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CONVERSION OF THE C_3 COMPONENT OF COMPLEMENT IN MICE INFECTED WITH TRYPANOSOMA CRUZI (*)

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SUMMARY

The levels of total C_3 (native C_3 plus its degradation products) and the degree of conversion of native C_3 into its breakdown products were studied in sera of mice at different phases of infection by **Trypanosoma cruzi** (8th, 10th, 16th days and over 3 months of infection). Sera from a group of normal mice were also analysed. While the levels of total C_3 were not significantly different among these groups, the conversion of C_3 into its degradation products were significantly greater for the 10 days of infection group than for all the others, excepting the group infected for more than 3 months, which presented a moderate C_3 conversion.

INTRODUCTION

BUDZKO et al.[2] have demonstrated that the depletion of complement in mice, by cobra venon factor (CoF), caused an exacerbation of **Trypanosoma cruzi** infection, as reflected by increased parasitemia and mortality, which shows the importance of the complement system in the control of the infection.

CUNNINGHAM et al.[7] have observed a progressive decrease in the levels of hemolytic complement in 2 strains of mice infected with **T. cruzi,** which presented different susceptibilities to the parasite. In the same study, these authors observed that the supernatants from trypanosomes cultures had a soluble factor, produced by the parasites, with high capacity for decomplementing sera.

The observation, by SIQUEIRA et al.[16], that the liberation of a soluble antigen on the 5th and 6th days after inoculation of rats with **T. cruzi** was followed by complement depletion on the 7th and 8th days, suggests a possible correlation between the presence of the soluble antigen in serum and complement activation.

In a previous study[3] we have observed the presence of circulating immune complexes (IC) in mice infected with the FL strain of **T. cruzi,** on the 10th and 15th days after infection (10 d. and 15 d.). Immune complexes are activators of the complement system by the classical pathway mainly, leading to the cleavage, among other components, of C_3.

This induced us to look for a possible coincidence between the phases of infection in which circulating IC are present[3] and those in which there may be complement activation.

MATERIALS AND METHODS

T. cruzi — The strain used to inoculate the animals was the FL strain, which was isolated and studied by BRENER[1]. Its blood trypomastigotes consist mainly of broad forms a few days after infection.

Antiserum — Antiserum to mouse C_3 was raised in rabbits by the method of MARDINEY and MÜLLER-EBERHARD[11].

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Collection of sera — SW male mice, 20-25 g in weight, were inoculated intraperitoneally with 10^5 trypomastigotes of the FL strain. The sera were collected on the 8th, 10th and 16th days of infection and, from the mice which survived the acute phase, above 3 months after infection (chronic phase). No longer than 1 hour was run between bleeding and separation of the sera. The samples which were not analysed immediately after collection were kept at -70°C. The same procedure was used for the sera of the control mice.

Radial immunodiffusion (RID) — was performed by the method of MANCINI et al.[10], in plates having anti-mouse C_3 incorporated to the agarose. This antiserum reacted with mouse native C_3, and with its breakdown products, and consequently this method measured the total C_3 (native C_3 + C_3 breakdown products) of the sera. A unique pool of 10 sera from normal mice was used as standard in all plates.

Immunoelectrophoresis — LAURELL's crossed immunoelectrophoresis (CIE) was performed in a micro-technique, as described by WEEKE[17], with slight modifications. The same antiserum to mouse C_3 was used in all CIE plates. The measuring of the area enclosed by the precipitates was performed by planimetry, after a 5.2 times enlargement. Since this area is related to the concentration of the antigen reacting with the antiserum incorporated to the agarose, the % of C_3 conversion for each sample was calculated according to the formula:

$$\% \text{ of conversion} = \frac{\Sigma\ C_3B.P.\ X\ 100}{\Sigma\ C_3B.P. + NC_3}$$

Where $C_3B.P.$ = area enclosed by the precipitate formed by each C_3 breakdown products and the antiserum incorporated to the agarose.

NC_3 = area enclosed by the precipitate formed by native C_3 and the antiserum incorporated to the agarose.

