Rev. Inst. Med. trop. São Paulo
23(3):122-126, *maio-junho,* 1981

UDC 593.161.13

IMMUNOSUPPRESSION IN MICE INFECTED WITH TRYPANOSOMA CRUZI (CHAGAS, 1909)

II — Trypomastigote Crude Extract (TCE) suppress the humoral immune response in mice

Antonio C. CORSINI and Marcos G. COSTA (1)

SUMMARY

Balb/C mice infected i.p. with 100 parasites of **Trypanosoma cruzi** strain Y had their humoral response to SRBC suppressed in contrast to normal delayed type hypersensitivity reactions to DNFB. An extract prepared from circulating trypomastigotes (TCE) suppressed primary and secondary immune response to SRBC when injected 3-4 days before antigen. This fact may reflect a depletion of Antigen Reactive Cells in the spleen as a consequence of a mitogen driven exhaustion of B cell potential similarly to infections with African trypanosomes.

INTRODUCTION

In the preceding paper we reported the evidences of a B cell polyclonal activation in **Trypanosoma cruzi** infected Balb/C mice. Moreover, an extract prepared from circulating trypomastigotes (TCE) exhibited polyclonal B cell activation "in vivo".

This phenomenon previously described in **Trypanosoma brucei** infections [10,13], was shown to be an important mechanism of suppression in African trypanosomiasis [1,5,10].

In this paper we report the suppression of the humoral immune response to SRBC either in mice infected with **T. cruzi** or injected previously with TCE. The suppression mediated by the extract is time dependent since it manifested itself only when TCE was injected before antigen. This fact correlates well with suppression of the humoral response to SRBC in LPS injected mice [7] and is a further evidence of a mitogenic activity in TCE.

Delayed type hypersensitivity reactions to DNFB were not affected either in infected animals or in TCE injected mice.

MATERIAL AND METHODS

1. **Animals** — Female and male Balb/C mice bred and kept in our animals facilities were used with 6-8 weeks of age.

2. **Trypomastigote crude extract (TCE)** — The extract was prepared by freezing-thawing (3x) a suspension containing $2x10^6$ trypomastigotes/ml as described in the preceding paper. Mice were injected intravenously (i.v.) with 0.2 ml of the extract.

3. **Immunization of mice with SRBC** — Mice were immunized i.v. with $2x10^8$ SRBC in 0.2 ml of PBS. Red cells harvested in Alsever solution were washed previously (3x) with PBS and were counted in a Neubauer haemocytometer. Boost was done 26 days after primary immunization by the i.v. injection of $2x10^8$ cells.

4. **Plaque forming cells (PFC)** — Jerne PFC were performed as described by DRESSER [8] on the 4th day after primary immunization or boost. Preliminary experiments had shown the peak response to occur on those days.

(1) Departamento de Microbiologia e Imunologia da Universidade Estadual de Campinas — UNICAMP. 13.100 — Campinas, São Paulo, Brasil
Correspondence to A. C. Corsini at the above address.

5. **Sensitization with dinitrofluorobenzene** (DNFB) — Mice were sensitized to DNFB as described by VADAS et al.[18]. Mice were shaved in the abdomen and painted with 50 μl of a solution containing 10 mg/ml of 2.4 dinitro-1-fluorobenzene (DNFB, British Drug Houses Chemicals, Poole, Dorset) in acetone-olive oil (1:1). Challenge was done 5 days later painting the left ear with 5 μl of the same solution.

6. **Assessment of delayed type hypersensitivity (DTH) reactions** — Twenty four hours after challenge animals were killed by cervical dislocation and both ears cut off at their roots using scissors while pulling with forceps. Hair and any extraneous tissue were removed from both ears. Usually, we obtained the same amount of tissue from both ears. The ears were weighed on a Mettler H-54 balance. Results are expressed by the left (challenged)/right (unchallenged) ear weight ratio as described previously[6].

7. **Statistical analysis** — Student "t" test was used to analyse the data.

