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Experimental *Trypanosoma rangeli* infection in a murine model

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Abstract: *Trypanosoma rangeli* experimental murine infections were performed in order to study parasitemias and anti-parasite antibody levels. Three groups of mice were used: a) mice infected with metatrypomastigotes derived from infected bugs; b) mice which received four reinoculations of metatrypomastigotes and c) mice immunosuppressed with cyclophosphamide. The results showed that bloodstream parasites can be found from the first day post inoculation reaching a peak at day 5 or 7 and then start to decline. Parasites disappeared completely from the circulation after 20-25 days. However in the imunosuppressed group, parasites were found in blood up to 45 days post infection. The humoral immune response was monitored using an ELISA test and low levels of specific IgG and IgM immunoglobulins were found. However the IgG titers were lower than the IgM. One could conclude that IgM was the predominant immunoglobulin isotype induced in a *T. rangeli* experimental infection because the highest titers were observed in the reinoculated group. IgM antibodies also showed the most prominent crossreactivities with *T. cruzi* antigens.

Key words: *T. rangeli*, parasitemia, antibodies levels, *T. cruzi*, cross-reactions.

There are two species of trypanosomes on the American Continent which can infect man and many other vertebrate hosts. *Trypanosoma cruzi* capable of inducing a life-threatening condition named Chagas' disease and *T. rangeli* which is considered to be entirely non-pathogenic to humans (D'Alessandro 1976).Experimental studies have stablished that these parasites share common and specific antigenic components (Afchain *et al.* 1979, Guhl & Marinkelle 1982, Basso *et al.* 1989, Saldaña & Sousa 1991, Saldaña *et al.* 1993, O'Daly *et al.* 1994) Because of this and since double infections are common in man and animals (Sousa & Johnson 1971, Sousa 1972) cross-reactivity could be found causing distortions in Chagas' disease epidemiological surveys (Hudson *et al.* 1988).

The aim of the present work was to monitor the parasitemia and humoral immune response induced by bug derived metacyclic trypomastigotes of *T. rangeli* in a murine model. The approximate number of bloodstream parasites was recorded by direct microscopic examination and the antibody levels and isotypes by an ELISA test.

Our results strongly support the notion that a *T. rangeli* infection in immunocompetent mice is characterized by a low and short lasting parasitemia. As well we suggest a significant role for immunoglobulins, mainly IgM, in the clearance of bloodstream trypomastigotes. Finally it was demostrated that those IgM antibodies recognize *T. cruzi* antigens after several reinoculation with *T. rangeli* metacyclic trypanosomes.

MATERIALS AND METHODS

Parasites: The *T. rangeli* clone PG CL2 and *T. cruzi* clone IR-116B isolated from panamenian human patients were provided by Dr. O.

Sousa and are maintained in culture forms at the Center for Research and Diagnostics of Parasitic Diseases (CIDEP), Faculty of Medicine, University of Panama. *T. rangeli* metacyclic trypomastigotes were isolated from the salivary glands of experimental infected *Rhodnius pallescens* (fourth and fifth instars) maintained in a colony (at CIDEP) originated from field material from central Panama. Salivary glands were removed at day 10 to 11 post inoculation of *T. rangeli* culture forms in the insect hemocele, disrupted with a fine needle to obtain the metacyclic forms which subsequently were washed in saline.

Serum collection and parasitemia measurements: Groups of 12 Carworth Farms white mice (CFW) between 9-15 g weight and 20-25 days old were inoculated intraperitoneally with $4x10^5$ metacyclic trypomastigotes from bugs dissected salivary glands. Three experimental groups were studied. The first group (A) received only one parasites injection at day 0, the second (B) was reinoculated with the same number of parasites every 15 days for 2 months and the third group (C) was immunosuppressed before infection at day 0. The immunosuppression was obtained by injecting cyclophosphamide (350mg/Kg body weight) 6hr prior to the intraperitoneal metacyclic injection. The parasitemia was determined daily for 15 days and after that every 5 days for 2 months using an standard microscopic technique (Brener 1962). To obtain the serum samples for Igs determinations, the mice from group A were bled from the ophtalmic plexus at day O, 3, 5, 7, 10, 15, 20, and 30 and mice from group B at day 0, 7, 10, 20, 35, 50 and 65 following infection. The sera were pooled and kept at -20°C until used.

