Rev. Inst. Med. trop. São Paulo
34 (1): 9-13, janeiro-fevereiro, 1992

TRYPANOSOMA CRUZI: *COURSE OF INFECTION IN PLATELETS-DEPLETED MICE*

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SUMMARY

The effect of platelet depletion on the course of **Trypanosoma cruzi** infection in BALB/c mice was investigated. Thrombocytopenia was achieved by inoculation of rabbit anti-platelet IgG during the parasitemic phase of the infection. The number of parasites in the blood of anti-platelet IgG treated was significantly higher than that of non-treated control mice, during the phase of high parasitemia.

Cumulative mortality of platelet-depleted mice was consistently but not significantly higher than that of control mice up to the 32nd day of infection; from the 33rd day on they were equivalent, no mortalities occurring from then on, until observations were discontinued on the 60th day. These results suggest that platelets participate of the mechanisms of parasites removal from the bloodstream, but do not have an effective role in the mechanisms of defence against **T. cruzi**, during the acute phase of infection.

KEY WORDS: **Trypanosoma cruzi**, infection; platelets depletion.

INTRODUCTION

Host defence mechanisms against trypanosoma are complex and not well understood. The participation of platelets has been suggested in the destruction of **Trypanosoma lewisi**[1] and **T. musculi**[12].

In vitro lysis of **T. cruzi** bloodstream trypomastigotes (tryps) in the presence of platelets, anti-**T. cruzi** immune serum and C5-deficient fresh mouse serum was described by UMEKITA & MOTA[10]. The same authors observed a significant reduction in the rate of antibody-dependent removal of tryps from the circulation of A/Sn mice depleted of platelets by inoculation of anti-platelet IgG antibody[11]. These results point to a possible participation of these cells in the mechanisms of protection of the host against **T. cruzi**. This is investigated now, by following the course of the infection by **T. cruzi** Y strain, in mice partially depleted of platelets during the acute phase of the disease.

MATERIALS AND METHODS

Parasites

The Y strain of **T. cruzi** was maintained by serial passages in BALB/c mice. Tryps used for infection were collected from mice treated with cyclophosphamide (4 mg/mouse, i.p., 48 hours after i.p. inoculation of 5 x 10^4 tryps), for suppresion of antibody production, and isolated by the method of ALCANTARA & BRENER[2]. The method of Brener[5] was used for determing the levels of parasitemia in blood samples.

Platelet depletion

Anti-platelet serum was obtained by a series of six inoculations (i.v.) of rabbits with 1.0 ml of a platelet-rich suspension, every other day. The animals were bled 5 days after the last inoculation. The anti-serum was absorbed with leukocytes and red blood cells; the IgG fraction was subsequently

This project was sponsored by "Fundação de Amparo à Pesquisa do Estado de São Paulo" - FAPESP
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isolated with protein A-Sepharose. Inoculation of a 0.26 mg dose (i.v.) of this anti-platelet IgG (a-plt. IgG) in normal mice induced a fall of approximately 95% in platelet blood counts, after six hours, which persisted up to 24 hours after inoculation, gradually increasing afterwords, to attain normal values 48 hours after inoculation. Five µl blood samples, collected from the mice tail vein, were added to 200 µl of 1% ammonium oxalate for platelets counting in a Neubauer chamber.

Complement levels

Complement was determined as CH50 units in mice depleted of platelets in order to evaluate the magnitude of C_3 consumption by the immune-complex "platelet-anti-platelet" and the time for its recovery to normal levels. For this purpose mice were inoculated with 0.26 mg doses of a-plt.IgG during three consecutive days. Blood samples were collected before (controls), 30 minutes, 2 and 5 hours after the last inoculation. CH50 was determined in a pool of three mice for each interval of time and for controls, with the use of 5 x 10^8 erythrocytes in a final dilution of 2.5 ml for each assay.

Course of infection

For observation of the parasitemic levels, two groups of ten, 5 to 6 weeks old, male, BALB/c mice were infected (i.p.) with 10^2 tryps of the Y strain. They were examined daily for the presence of parasites in blood. When tryps were first observed in one or more blood samples, treatment of animals, from one of the groups with a-plt.IgG and from the other with normal rabbit IgG (NRIgG; control group), was iniciated. This treatment consisted of i.v. inoculations of 0.26 mg/day during 3 consecutive days and of 0.52 mg/day per mouse in the 4 subsequent days.

An experiment was performed for comparison of blood platelet counts and cumulative mortalities between control and thrombocytopenic groups during the acute phase of the infection. Mice from the latter were inoculated (i.v.) every other day, from the day of infection on, with 0.26 mg of a-plt. IgG in the first 4 inoculations and with 0.52 mg in the subsequent ones, up to the 16th day of infection, when the number of parasites in blood became null and inoculations were interrupted. Mice from the control group were similarly treated, excepting that NRIgG was inoculated instead of a-plt.IgG.