RESULTS

1. **Suppression of the humoral response in infected mice** — A severe immunosuppression has been reported in **T. cruzi** infections[4,16]. To evaluate the humoral response to SRBC, mice were immunized on different days after infection with a optimal dose of antigen ($2x10^8$ cells) and their spleens assayed for PFC 4 days after immunization. Results presented in Table I show a 63% suppression of PFC after 7 or 11 days of infection. Later days were not verified since mortality was very high. On the other hand immunization at the beginning of the infection (up to 3 days) did not interfere with PFC number.

TABLE I

Immunosuppression in Balb/C mice infected with **Trypanosoma cruzi**

Days of infection	PFC/Spleen $x10^3$	PFC/10^6 viable cells	(%) Suppression
0	135 ± 5.2	1120 ± 180	
3	120 ± 5.0	1195 ± 140	0
7	50 ± 3.5	288 ± 22	63
11	32 ± 1.3	313 ± 19	76

Mice infected i.p. with 100 parasites. PFC determined 4 days after SRBC. Arithmetic mean ± 1 SEM (n = 5)

2. **Delayed type hypersensitivity (DTH) reactions to DNFB in infected mice** — The cell-mediated immune response in infected mice was evaluated by their capacity to mount a response to DNFB. Mice were skin painted on days 6 or 11 after infection on challenged 5 days later. Note that mice were then sensitized or challenged at the peak of parasitaemia. Results in Table II express the left (challenged)/right (unchallenged) ear weight ratio. No suppression of cell-mediated immunity was noticed in infected mice compared to controls.

TABLE II

Delayed type hypersensitivity reactions to DNFB in Balb/C mice infected with **T. cruzi**

Days of infection	L/R ear ratio
0	2.00 ± 0.2
6	2.05 ± 0.1
11	2.17 ± 0.1

DTH reaction assessed by the left/right ear weight ratio as described previously. Animals challenged 5 days after sensitization. Arithmetic mean ± 1 SEM (n = 5)

3. **Suppression of the primary humoral response to SRBC by TCE** — We had previously shown the mitogenic effects over the immune system of TCE. Since polyclonal B cell activators such as LPS may enhance or suppress the antibody response to SRBC depending on the time of antigen presentation[7] we looked for this phenomenon regarding TCE and SRBC.

Thus, mice were injected i.v. with 0.2 ml of TCE prepared from $2x10^6$ trypomastigotes/ml before or after antigen. A 40% inhibition of PFC numbers was obtained when TCE was given up

to 6 days before SRBC. Such inhibition was greater for smaller doses of antigen (Table III).

TABLE III

Suppression of the primary immune response to SRBC by Trypomastigote Crude Extract (TCE)

TCE before SRBC in days	PFC/spleen x10^3	(%) Suppression
Control	234 ± 15.0	
— 3	152 ± 16.0	35
— 4	167 ± 14.0	29
— 5	126 ± 9.0	46
— 6	142 ± 7.0	39

TCE prepared as described previously. Mice immunized i.v. with 2x10^8 SRBC. PFC assayed in the 4th day after immunization. Results express the arithmetic mean ± 1 SEM (n = 5). Statistical significance: $p < 0.01$

On the other hand, if TCE was given together with or 24 hr after SRBC the primary immune response was not affected (data not shown).

4. **Suppression of the secondary humoral response to SRBC by TCE** — Since TCE suppressed the primary response to SRBC when given before the antigen we looked for suppression of the secondary response when TCE was injected before boost.

Both, direct (IgM) and indirect (IgG) responses were suppressed when TCE was given again 3-4 days before boost (Table IV). Mice were injected i.v. with 0.2 ml of TCE prepared from 2x10^6 trypomastigotes/ml.

