

# THE EFFECT OF A DIALYZABLE TRANSFER FACTOR (TFd) IN THE EXPERIMENTAL AMERICAN TRYPANOSOMIASIS OF MICE\*

(Preliminary report)  
Humberto Menezes\*\*

*A dialyzable transfer factor (TFd) was obtained from spleen cells, of mice vaccinated with the avirulent PF strain of Trypanosoma cruzi.*

*This factor reduced significantly the parasitemia of animals treated before or after the infection with a virulent strain of the same parasite, but does not reduced the mortality rate to a level lower than that of the control mice.*

*It is expected that in a next future, new techniques in the use of such factor will bring better results.*

The knowledge of the cellular immunity has progressed greatly since Landsteiner and Chase (in 6) demonstrated that the delayed hypersensitivity could be transferred by cells.

In spite of the opinion of some investigators<sup>1</sup> that delayed hypersensitivity and cellular immunity are not identical, it is accepted by others<sup>2,3,6</sup> that both are alike.

In 1973, Ribeiro dos Santos<sup>9</sup> has demonstrated experimentally that the immunity in the american trypanosomiasis is predominantly cellular and could be transferred by cells. Identical conclusion was drawn, independently, from the experiments of Roberson and Hanson<sup>10</sup>.

The use of a dialyzable extract of lymphocytes in the transfer of cellular immunity by Lawrence et als.<sup>4</sup> and the observation above mentioned stimulated the realization of this work in which I tried to verify the existence of any possible stimulation of the immunologic defenses of experimental animals by a dialyzable extract of sensitized cells.

## MATERIAL AND METHODS

*Trypanosoma cruzi* of the avirulent strain PF<sup>7</sup>, harvested in Warren liquid medium<sup>11</sup>

following the Nakamura technique<sup>8</sup> was washed three times in sterile saline solution (NaCl 0,15M) and finally suspended in identical solution.

About 50% of the parasites were mobile forms and only 2% of trypomastigotes. From this suspension 3.500 flagellates per g/body weight were injected, subcutaneously, in 40 albino mice with 10g each.

Fifteen days after, all the animals were killed by cerebral concussion and the spleens collected aseptically.

The total weight of the material was 6g that was grinded in 60 ml of distilled water, in a Potter-Elvehjen homogenizer. This suspension was placed in a cellophane sac and dialyzed, against 60 ml of NaCl (0,15M) plus BPS (0,05M), pH 7,2, during 24 hours at 4°C.

The dialyzate was injected, by subcutaneous route, in 10 mice with 10g of body weight, 0,2ml, 0,2ml and 0,1ml in the three subsequent days, respectively.

Twelve days later the animals were challenged with 3.500 parasites/g of the virulent Y strain, blood forms from mice in the 8<sup>th</sup> day of infection. The infection with the virulent parasites was by subcutaneous route.

\* Presented partially to the 6<sup>o</sup> Cong. Latinoamericano de Farmecologia, 04/12/1976. Buenos Aires. Argentina.

\*\* Depart. Genetica, FMRP, USP, 14.100, Ribeirão Preto, S.P. — Brasil.  
Submitted to publication on March, 1977.

Ten mice controls were inoculated as above and ten others, the second experimental group, were infected in the same way. This later group, 5 days after the infection, was treated with the dialyzate, 0,2ml every day, during the following 10 days.

Parasitemia of all groups was done 8,15 and 30 days after the virulent infection. The cumulative mortality rate was settled on the same days, too.

RESULTS

The results were meager if we consider the mortality rate of the TFd treated animals but good according to the parasitemia that was statistically significant in the 15<sup>th</sup> day (Graph I) to both treated groups.