Statistical Analysis

Due to the great variability observed in the levels of parasitemia in each group, the median instead of the mean was determined to verify the central tendency of these values. The Wilcoxon test was used for comparing the parasitemic values between platelet-depleted and control groups.

The Chi-Square test was used to compare the cumulative mortalities ($\alpha = 0.05$).

RESULTS

The platelet counts of blood samples from both a-plt.IgG and NRIgG inoculated mice groups are presented in Figure 1. Thrombocytopenia was observed in the NRIgG inoculated controls soon after infection with **T. cruzi**, which persisted up to the 16th day, when platelet countings were discontinued. Nevertheless, the platelet blood levels in this group remained 60%, while the a-plt.IgG treated group remained 15%, that of non-infected mice, in the mean, during the period of observation.

The number of tryps in the blood of mice treated with a-plt.IgG was higher than that of NRIgG treated controls, during the whole time course of the parasitemic phase of the infection (Fig. 2). The Wilcoxon statistics test indicated this defference to be significant, at the 0.05 level, on days 10 to 12 of infection, when parasitemia was high. The parasitemic phase in platelets-depleted did not persist longer than that of control mice, though.

Hemolytic complement levels (CH50) were 1.9, 3.9 and 5.3 units/ml in sera collected 30 minutes, and 2 and 5 hours after the last of three 0.26 mg a-plt.IgG daily inoculations of mice, respectively. CH50 serum levels of non-inoculated controls were 4.6 units/ml. So, serum CH50 levels had already raised to levels close to the normal ones (84.7%) 2 hours after a-plt.IgG inoculation.

Cumulative mortality of the platelet-depleted group was consistently but not significantly higher ($\alpha = 0.05$) than that of the control group (Fig. 3).

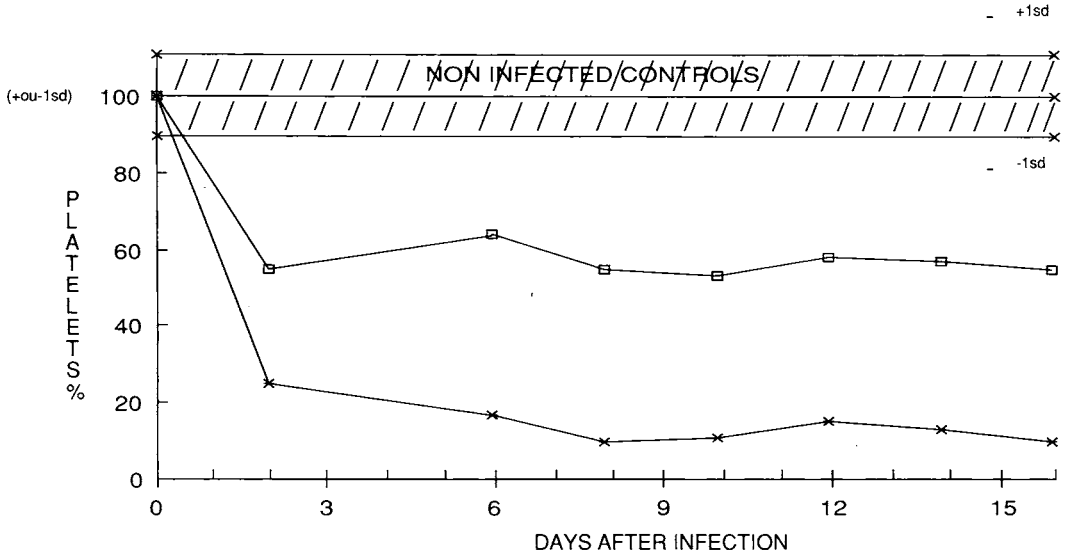

Figure 1 - Blood platelet counts (mean ± 1 S.D.) of mice (10/group) inoculated every 48 hours from the first day of infection on with **T. cruzi**, up to the 16th day, either with anti-platelet IgG (✻——✻) or with normal rabbit IgG (□——□) (0.26 mg of IgG in the first 4 inoculations and 0.52 mg in the subsequent ones).