TABLE IV

Suppression of the secondary immune response to SRBC by Trypanosoma Crude Extract (TCE)

TCE before boost in days	PFC/Spleen x 10^3			
	Direct		Indirect	
Control	78 ± 3.7 [a]		898 ± 62 [d]	
— 3	57 ± 7.1 [b]	(27)	537 ± 28 [e]	(40)
— 4	48 ± 5.8 [c]	(40)	647 ± 77 [f]	(28)
No Boost	0.4 ± 0.5		3.5 ± 1.3	
TCE only	2.5 ± 4.2		3.6 ± 1.2	

Mice immunized as described in **Material & Methods.** PFC assayed on the 4th day after boost. Results express the arithmetic mean ± 1 SEM (n = 5). In brackets % of suppression. a — b $p < 0.05$; a — c $p < 0.01$; d — e $p < 0.01$; d — f $p < 0.05$

We concluded that both primary and secondary humoral response to SRBC were suppressed by TCE provided mice were injected with the extract 3-4 days before antigen. On the other hand, no enhancement of the responses was detected when the extract was given together with or immediatly after the antigen (data not shown).

5. **Delayed type hypersensitivity to DNFB in mice injected with TCE** — It had been shown previously that an extract from **T. cruzi** epimastigotes depresses DTH reactions to DNFB in mice (CORSINI et al., Immunology in press). Since DTH reactions were not affected in infected mice we decided to look at the effects of TCE on cell-mediated immunity. Mice were sensitized to DNFB and were injected i.v. with 0.2 ml of the extract during 3 days after sensitization or with a single shot on the day of antigen challenge.

Results in Table V express the L/R ear weight ratio for groups of 5 animals (arithmetic mean ± 1 SEM). No significant ($p < 0.05$) reduction in L/R ratios was noticed in TCE injected animals. We concluded that TCE does not interfere with DTH reactions to DNFB in mice similarly to experimental infections.

TABLE V

Delayed type hypersensitivity reactions to DNFB in mice injected with Trypomastigote Crude Extract (TCE)

Groups	L/R ear weight ratio
Control not injected	1.70 ± 0.1
TCE on day of challenge	1.46 ± 0.1
TCE during 3 days after sensitization	1.60 ± 0.1

Arithmetic mean ± 1 SEM (n = 5). The differences between the groups were not significant

DISCUSSION

We had previously shown in the preceeding paper that Balb/C mice are susceptible to an infection with 100 parasites of **Trypanosoma cruzi** strain Y. During the pre-patent period i.e. before parasites can be detected in peripheral blood there was a splenomegaly and a tremendous increase in background PFC against SRBC.

These important alterations in the spleen during the pre-patent period of the infection

were reflected in the immune responses towards unrelated antigens. Thus, mice immunized i.v. with SRBC or skin sensitized to DNFB during this period were not suppressed. Conversely, after 7 days of infection mice were dramatically unable to mount an humoral response to SRBC although DTH reactions to DNFB remain unimpaired.

These results regarding humoral suppression to SRBC confirm previous results obtained in heavily infected (10^4) mice [4,16]. On the other hand we could not confirm the results of impaired DTH reactions in **T. cruzi** infections described by REED et al. [17]. This might be due to the large inoculum (10^4 parasites) used by those Authors which makes a heavy load to the immune system.

The humoral suppression detected in **T. cruzi** infections might be due to a decrease in Antigen Reactive Cells (ARC) [15] since those cells were driven to proliferation and differentiation at the beginning of the infection raising consequently the background number of IgM PFC. This could explain as well the existing timing between antigen administration and suppression.

Similar phenomenons have been described in rodent **T. brucei** infections [10] and the existence of a postulated mitogen [9] could explain suppression.

The existence of a polyclonal B cell activator (PBA) was well characterized in TCE. The preceding paper described its action over the immune system including primary lymphoid organs i.e. the bone marrow. This latter fact is important if we consider that primary uncommited stem cells were driven to proliferation by the extract. If differentiation took place at the end of the proliferative phase an exhaustion of clones might have occurred in those TCE injected mice.