Incubation of the mouse sera with IC — An experiment was performed for identification of the individual precipitate curves on the CIE plates. Aliquots of a pool of fresh mouse sera were incubated for 10 minutes, 30 minutes and 24 hours at 37°C, with particulate human IgG-rabbit anti-human IgG complex. The supernatants were used for CIE analysis.

Number of sera analysed — Table I shows the number of sera of mice at each phase of infection, analysed by RID and by CIE.

TABLE I

Number of mouse sera analysed

Phase of infection by **T. cruzi**	Number of sera analysed	
	RID	CIE
8 days after infection	15	19
10 " " "	35	28
16 " " "	18	15
Chronic phase	21	9
Controls	22	19

Statistical analysis — Was performed by using the KRUSKAL-WALLIS test[5] at the 0.001 level of significance. When the analysis lead to rejection of the null hypothesis, groups of two samples were analysed using the same statistic until the difference between populations had been satisfactorily detected. In these cases the conclusions must be taken with care, because the overall level of significance is distorted. However, in order to avoid a large distortion, the same critical value for the general test was used when comparing two groups. It is important, also, to point out that we have worked with a very conservative rule of decision, since we have chosen a small level of significance.

RESULTS

The results of the quantitation of total C_3 by RID are presented in Fig. 1. The mean value and S.D. for each group was calculated as % of the control's mean value. The KRUSKAL-WALLIS T statistic (= 15,59) indicates that there are no differences among the groups at different phases of infection for total C_3($P=0.0036$).

Mouse sera incubated for different periods of time with human IgG-rabbit anti-human IgG complex gave different profiles of CIE against anti-mouse C_3 (Fig. 2). The electrophoretic mobilities of native C_3 and of its products of cleavage by EAC $\overline{142}$ in mouse plasma were described by MARDINEY & MÜLLER-EBER-

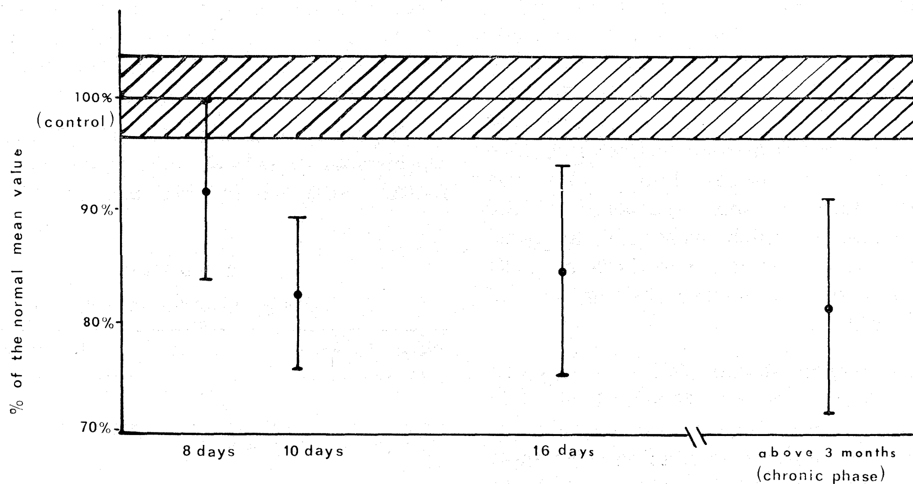

Fig. 1 — Total C_3 of mice at different phases of infection by **T. cruzi**, as measured by RID. The mean and S.D. for each group were calculated as % of the mean of the control group

HARD[11]. They observed that native C_3 (β1C) was initially cleaved to a faster mobility product, which they designated β1G. Subsequently, β1G underwent conversion into a fragment with slower mobility than native C_3, which they designated β1D. These authors found, further, by sedimentation rate studies, that βID (6.5S) was substantially smaller than native C_3 (9,5S), with β1G in between. PEPYS et al.[13] have done identical observation using either EAC$\overline{142}$ or the CoF-dependent C_3 convertase for conversion of C_3, and by estimating the molecular weights by gel filtration.