Antigens: PGCL2 epimastigotes were grown in Seneckjie' medium plus 15% rabbit blood and one overlay of Brain Heart Infusion (Difco). The period of incubation was 8-10 days at 27°C. IR-116B epimastigotes were grown at 27 °C in Liver Infusion Tryptose medium (LIT) supplemented with 10% fetal bovine serum . Parasites were harvested by pelleting them at 1000 g for 15 min, washed 2 times in cold PBS and finally lyophilized following procedures early described. (Vattuone & Yanousky 1971).

ELISA test: The procedure used for this test was performed as described by Saldaña (1990). Briefly, microwell assay plates for ELISA were coated overnight at 4°C with 50µl of carbonate-bicarbonate buffer pH 9.6 containing 30µg/ml of *T. rangeli* or *T. cruzi* sonicated epimastigotes. After washing 3 times with PBS pH 7.4 containing 0.05% Tween 20, the plates were blocked with 100 µl of 5% (w/v) nonfat dry milk in PBS for 2h at 37°C. They were again washed 3 times with PBS-Tween and 50µl of sera diluted 1:20 in milk solution was added and allowed to bind for 2h at 37°C. The plates were washed thrice with PBS-Tween and the bound antibodies were detected by incubation at 37°C with 50µl of antimouse IgG or IgM peroxidase conjugates (SIGMA) diluted 1:2800 in PBS for 1h. After washing, 50µl of OPD/H_2O_2 in 0.1 M citric acid-phosphate buffer was added as substrate. The color reaction was allowed to develop for 20 min and quantitated in a ELISA reader at 492nm.

To determine the cut off, the absorbances of each wells with 50 negative control sera (day 0) were determined and the mean plus two standard deviations were calculated.

Statistical analysis: For the analysis of the data a non parametric test (Wilcoxon/Kruscal-Wallis test) was used for the parasitemia and a Student's t test for paired data for the antibodies responses.

RESULTS

Parasitemia in mice infected with *T. rangeli* : The kinetics of blood parasitemia was first studied in mice that received an intraperitoneally inoculation of $4x10^5$ *T. rangeli* metacyclic trypomastigotes. The peak of parasitemia in blood ($2.8x10^4$ parasites/ml) was reached by day 5 post inoculation. From that day the parasite levels started to decline and between 20 and 25 days post inoculation no parasites could be detected in blood (Fig. 1A). In the imunosuppressed group, a maximun in parasitemia ($3.8x10^4$ parasites/ml) was obtained after 7 days and the parasites could be found as long as 45 days after the inoculation (Fig. 1A). Comparing parasitemias of these two groups we found that

they were statistically similar from day 1 to 5. From days 6 to 35, however they were statistically different.

Another group of mice, which was reinoculated 3 times, every fifteen days with $4x10^5$ parasites, showed a similar parasitemia compared to the mice receiving only one infective dose. No parasites were observed in blood after day 20 eventhough the same dose of metacyclics were injected in each reinoculation (Fig. 1A).

ELISA test with sera from mice infected with *T. rangeli* : Both IgG and IgM specific imunoglobulins were detected in these samples. Although the level of recognition with IgG was lower than with IgM for both groups (Fig. 1B and Fig. 1C). *T. rangeli* metatrypomastigotes were also able to induce humoral immune responses detectable with *T. cruzi* antigens. This was most overt in the IgM determinations (Fig. 1D). However, we were unable to detect any IgG crossreaction activity (Data not shown).

