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THE COMPLEMENT SYSTEM AND THE INFLAMMATORY RESPONSE IN EXPERIMENTAL CHAGAS' DISEASE

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SUMMARY

Male SW mice infected with either the FL or the Y strain of **Trypanosoma cruzi** were decomplemented with Cobra Venom Factor. The parasitemia of decomplemented mice infected with the FL strain was significantly higher on the 10th day of infection, as compared to the normocomplementemic infected control group, but not on the 12th day. Parasitemia of mice decomplemented and infected with the Y strain was not significantly different from the controls, neither on the 5th, nor on the 7th day of infection. Histological examination showed the inflammatory response of the mice infected with the Y strain to be slightly milder in the decomplemented animals, as compared to the controls. No significant differences in the degree of the inflammatory response was observed between decomplemented and normocomplementemic mice infected with the FL strain of **T. cruzi.**

INTRODUCTION

In a previous work [8] it was observed, in mice infected with the FL strain of **Trypanosoma cruzi,** a marked activation of complement (C) on the 10th day after infection, and a moderate activation in the chronic phase of the disease. This activation might be due to the presence of soluble immune complexes (IC) which on the 10th day of infection were already detected in the circulation [4] and deposited in the heart [5], as well as to the presence of particulate IC (parasites coated with antibodies). Such C activation could induce a localized concentration of the C3a and C5a fragments, which are chemotactic for polymorphonuclear leukocytes and macrophages. For this reason, we decided to investigate the possible contribution of C to the formation of the inflammatory infiltrate in Chagas' disease. The present study is limited to the acute phase of the infection.

On the other hand, results in the literature as to the protection given by C "in vivo" to **T. cruzi** are conflicting. BUDZKO et al. [3] observed that decomplemented mice, infected with the Tulahuén strain, a reticulotropic strain of **T. cruzi,** had significantly increased parasitemia levels and early mortality, as compared to those of the normocomplementemic infected controls. Similar results were obtained by KIERSZENBAUM [9], with mice infected with the Y strain, another reticulotropic strain. On the other hand, DALMASSO & JARVINEN [7] observed no significant differences in the parasitemia levels and mortality rates between genetically C-deficient mice or guinea pigs, infected with the H-510 strain, a myotropic strain of **T. cruzi,** and the normocomplementemic infected controls. Due to these conflicting results we extended our observations in the present study to the levels

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of parasitemia, and two strains of **T. cruzi** of different tropism and shape were used for the investigation of the role of C in the protection against the infection and in the inflammatory response of the host: the FL and the Y strains.

MATERIALS AND METHODS

T. cruzi strains: The FL strain, isolated from naturally infected **Triatoma infestans** and studied by BRENER [1,2], is a predominantly stout and miotropic strain. The Y strain, isolated by means of xenodiagnosis from a patient in the acute phase of the infection by SILVA & NUSSENZWEIG [12] and studied by BRENER [1] and by MELO & BRENER [10] is a predominantly slender and reticulotropic strain.

Infection: SW male mice, 20-25 g in weight, were inoculated intraperitoneally with 10^5 trypomastigotes either of the FL or of the Y strain. Evaluation of the natural protection given by C was limited to the levels of parasitemia and of the inflammatory infiltrate, since the cumulative mortality of the animals were still very low the day we sacrificed them. Parasitemia was determined in a Newbauer counting chamber in adequate dilutions of blood, on the 5th and 7th days of infection with the Y strain, and on the 10th and 12th days of infection with the FL strain of **T. cruzi.**

Complement depletion: Preliminary experiments demonstrated that the intraperitoneal administration of a series of four doses of Cobra Venom Factor (CVF), containing 68μg of protein in 0.1 ml of saline, distributed in a period of 48 hours, would maintain the mice decomplemented for 5 days. This series was initiated on the 1st day after infection with **T. cruzi,** and was repeated every 6th day, so that the animals were kept decomplemented during the whole course of infection. Control mice were injected, on the same schedule, with 0.1 ml of saline.

The CVF was prepared from the venom of Naja-naja snake (supplied by Enzymfarma Ltda, S. Luiz, Maranhão, Brazil), according to the method of MÜLLER-EBERHARD & FJELLSTRÖM [11].

Histology: Mice infected with the FL strain of **T. cruzi** were sacrificed on the 12th day and those infected with the Y strain on the 7th day after infection. Fragments of heart were collected immediately after sacrifice and fixed in 10% Formalin. Specimens were routinely embedded in paraffin, sectioned and stained with hematoxylin-eosin. Lesions were evaluated according to the intensity and diffusibility of the inflammatory infiltrate. Amastigotes nests were also taken in consideration in the evaluation. Classification ranged from + to +++ according to these parameters.

RESULTS

Parasitemia: The parasitemia of control and decomplemented mice, infected with the FL strain of **T. cruzi,** on the 10th and 12th days after infection are presented in Table I, and those of mice infected with the Y strain are in Table II.

The Wilcoxon statistics test indicated that the levels of parasitemia in mice infected with the FL strain are significantly higher on the 10th day after infection, in the complement depleted group, as compared to the control (normocomplementemic) group, at the 0.05 level of significance, while they are not significantly different for these groups on the 12th day after infection. The parasitemia of mice infected with the Y strain was not significantly different in the decomplemented and control groups, neither on the 5th, nor on the 7th day after infection, at the same level of significance.