The peaks, we have obtained for fresh murine serum and for sera incubated with particulate IC, gave identical electrophoretic mobilities (Fig. 2) as those mentioned in these two studies. We have also observed two small peaks, with mobilities between those of β1G and β1C, which were not mentioned by these authors. We have designated these two peaks fast and slow inter-β, respectively. The slower inter-β peak was present only in sera incubated for 10 and for 30 minutes with IC, while the faster inter-β peak was present in sera incubated for 24 hours, with IC.

Typical profiles of CIE against anti-murine C_3 are presented in Fig. 3. Most 8 d. and some 16 d. sera show a very similar profile to that of the controls, with very low β1G peak and high β1C peak. None of the sera of animals in those two phases of infection presented the β1D or inter-β. Most 10 d. and some of the chronic phase sera revealed a completely altered profile, with very low native C_3 (β1C) and high β1G peaks. β1D and slow inter-β were only present in sera having high β1G. The fast inter-β, observed in sera incubated with IC for 24 hours, was not present in any of the sera of the different groups studied.

As one could expect, the % of the C_3 breakdown products is higher in the phases of infection in which the % of native C_3 is low. This can be observed in Fig. 4, which depicts the % of C_3 and of its cleavage products for each of the different groups studied. Native C_3 is extremely low, while its breakdown products are higher in the 10 d. group. The same occurs in a milder way with the chronic phase group.

The % of C_3 conversion, at the different phases of infection studied is presented in Table II.

TABLE II

Mean ± Standard Deviation of % of C_3 conversion in groups of mice at different phases of infection by **T. cruzi**

Groups of infection				
Control	8 d.	10 d.	16 d.	chronic
27 ± 10	26 ± 10	85 ± 8	38 ± 13	68 ± 16

The overall KRUSKAL-WALLIS statistic, for the test of no difference among the groups

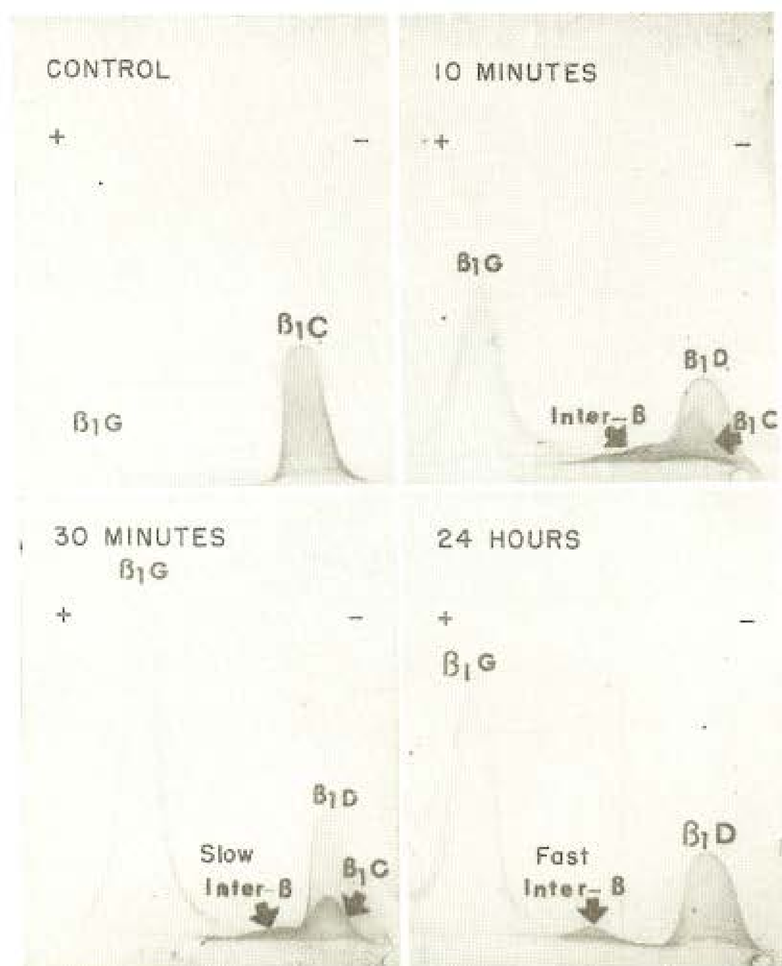

Fig. 2 — Conversion of murine C_3 by IC. Undiluted mouse fresh serum was incubated for different times with human IgG-rabbit anti-human IgG particulate complex, at 37°C. The time of incubation is indicated on each profile. CIE was performed, after centrifugation, against rabbit anti-mouse C_3

studied, for the C_3 conversion was equal to 53.74 ($P < 10^{-9}$).

