (FALK et al [13], STEINER & WATNE [26]) and in mice (AL-ASKARI et al.[1], PAQUE [17], HALLIDAY [16]). Almost all of these investigations used the capillary tube technique introduced by GEORGE & VAUGHAN [14] and developed by DAVID et al. [10].

Delayed hypersensitivity has been shown to occur in many microbial, fungal and protozoal diseases. In protozoal diseases caused by haemoflagellates, this type of hypersensitivity has been recently examined by GONZALES-CAPPA et al. [15] using the skin test technique by SEAH [22] using the macrophage spreading inhibition technique and by SCHMUNIS et al. [21] using the macrophage migration inhibition. In human *Trypanosoma cruzi* infection the delayed hypersensitivity response is proved to occur as demonstrated by inhibition of leukocyte migration (YANOVSKY & ALBADO [29]) and by specific lymphoblastic transformation (TSCHUDI et al. [28]). In the study reported here, the leukocyte migration inhibition test with spleen cells is used to demonstrate the presence of delayed hypersensitivity in mice infected with different strains of *T. cruzi*, and the predominance of this type of hypersensitivity in mice inoculated with strains PNM and CL is shown.

MATERIALS AND METHODS

Trypanosomes — Groups of male albino mice weighing 18-20 gm were inoculated intraperitoneally with 50,000 bloodstream trypomastigotes of the following strains: 1) *Berenice* strain, isolated by xenodiagnosis from a woman considered to be the first human case of Chagas' disease described by Chagas (SALGADO et al. [19]); 2) *PNM* strain, isolated by xenodiagnosis from a patient with chronic Chagas' disease (BRENER [7]); 3) *Y*

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Instituto de Ciências Biológicas. Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.

strain, isolated by SILVA & NUSSENZWEIG [23] using xenodiagnosis, from a patient with acute Chagas' disease; 4) *CL* strain isolated from naturally infected *Triatoma infestans* collected in Rio Grande do Sul, Brazil (BRENER [7]); 5) *Brazil* strain, isolated in 1942 in Brazil from a patient with Chagas' disease (SALGADO et al. [19]).

All infected mice were examined on the 7th day of inoculation and presented trypomastigotes in the blood.

The five strains have been kept in male albino mice by regular intraperitoneal blood passages. Some morphological and biological characteristics of these strains have been previously reported (BRENER & CHIARI [8], BRENER [7]).

Preparation of antigen — Culture of four *T. cruzi* strains (Y, MR, CL and Berenice), cultivated in LIT (liver infusion-tryptose) by weekly passage, kept at 28°C, were washed twice in 0.01 M phosphate buffer to 10% (v/v) and subjected to three cycles of freezing (−70°C) and thawing in a 37°C water bath. Thereafter the suspension of organisms was ultrasonicated for 1 minute and finally centrifuged for 20 minutes at 1200 x G and 4°C. The supernatant obtained was sterilized by millipore filtration, and after protein determination, used at a final concentration of 100 g/ml protein in all experiments, according to previous dose response.

MIF (Migration Inhibitory Factor) — Mice 60 days after inoculation with *T. cruzi* and normal mice, were shaved and washed with alcohol on the ventral side and killed with ether. The skin was cut from pubis to sternum and deflected. The spleen was explanted through a midline incision and transferred to a sterilized Petri dish. The spleens were minced with sterilized scissors and washed three times in Hanks (HBSS) solution. Trypanosomes were not found in the preparation but a few amastigotes were observed. In order to disrupt the erythrocytes, the cell suspension from each spleen was mixed with 2 ml of Tris ammonium chloride solution. After washing, the remaining white cells were suspended in 199 medium containing 10% heat-inactivated horse serum and standardized to a final concentration of 7.0×10^7 cells/ml. The amount of cells collected did not vary markedly from animal to animal or from group to group. For the cell migration assay, the ROSEMBERG & DAVID test [18] was employed with minor modifications. The cells were withdrawn into 3 polypropylene capillaries 1 mm inner diameter and centrifuged 150 x G for 5 minutes. The tubes were cut at the cell-liquid interface and placed in Mackaness chambers. The basic migration media consisted of TC-199 enriched with 10% heat-inactivated horse serum, 100 units/ml of penicillin, 50 mg/ml of streptomycin and 0.15M hepes solution. This media was used with and without antigen at a final pH of 7.2 throughout.

Migration chambers were incubated 18 to 24 hr at 37°C without CO_2. The migrating areas, enlarged in a projection microscope, were outlined on paper and measured by planimeter. Triplicate determinations were performed for each sample. The controls without antigen were also run in triplicate. The results were expressed as a "migration index", the average area of migration with antigen divided by average area of migration without antigen.

RESULTS

All mice infected with different strains of trypomastigotes developed parasitaemia at the 7th day of infection as noted by direct microscopy examination of tail blood. Inhibition of leukocyte migration *in vitro* in the presence of *T. cruzi* antigens was demonstrated in animals that had been inoculated intraperitoneally with different strains of *T. cruzi*. Figure 1 shows the results obtained in the normal and in the different groups of inoculated mice. Each point represents the average migration index for three experiments. The horizontal bars represent the mean of the groups. It can be seen that compared with normal groups of mice (group A), there was significant inhibition of leukocyte migration in the presence of *T. cruzi* antigen in all groups of mice infected with different strains of *T. cruzi* (Y, Berenice and Brazil strains, $P < 0.05$; PNM and CL strains $P < 0.001$).