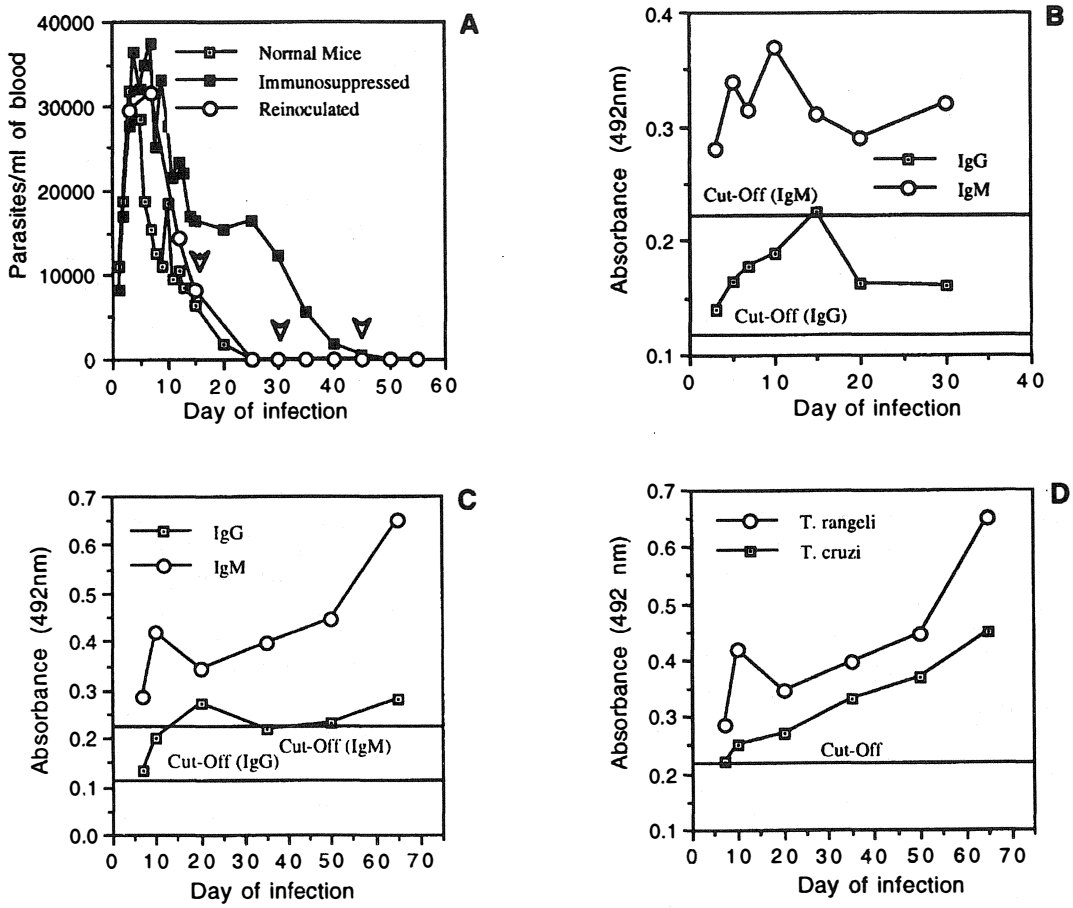

Fig. 1. Parasitemia and specific immunoglobulins kinetics in mice experimentally infected with *Trypanosoma rangeli*. A similar infective dose ($4X10^5$ metacyclic trypomastigotes) was used in normal, immunossuppressed (cyclophosphamide treated) and reinoculated mice. Reinoculations were done with an identical parasites injection at time points arrowed (A). The kinetics of specific IgG and IgM levels anti-*T. rangeli* were determined by an ELISA test using a *T. rangeli* epimastigotes extract as antigen: normal mice (B) and reinoculated mice (C). The specific and *T. cruzi* cross-reactive IgM levels were determined in mice that received reinoculations of parasites at days 15, 30, 45 and 60 post initial infection (D).