TABLE I

Comparison of the levels of parasitemia (**T. cruzi**/ml) x 10^{-5} between control and C-depleted mice, infected with a 10^5 trypomastigotes dose of the FL strain of **T. cruzi,** on the 10th and 12th days after infection

Experimental group	10th day after infection				12th day after infection			
	n	Mean ± S.D.	Median	Amplitude of Variation	n	Mean ± S.D.	Median	Amplitude of Variation
Normocomplementemic	26	16.8 ± 20.6	11.5	0 — 98.3	23	71.6 ± 59.5	76.0	2.0 — 223.0
Complement depleted	35	34.8 ± 28.9	27.5	0.75 — 127.8	32	74.6 ± 40.9	70.0	3.0 — 161.0

n = No. of mice in the group
S.D. = Standard deviation

T A B L E II

Comparison of the levels of parasitemia (**T. cruzi**/ml) x 10^{-5} between control and C depleted mice, infected with a 10^{-6} trypomastigotes dose of the Y strain of **T. cruzi**, on the 5th and 7th days after infection

Experimental group	5th day after infection				7th day after infection			
	n	Mean ± S.D.	Median	Amplitude of Variation	n	Mean ± S.D.	Median	Amplitude of Variation
Normocomplementemic	28	6.3 ± 4.0	6.8	0 — 15.3	28	64.6 ± 68.6	52.5	0 — 296.5
Complement depleted	30	6.5 ± 6.4	4.7	0 — 29.8	26	51.2 ± 57.3	33.5	0 — 261.0

For abbreviations, see footnotes under Table I

Histopathology: Most of the infected mice exhibited myocarditis characterized by focal inflammatory infiltrate made up mostly by histiocytes and few lymphocytes. Rarely, neutrophils and eosinophils were part of the inflammatory response. Vessels of the heart microcirculation were frequently dilated with prominent endothelial lining. Myocardial fibers were also focally damaged. The lesion ranged from a degenerative process to fiber death and its local replacement by collagen tissue. Except for this cicatricial lesion, which was more common in the myocardium of the ventricles, the myocarditis as described above was more frequent and intense in the myocardium of the atria and atrioventricular region. Focal involvement of the cardiac neuronal plexus by the inflammatory infiltrate was also observed.

In the mice infected with the FL strain the inflammatory infiltrate was less intense and the number of amastigotes nests was lower than in mice infected with the Y strain. However, the degree of lesion for the mice infected with the Y strain was significantly lower in the decomplemented than in the normocomplementemic group. This difference was not significative in mice infected with the FL strain. The composition of the inflammatory infiltrate was similar for decomplemented and normocomplementemic animals, in the infection with either the FL or the Y strain.

DISCUSSION

The only significant difference in the levels of parasitemia between decomplemented and normocomplementemic mice we had in our results, occurred on the 10th day of infection with the FL strain, which on the 12th day became equivalent for both groups. This difference on the 10th day might be explained by supposed sufficient C levels for natural protection to low parasitemia on this day of infection. Another factor of the C sufficience on the 10th but not the 12th day could be a C consumption by the IC formed [4,5]. Complement activation was evident on the 10th but not on the 8th, which would cause a depletion on the 12th day [8]. In fact, CUNNINGHAM et al. [6] observed a gradual and continuous fall in the levels of hemolytic C in mice infected with another strain of **T. cruzi.** Different from mice infected with the FL strain, those infected with the Y strain presented equivalent parasitemia levels for the decomplemented and normocomplementemic groups, both on the 5th and 7th days of infection. BRENER [2] observed that the stout forms of **T. cruzi,** when injected in the circulation, persisted in the blood for some days, whereas most of the slender forms injected, rapidly disappeared from circulation, penetrating the tissue cells. This would expose the stout forms to the action of C for a much longer time than the slender ones, and could be the cause of the sensibility of the FL strain, in which the stout forms predominate, and of the apparent insensibility to C of the Y strain, a predominantly slender strain.

It is difficult to compare our results of parasitemia levels to those of other authors, because they either used different strains of **T. cruzi,** or determined these levels in different days of infection than those in which we did.

Histopathology did not disclose differences in the heart inflammatory response between normocomplementemic and decomplemented mice infected with the FL strain of **T. cruzi.** However a slight difference was observed between both groups when the infection was due to the Y strain. The cell composition of the inflammatory infiltrate was similar in both groups and the slight difference observed was quantitative. We cannot explain, with the data we have at present, this different histopathologic picture observed in mice infected with the

Y and those infected with the FL strain of **T. cruzi.**

The overall results apparently point to a secondary role of the chemotactic factors of C, in the heart inflammatory response observed in experimental Chagas' disease.

These results are in agreement with a previous work by DALMASSO & JARVINEN [7], as well as with those of WILSON et al. [13] in which these later Authors conclude that C chemotactic factors are not important in the induction of the kidney inflammatory infiltrate in mice infected with **E. coli.**

RESUMO

O sistema complemento e a resposta inflamatória na moléstia de Chagas experimental

Camundongos SW, machos, infectados com a cepa FL ou com a Y do **T. cruzi** foram decomplementados com CVF.

A parasitemia dos camundongos decomplementados e infectados com a cepa FL foi significativamente mais alta no 10.º dia de infecção do que a do grupo controle, infectado mas normocomplementemico, mas não no 12.º dia. A parasitemia dos camundongos decomplementados e infectados com a cepa Y não foi significativamente diferente da dos controles nem no 5.º, nem no 7.º dia de infecção.

O exame histopatológico do coração demonstrou que a intensidade da resposta inflamatória dos camundongos infectados com a cepa Y é pouco menor para os animais decomplementados do que para os controles. Não foram observadas diferenças significativas no grau da resposta inflamatória entre os camundongos decomplementados e os não decomplementados, infectados com a cepa FL do **T. cruzi.**

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