Although only half of the spleen cells respond to LPS [11] "in vitro" antibody secreting cells represent the end product of several cell divisions [2,12]. Since the B cell potential is limited [19] we may assume that polyclonal B cell activators as those described in trypomastigotes extract may act on B cells at different levels of differentiation pushing them towards a proliferative-differentiative pathway resulting in an exhaustion of B cell clones.

This fact implies that ARC would be activated in the absence of antigen which in later periods would face the spleen depleted of ARC and as a consequence suppression results.

This PBA mediated suppression against humoral SRBC responses was previously described, in "in vivo" experiments using LPS [7]. The effects of TCE are quite similar to those described for LPS since a suppression was verified only when TCE was administered before antigen.

On the other hand we could not demonstrate an adjuvant effect of TCE when given at the same time as antigen or on days 1 or 2 following immunization, a phenomenon well known for LPS [7]. However, evidences of the adjuvant effect of the infection in SRBC responses have been accumulated. Thus, (CBA x C_{57} B1/10) F_1 mice, a strain resistant to **T. cruzi**, immunized together with the infective dose gave an enhanced response to SRBC (CORSINI et al., submitted).

DTH reactions of DNFB were not impaired either in infected or in TCE injected mice. Similar results were verified in **T. brucei** infections although T cell functions were profoundly suppressed in experimental African trypanosomiasis [1,14].

Unimpaired DTH reactions in infected or TCE injected mice could be due either to a resistant mediating T lymphocyte subset or due to an increase in monocytes an macrophages at the challenge site compensating the defective but still lymphokine (such as MIF) producing T cell [3].

In conclusion: we presented evidences of polyclonal B cell activation in an extract prepared from circulating trypomastigotes of **T. cruzi** that can be related to the humoral suppression towards unrelated antigens in infections with this stercorarian trypanosome. As in infected mice DTH reactions to DNFB were not affected. An exhaustion of B cell potential thanks to an un specific polyclonal activation decreasing ARC may explain suppression depending on the timing between TCE and antigen presentation.

CORSINI, A. C. & COSTA, M. G. — Immunosuppression in mice infected with **Trypanosoma cruzi** (CHAGAS, 1909). II — Trypomastigote Crude Extract (TCE) suppress the humoral immune response in mice. **Rev. Inst. Med. trop. São Paulo** 23:122-126, 1981.

RESUMO

Imunosupressão em camundongos infectados com Trypanosoma cruzi (Chagas, 1909)

II — Supressão da resposta humoral em camundongos inoculados com extrato de tripomastigotas

Camundongos Balb/C infectados em 100 parasitas da cepa Y do **T. cruzi** tiveram sua resposta humoral a hemácias de carneiro suprimida. Este fenômeno foi também verificado quando os camundongos foram inoculados, previamente à inoculação do antígeno, com um extrato de tripomastigotas. Ambos os fenômenos poderão representar uma diminuição das "Células Virgens Reativas ao Antígeno" em conseqüência da ativação policlonal do sistema linfóide. A resposta imune mediada por células não foi comprometida em ambas as situações.

ACKNOWLEDGEMENTS

We thank Mrs. Ismália M. Doné for her assistance in breeding the mice and Miss Dirce Lima Gabriel for her technical assistance. The suggestions of Dr. A. F. Pestana de Castro are fully appreciated.

REFERENCES

1. ASKONAS, B. A.; CORSINI, A. C.; CLAYTON, C. E. & OGILVIE, B. M. — Functional depletion of T and B memory cells and other lymphoid cell subpopulations during trypanosomiasis. **Immunology 36:** 313-321, 1979.

2. ASKONAS, B. A. & NORTH, R. J. — The lifestyle of B-cells. Cod Spring Harbor Symp. on Quant. **Biology 41:** 749-754, 1976.

3. CLAYTON, C. E. — **The Role of the Spleen in the Immunology of Parasitic Diseases.** 1a. ed. Basel, Tropical Diseases Research Series n.º 1, 1979, Cap. 8, pp. 95-119.