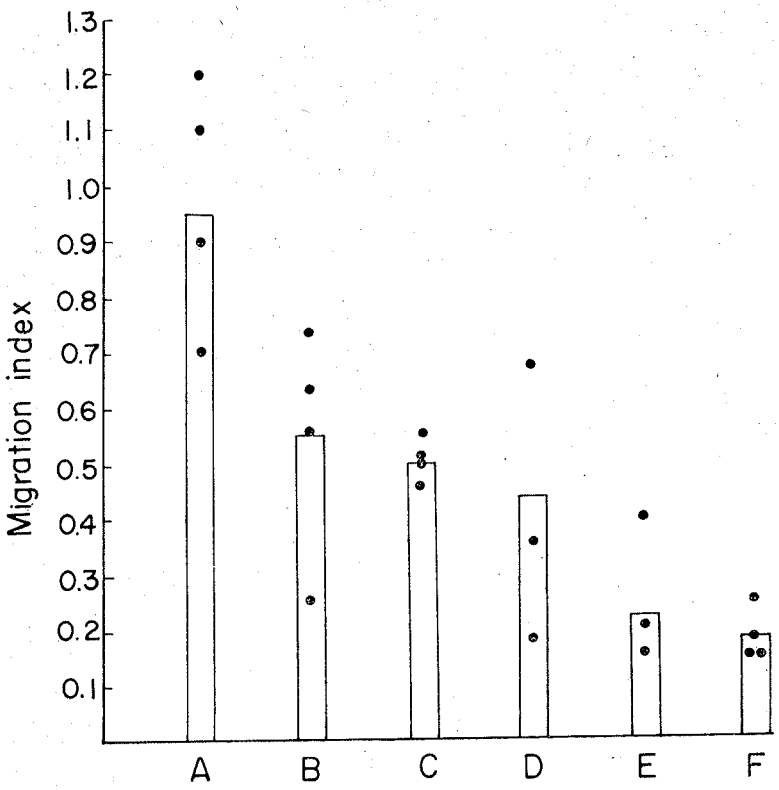

Fig. 1 — Leukocyte migration inhibition of mouse spleen cells by *Trypanosoma cruzi* antigen.

A. Normal mice cells + *Trypanosoma* antigen
B. Mice infected with *T. cruzi* strain Y + T antigen
C. Mice infected with *T. cruzi* strain Berenice + T antigen
D. Mice infected with *T. cruzi* strain Brazil + T antigen
E. Mice infected with *T. cruzi* strain PNM + T antigen
F. Mice infected with *T. cruzi* strain CL + T antigen.

DISCUSSION

It is well documented that on contact with antigen, lymphocytes specifically sensitized will develop a soluble factor which inhibits the migration of leukocytes (AL-ASKARIS et al.[1]), and several studies have shown that the migration assay correlates well with the cellular immune response *in vivo* (BLOOM & GLADE[6]). In *T. cruzi* infections (SEAH[22]) and SCHMUNIS et al.[21] have demonstrated that in mice the migration assay is well established 60 days after the inoculation with trypomastigotes, or 150 days after inoculation with epimastigotes. Cellular immune response has been demonstrated in human *T. cruzi* infection by the lymphocyte transformation and migration inhibition assays (SEAH[22]; SHUDI et al.[28]).

The results presented demonstrate that mice infected with five different strains of trypanosomes show leukocyte migration inhibition in the presence of *T. cruzi* antigens. The inhibition of migration was significantly higher in mice infected with strains of *T. cruzi* which show predominance of stout forms, a gradual ascending parasitaemia, and trypomastigotes being detected in the bloodstream for a few days (CL and PNM strains). In mice inoculated with strains showing predominance of slender forms (Y and Berenice strains) the parasites are not easily detected in the blood during

the very early period of infection. In these mice two peaks in the parasitaemia are noted followed by a sharp decline in number of parasites (BRENER [7]). The *in vitro* cellular response of these animals in the leukocyte migration inhibition test was less evident than in mice showing predominance of stout forms in the blood. BRENER et al. [9] suggest that the increase in the number of trypomastigotes in the bloodstream of mice infected with strains of *T. cruzi* showing predominance of broad and stout forms could result from an enhanced resistance of these forms to the host's immune mechanism. Our data seems to support the hypothesis raised by BRENER et al. [9].

The most important biological implication of the data presented here is the possible correlation between the level of specific host resistance and the degree of specific cellular migration inhibition *in vitro* which varies according to different strains of *T. cruzi*. As cellular immunity is probably related to protection in *T. cruzi* infections (TALIAFERRO & PIZZI [27], SCHMUNIS et al. [20]) the leukocyte inhibition migration test seems to be an important tool for the selection of strain of *T. cruzi* in vaccine attempts, and strains PNM and CL seem to be the most suitable for such procedures.

RESUMO

Imunidade celular na infecção do camundongo pelo Trypanosoma cruzi

A resposta *in vitro* das células de baço de camundongos experimentalmente infectados com diversas cepas de *Trypanosoma cruzi* em presença de antígeno específico foi examinada, empregando-se a técnica de inibição de migração de leucócitos. Com pequenas modificações que são mencionadas no texto, o teste de Rosemberg e David mostrou-se apropriado para o estudo de imunidade do tipo celular em infecção com *T. cruzi*. Nos camundongos inoculados com cepas que mostram a predominância de formas largas (cepas CL e PNM) houve significante aumento de inibição de migração dos leucócitos do que em camundongos inoculados com cepas que mostram predominância de formas finas (cepas Y e Berenice).

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