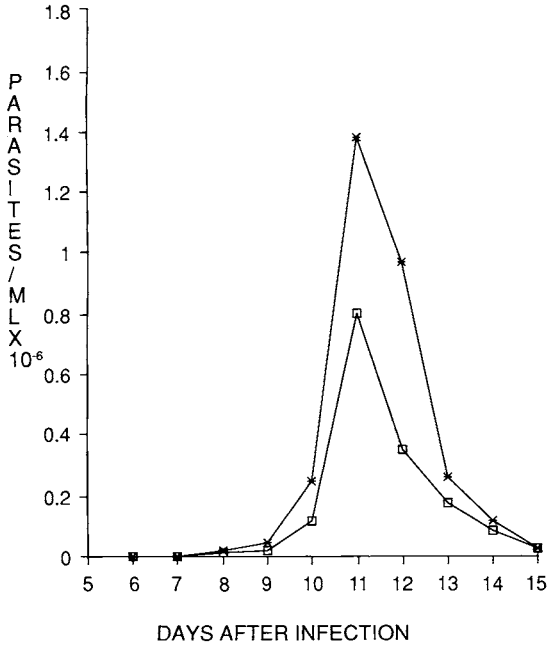

Figure 2 - Levels of parasitemia in BALB/c mice daily treated, with either anti-platelet IgG (✻——✻) or with normal rabbit IgG (□——□) (0.26 mg/day during 3 consecutive days and 0.52 mg/day in the 4 subsequent ones), during the parasitemic phase of infection (days 6 to 12) with **T. cruzi**. Each point represents the median of samples from 10 mice. The difference in parasitemias between the two groups were significant on days 10 to 13 after infection.

DISCUSSION

A decline in blood platelet levels, soon after infection, was described by Cardoso and Brener[4] in experimental Chagas' disease and by a number of authors[5-7] in other experimental trypanosomiasis. A significant decrease in platelet levels to approximately 60% those of non-infected controls was detected, in the current study, in animals not treated with a-plt.IgG, before and during the parasitemic phase, which lasted up to the 16th day of infection, when platelet coutings were discontinued (Fig. 1), Contrary to experimental data, platelet counts within normal limits were observed in patients in the acute phase of Chagas' disease by JAMRA et al.[8]. The increased aggregation of platelets to different agonists and the elevated plasma levels of thromboxane A2 of mice infected by **T. cruzi**, observed by TANOWITZ et al.[9], may account, at least in part, for this decrease in platelet levels which occurs even before the parasitemic phase of murine Chagas' disease. CARDOSO and BRENER[4] suggested that the injury of precursor cells, by heavy parasitism of bone marrow and spleen, might be one of the causes of this decline, but these authors did not exclude disseminated intravascular coagulation or the participation of immune-complexes and complement in the process of platelets sequestration, as other possible concurrent causes of thrombocytopenia.

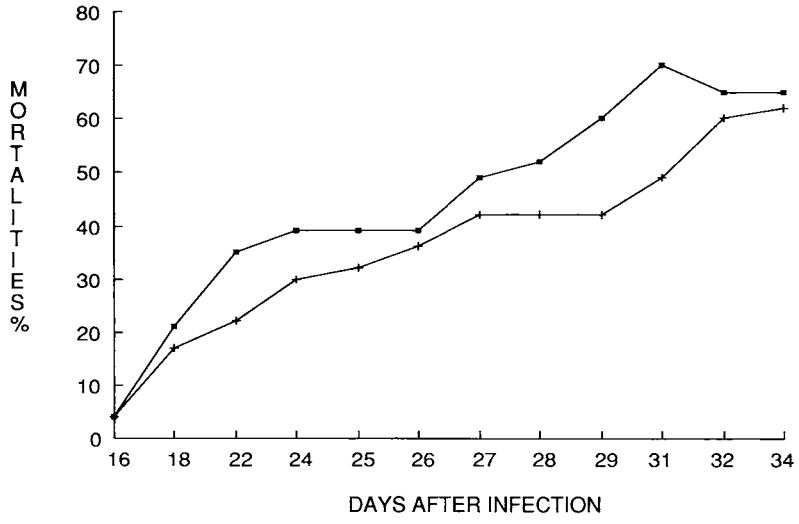

Figure 3 - Cumulative mortalities of the mice groups described in Figure 2.

The reduced rate of clearence of tryps from blood of mice depleted of platelets reported by UMEKITA and MOTA[11], as well as the significantly higher parasitemia observed in mice treated with a-plt.IgG and presenting around 15% of the platelets levels of non infected controls, in the current study (Fig. 2), point to the participation of platelets in the removal of tryps from the circulation. This participation seems to be restricted to the first days of infection, when the specific mechanisms of defence are not fully developed yet, since both groups, platelet-depleted and control, present a decline in parasitemia, starting on the 11th day, down to equivalent levels in a coincident day of infection.

Since CH50 levels in mice daily inoculated with a-plt.IgG were aquivalent to those of normal mice during most of the period of time between two of these inoculations, we assume that this decrease in the complement levels did not consistently contribute to the higher parasitemias observed in the platelet-depleted as compared to the control group. Our results suggest also that, as the specific immunity develops, platelet-independent mechanisms of trypomastigotes removal from circulation become more effective (11th day of infection on) and compensate for the hampering of the platelet-dependent ones. Besides, platelets do not seem to confer an effective protection to the host in the acute phase of the infection, since only a slight and not significantly higher mortality was observed in the platelet-depleted group (Fig. 3), as compared to the control group. Further study is necessary to understand the mechanisms of removal of tryps from blood circulation by platelets.