DISCUSSION

Our results are in line with those obtained by Añez *et al.* (1985) and Urdaneta Morales and Tejero (1985) in which, using a *T. rangeli* strain isolated in Venezuela, a progressive increase and decrease of parasitemia was observed from day 1-20 post inoculation (Fig.1A). After this period of time, parasites were hard to demonstrate in blood samples, probably as a result of specific and non specific immune mechanisms. However, as in American trypanosomiasis, a premunition situation is possible to occur due to the chance of recuperating parasites from blood after long time of infection (D'Alessandro 1976). This is also insinuated by the finding that reinoculations of metatrypomastigotes in a group of mice, did not increase the parasitemia (Fig. 1A). Maybe the increase was so low and short lasting that it was impossible to detect with our microscopical methodology. Moreover this finding suggest that mice are apparently resistant to re-infections with the same parasite clone after the primary inoculation, nevertheless other factors such as host age could be implicated (Tejero *et al.* 1988).

The above results were extended by the demonstration that the humoral immune response during these infection and re-inoculations by *T. rangeli* are constituted of both specific IgG and IgM (Figs. 1B and 1C). However, from these data it appears that IgM is the predominant immunoglobulin observed during a *T. rangeli* murine infection.

Previous studies (Añez *et al.* 1985) demonstrated the existence of agglutinating immunoglobulins in a *T. rangeli* experimental infection and suggested that these antibodies are responsable for the clearance of circulating parasites. These findings, support the hypothesis that the sudden and fast disappearance of *T. rangeli* blood parasites is due to the lytic action and/or increase of phagocytosis promoted by IgM molecules and complement factors. In addition an immunosuppresed mice group, treated with cyclophosphamide, an antiproliferative and lymphocytic agent which suppresses initial host response to several antigens and inhibit subsequent antibody synthesis, showed a parasitemia not enhanced but prolonged (Fig. 1A). This results is consistent with the consideration of a possible role for specific immune responses in a *T. rangeli* infection, furthermore additional work is required to clarify the immunological events that guide a *T. rangeli* murine infection.

On the other hand, Guhl *et al.* (1985 and 1987) suggested the possibility that although trypomastigotes are considered to be the infective and demonstrable stage in the vertebrate host, it is much less immunogenic than the epimastigotes stage. Regarding this information it should be kept in mind that our inocula consisted in a high number of metatrypomastigotes and a very low proportion of epimastigotes. These last parasites could have a significant role to provide the antigenic determinants responsable by the observed antibody response. It is also possible that the specific immunoglobulins (IgG and IgM) produced in infections by metacyclic trypomastigotes from vector salivary glands, failed to recognize many epimastigotes epitopes, which represents the antigen used in the immunological tests employed by us. In view of this, the use of metatrypomastigotes antigens could be necessary in order to define these possibilities, even if probably more than 90% of the antigens are similar.

Aditional it was found that inoculations of mice with *T. rangeli* metatrypomastigotes resulted in the production of antibodies which bound *T. cruzi* epimastigotes antigens. A similar finding was reported by Guhl & Marinkelle (1982) using an immunofluorescence assay, however in that experiment the antibody isotypes were not demonstrated. Our results show that in mice that received several reinoculations, an increase of both anti-*T. rangeli* and anti-*T. cruzi* IgM titers occurred.

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RESUMEN

Se estudió la parasitemia y la respuesta inmune humoral en ratones experimentalmente infectados con *Trypanosoma rangeli* . En ratones inmunocompetentes los parásitos sanguineos mostraron una máxima parasitemia a los 5-7 días post-inoculación para luego desaparecer de la circulación a los 20-25 días. Sin embargo en ratones tratados con ciclofosfamida la parasitemia se prolongó hasta los 45 días post-inoculación. Durante este tiempo se detectaron, por medio de una prueba de ELISA, anticuerpos específicos IgG e IgM siendo estos últimos los predominantes y de posible importancia en el control de la infección. De forma similar los anticuerpos IgM mostraron mayor reconocimiento de los antígenos de *Trypanosoma cruzi* . Esta observación enfatiza la necesidad de considerar las infecciones por *T. rangeli* en el diagnóstico y patología de la enfermedad de Chagas.

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