4. CLINTON, B.; ORTIZ-ORTIZ, L.; GARCIA, W.; MARTINEZ, T. & CAPIN, R. — **Trypanosoma cruzi:** early immune responses in infected mice. **Exp. Parasitol. 37:** 417-425, 1975.

5. CORSINI, A. C.; CLAYTON, C. E.; ASKONAS, B. A. & OGILVIE, B. M. — Suppressor cells and loss of B-cell potential in mice infected with **Trypanosoma brucei. Clin. Exp. Immunol. 29:** 122-131, 1977.

6. CORSINI, A. C.; BELLUCCI, S. B. & COSTA, M. G. — A simple method of evaluating delayed type hypersensitivity in mice. **J. Immunol. Methods 30:** 195-200, 1979.

7. DIAMANTSTEIN, T.; KEPPLER, W.; BLITSTEIN-WILLINGER, E. & BEN-EFRAIM, S. — Suppressor of the primary immune response **in vivo** to sheep red blood cells by B-cell mitogens. **Immunology 30:** 401-407, 1976.

8. DRESSER, D. W. — **Handbook of Experimental Immunology.** 2a. ed. Oxford, D. M. Weir (ed). Cap. 28, 1978, pp. 28.1-28.5.

9. GREENWOOD, B. N. — **Parasites in the Immunized Host: Mechanisms of Survival.** 1a. ed. Amsterdam, R. Porter and J. Knight (eds.). Cap. 9, 1974, pp. 137-146.

10. HUDSON, K. M.; BYNER, C.; FREEMAN, J. & TERRY, R. J. — Immunodeppression, high IgM levels and evasion of the immune response in murine trypanosomiasis. **Nature** (London) **264:** 256-258, 1976.

11. JANOSSY, G. & GREAVES, M. — Functional analysis of murine and human B lymphocyte subsets. **Transplant. Rev. 24:** 177-236, 1975.

12. ZAUDERER, M. & ASKONAS, B. A. — Several proliferative phases precede maturation of IgG-secreting cells in mitogen-stimulated cultures. **Nature** (London) **260:** 611-613, 1976.

13. KOBAYAKAWA, T.; LOUIS, J.; IZUI, S. & LAMBERT, P. H. — Autoimmune response to DNA, red blood cells, and thymocyte antigens in association with polyclonal antibody synthesis during experimental African trypanosomiasis. **J. Immunol. 122:** 296-301, 1979.

14. MURRAY, P. K.; JENNINGS, F. W.; MURRAY, M. & URQUHART, G. M. — The nature of immunosuppression in **Trypanosoma brucei** infections in mice. II. The role of the T and B lymphocytes. **Immunology 27:** 825-840, 1974b.

15. NOSSAL, G. J. V. & ADA, G. L. — **Antigens, Lymphoid Cells, and the Immune Response.** 1a. ed. New York, Cap. 6, 1971, pp. 85-106.

16. RAMOS, C.; LAMOYI, E.; FEOLI, M.; RODRIGUEZ, M.; PÉREZ, M. & ORTIZ-ORTIZ, L. — **Trypanosoma cruzi:** immunosuppressed response to different antigens induced in the infected mouse. **Exp. Parasitol. 45:** 190-199, 1978.

17. REED, S. C.; LARSON, C. L. & SPEER, C. A. — Suppression of cell-mediated immunity in experimental Chagas' disease. **Z. Parasitenkd. 52:** 11-17, 1977.

18. VADAS, M. R.; MILLER, J. F. A. P.; GAMBLE, J. & WHITELAW, A. — A radioisotopic method to measure delayed type hypersensitivity in the mouse. I. Studies in sensitized and normal mice. **Int. Archs. Allergy App. Immunol. 49:** 670-692, 1975.

19. WILLIAMSON, A. R. & ASKONAS, B. A. — Senescence of an antibody-forming cell clone. **Nature** (London) **238:** 337-339, 1972.

Recebido para publicação em 4/6/1980.