So, we concluded that the groups are not homogeneous. Table III shows the results of the statistical analyses for 2x2 comparisons.

So, we can summarize these conclusions by saying that the % of conversion of the 10 d. group is significantly higher than that of all other groups studied, excepting the chronic phase group.

T A B L E III

Statistical analysis of the results for the % of C_3 conversion for 2x2 comparison of the different groups studied (different phases of infection)

Comparison between groups	KRUSKAL-WALLIS statistic	P-value
N x 10 d.	28,21	$<10^{-3}$*
N x chronic	13,77	0.0081
N x 16 d.	4,48	0.3454
N x 8 d.	0,16	0.9971*
10 d. x 16 d.	21,75	0.0002*
16 d. x chronic	9,07	0.0594
10 d. x chronic	10,84	0.0284

* Significant at a 0.001 level

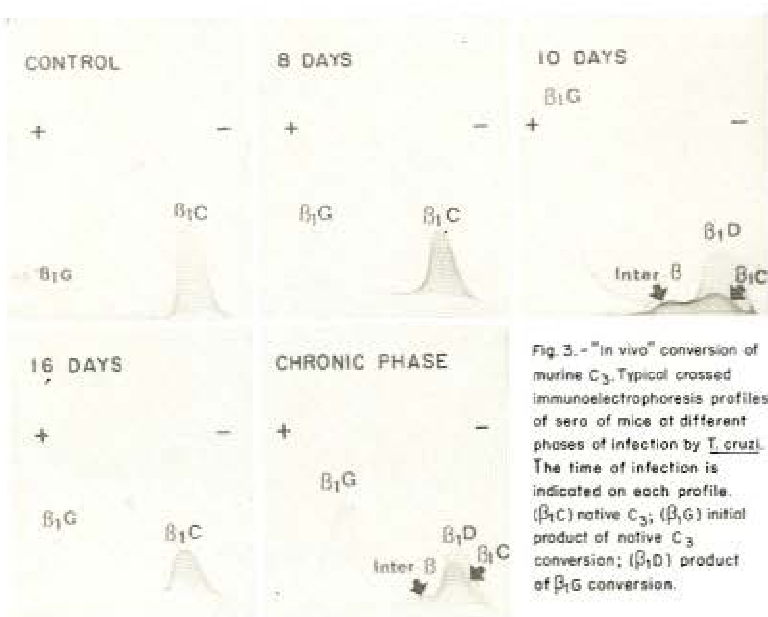

Fig. 3. - "In vivo" conversion of murine C_3. Typical crossed immunoelectrophoresis profiles of sera of mice at different phases of infection by T. cruzi. The time of infection is indicated on each profile. (β_1C) native C_3; (β_1G) initial product of native C_3 conversion; (β_1D) product of β_1G conversion.