RESUMO

Trypanosoma cruzi: curso da infecção em camundongos depletados de plaquetas

O efeito do esgotamento de plaquetas sobre o curso da infecção pelo **Trypanosoma cruzi** em camundongos BALB/c foi estudado. A trombocitopenia foi provocada por inoculação de IgG de coelho anti-plaquetas durante a fase parasitêmica da infecção. O número de parasitas no sangue dos camundongos tratados com IgG anti-plaquetas foi significantemente maior que aquele dos controles não tratados, no período de maior parasitemia.

A mortalidade cumulativa dos camundongos esgotados de plaquetas foi consistente mas não significativamente maior que a dos controles até o 32º dia de infecção; do 33º dia em diante elas foram equivalentes, nenhuma morte ocorrendo a partir de então, até o 60º dia, quando as observações foram interrompidas. Estes resultados sugerem que as plaquetas participam dos mecanismos de remoção dos parasitas da circulação, mas que não desempenham um papel efetivo nos mecanismos de defesa contra o **T. cruzi** na fase aguda da infecção.

ACKNOWLEDGEMENT

The authors thank Dr. Euthymia Brandão Almeida Prado for the CH50 determination as well as for the useful suggestions and Drs. Junko Takano Osaka and Luci Ebisui, of the Central Animal House of Faculdade de Medicina da Universidade de São Paulo, for the animals supplied.

REFERENCES

1. ALBRIGHT, J.W. & ALBRIGHT, J.F. - Basis of the specificity of rodent trypanosomes for their natural hosts. **Infect. Immun., 33:** 355-363, 1981.

2. ALCANTARA, A. & BRENER, Z. - The **in vitro** interaction of **Trypanosoma cruzi** bloodstream forms and mouse peritoneal macrophages. **Acta trop. (Basel), 35:** 209-219, 1978.

3. BRENER, Z. - Therapeutic activity and criterion of cure on mice experimentally infected with **Trypanosoma cruzi. Rev. Inst. Med. trop. S. Paulo, 4:** 389-396, 1962.

4. CARDOSO, J.E. & BRENER, Z. - Hematological changes in mice experimentally infected with **Trypanosoma cruzi. Mem. Inst. Oswaldo Cruz, 75:** 97-104, 1980.

5. DAVIS, C.E. - Thrombocytopenia: a uniform complication of African trypanosomiasis. **Acta trop. (Basel), 39:** 123-133, 1982.

6. DUFFEY, L.M.; ALBRIGHT, J.W. & ALBRIGHT, J.F. - **Trypanosoma musculi:** Population dynamics of erythrocytes and leukocytes during the course of murine infections. **Exp. Parasit., 59:** 375-389, 1985.

7. EMERIBE, A.O. & ANOSA, V.O. - Haematology of experimental Gambiense trypanosomiasis, I. Platelet and other haemostatic changes. **Centr. Afr. J. Med., 34:** 286-290, 1988.

8. JAMRA, M; AMATO NETO, V.; FREITAS, J.L. de; SILVA, L.H.P. da & TARTARI, J.T.A. - Aspectos hematológicos da doença de Chagas nas fases iniciais. **Rev. paul. Med., 45:** 52-60, 1954.

9. TANOWITZ, H.P.; BURNS, E.R.; SINHA, A.K.; KAHN, N.N.; MORRIS, S.A.; FACTOR, S.M.; HATCHER, V.B.; BIELEZIKIAN, J.P.; BAUM, S.G. & WITTNER, M. - Enhanced platelets adherence and aggregation in Chagas' disease: a potential pathogenic mechanism for cardiomyopathy. **Amer. J. trop. Med. Hyg., 43:** 274-281, 1990.

10. UMEKITA, L.F. & MOTA, I. - In vitro lysis of sensitized **Trypanosoma cruzi** by platelets: role of C3b receptors. **Paras. Immunol., 11:** 561-566, 1989.

11. UMEKITA L. & MOTA, I. - Role of platelets in the **in vivo** removal of **T. cruzi** from circulation. **Braz. J. med. biol. Res., 23:** 593-596, 1990.

12. VIENS, P.; DUBOIS, R. & KONGSHAVN, P.A.L. - Platelet activity in immune lysis of **Trypanosoma musculi. Int. J. Parasit., 13:** 527-530, 1983.

Recebido para publicação em 14/8/1991
Aceito para publicação em 5/12/1991