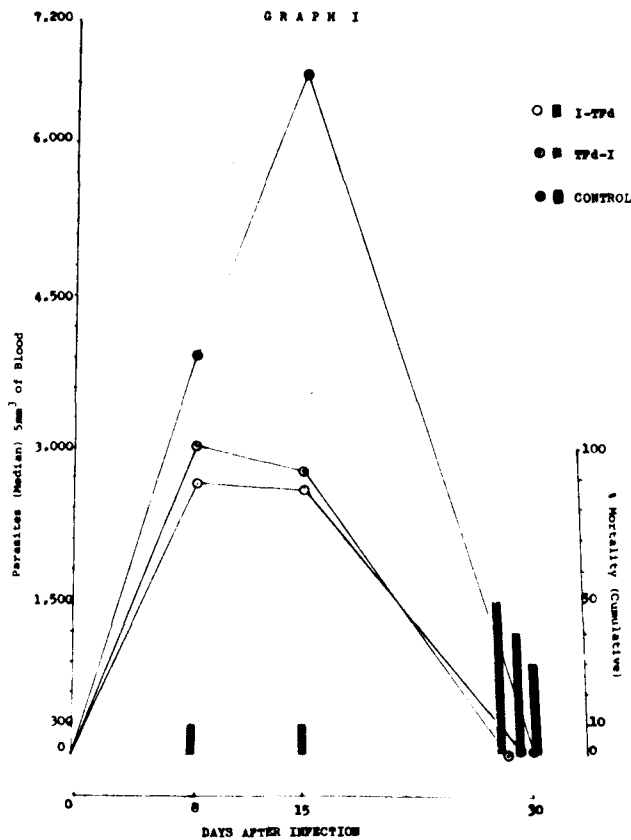
DISCUSSION

In spite of these poor results it seems to me that more research must be done, with new techniques of infection and treatment.

Using a small dose of the TFd before the infection I obtained almost the same results as treating the infected mice with a larger dose.

In a new schema, with a longer pre-treatment, the TFd will have, perhaps, a better achievement in the prevention of a experimental trypanosomiasis infection.

The nature and properties of the TFd were extensively examined by Lawrence et als, whose papers I recommend to those interested<sup>4,5,6</sup>.



Graph I - Parasitemia 15<sup>th</sup> day  
 C/I-TFd  $X^1 = 8,6$   
 C/TFd-I  $X^2 = 7,8$   
 $X^2 (df=1) = 3,8(5\%)$  and  $6,6(1\%)$ .

## RESUMO

Um fator dialisável capaz de transferir a imunidade de células sensibilizadas foi obtido de camundongos vacinados com a cepa PF.

Este fator foi capaz de reduzir significativamente a parasitemia de animais previamente tratados com o mesmo e depois infectados ou de camundongos infectados inicialmente e depois tratados.

Os resultados foram considerados pobres com respeito a mortalidade dos tratados, mas não desencorajadores, esperando-se melhores conclusões no futuro.

## REFERENCES

1. BARRETT, J.T. — Textbook of Immunology. 1970. The C.V. Mosby Co. St. Louis.
2. BELLANTI, J.A. — Immunology. 1971. W.B. Saunders Co. Philadelphia.
3. FUDENBERG, H.H.; STITES, D.P.; CALDWELL, J.L. & WELLS, J.V. — Basic and Clinical Immunology. 1976. Lange Med. Public. Los Altos. California.
4. LAWRENCE, H.S. — Transfer Factor, *Advances Immunol.* 11:195-1969
5. LAWRENCE, H.S. — Transfer Factor. In *Mediators of Cellular Immunity*. H.S. Lawrence & M. Landy. Ed. 1969. Academic Press. N. York
6. LAERENGE, H.S. — Transfer Factor. and Cellular Immunity. In *Immunobiology*. R.A. Good & D.W. Fischer. Ed. 1974. Sinauer Assoc. Inc. Publish. Sunderland, Massachusetts.
7. MENEZES, H. — The un-infectivity of the PF cultivated strain of *Trypanosoma cruzi* to mice. evaluation through a one year period by blood cultures and histopathology. *Rev. Soc. Bras. Med. Trop.* 9:1-15, 1975.
8. NAKAMURA, M. — Cultivation of *Trypanosoma cruzi* in a Protein-free dialyzate medium. *Proc. Soc. Exp. Biol. Med.* 125:779-780, 1967.
9. RIBEIRO DOS SANTOS, R. — Contribuição ao estudo da imunidade na fase aguda da Doença de Chagas experimental. Têse, 1973. Fac. Ciências Med. UNICAMP. Brasil
10. ROBERSON, E.L. & HANSON, W.L. — Passive transfer of immunity to *Trypanosoma cruzi*. *Trans. Roy. Soc. Trop. Med. & Hyg.* 68(4): 338. 1974 (Correspondence).
11. WARREN, L.G. — Metabolism of *Schizotrypanum cruzi*. Chagas. I-Effect of culture age and substrate concen-