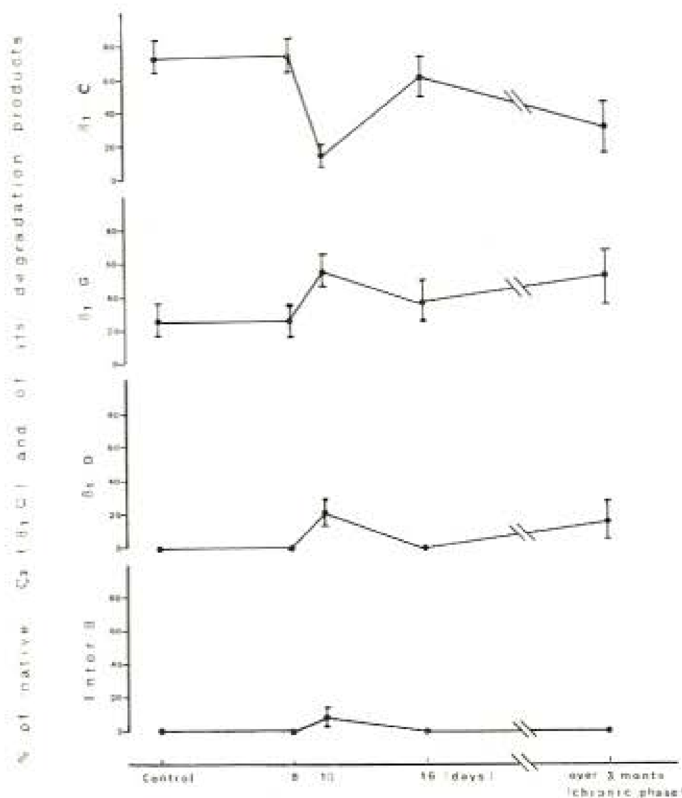

Fig. 4 — % of native C_3 ($\beta 1C$) and of its degradation products in sera of mice at different phases of infection by **T. cruzi** (Mean ± 1 S.D.)

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DISCUSSION

The activation of complement in mice infected with **T. cruzi** presented a marked peak on the 10 d., since none of the 8 d. and 16 d. sera studied had a significant C_3 conversion. The presence of circulating IC [3] in infected mice, in a phase with high, as well as in a phase with low complement activation, as we have observed on the 10 d. and 16 d. respectively, suggests that soluble circulating IC do not represent the main mechanism of complement activation in the acute phase of infection, though it probably contributes to it.

Other possible factors acting concomitantly for the complement activation might be the presence of circulating particulate IC (parasites containing antibodies on their surface) as well as components of the parasite membrane.

The moderate C_3 conversion in the chronic phase, observed in this study, when no circulating IC were detected [3], supports the view that other factors, besides circulating IC, are involved in the complement activation during the infection. A probable factor of complement activation during this chronic phase could be deposited IC.

BUDSKO et al. [2] have demonstrated the importance of the complement system in the resistance to the infection by **T. cruzi** during the acute phase, in mice. The moderate activation of complement we have observed during the chronic phase may be responsible, in part, for the control of parasitemia during this phase. This could be achieved by complement, through one of the following mechanisms: a) together with antibody it could cause lysis of the parasites, directly; b) it could facilitate destruction of the parasites by phagocytes, through its opsonic capacity; c) through its anaphylatoxin properties.

Another possible and undesirable consequence of the marked complement activation on the 10 d. for the host would be the immunosuppression, as a consequence of complement depletion. The impairment of the capacity of mice, depleted of complement with CoF, to mount an antibody response to a variety of thymus-dependent antigens, was stressed by PEPYS [12]. In fact, immunosuppression has been demonstrated in experimental Chagas' disease [6,8,9,14,15], which may be in part due to the depletion of complement as a consequence of its activation.

Finally, since activation of complement generates pharmacological active compounds, their action should be considered in the pathogenesis of the disease.

Abbreviations used in this paper: 8 d., 10 d. and 16 d. = 8 days, 10 days and 16 days of infection by **T. cruzi**, respectively; chronic = more than 3 months of infection by **T. cruzi**; N = controls; IC = immune complex (es); RID = radial immunodiffusion; CIE = crossed immunoelectrophoresis; S.D. = standard deviation; CoF = cobra venom factor.

R E S U M O

Conversão do componente C_3 do complemento em camundongos infectados com Trypanosoma cruzi

Os níveis de C_3 total (C_3 nativo mais seus produtos de degradação) e o grau de conversão do C_3 nativo em seus produtos de degradação, foram estudados em soros de camundongos em diferentes fases da infecção por **Trypanosoma cruzi** (8.°, 10.°, 16.° dias e mais de 3 meses de infecção). Os soros de um grupo de camundongos normais também foram analisados. Enquanto os níveis de C_3 total não diferiram significativamente nos grupos estudados, a conversão de C_3 em seus produtos de degradação foi significativamente maior no grupo com 10 dias de infecção do que em todos os outros, excetuando-se o grupo com mais de 3 meses de infecção, o qual apresentou conversão moderada.

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