Rev. Inst. Med. trop. São Paulo
13(1):31-36, janeiro-fevereiro, 1971

UDC 616.937.3-092.9

PROTECTIVE CROSS-IMMUNITY BETWEEN *TRYPANOSOMA CRUZI* AND *T. LEWISI*

Judith KLOETZEL [1] and Maria P. DEANE [2]

SUMMARY

Young rats previously infected with *T. lewisi* were not resistant to *T. cruzi*.

On the other hand a previous infection with *T. cruzi* rendered rats much less susceptible to a *T. lewisi* infection.

Mice inoculated with multiple doses of *T. lewisi* bloodstream forms developed pratically no resistance against *T. cruzi*.

INTRODUCTION

Garcia and Mühlpfordt reported to have obtained a partial protection against *Trypanosoma cruzi* infection in mice repeatedly injected with culture forms of *T. lewisi* [3]. Common antigens between culture forms of the two species were also detected by GARCIA et al. [4].

As is well known, *T. lewisi* does not normally multiply in mice and in its natural mammalian host, the rat, it causes an infection that ends spontaneously and produces a solid immunity against reinfection [2, 9]. Therefore, we thought it would be interesting to investigate if this immunity against *T. lewisi* would affect the course of infection by *T. cruzi* in the rat and vice-versa. We also investigated the possibility that repeated injections of the bloodstream forms of *T. lewisi* could afford to mice a better protection against *T. cruzi* than the one reported for the culture forms.

MATERIAL AND METHODS

The bloodstream strain of *T. lewisi* was isolated in our laboratory from a wild rat, 3 years ago, and has been maintained since then through passages in white rats. The *T. cruzi* strain used was the "Y" strain (8), originally isolated from a human case of Chagas' disease, and maintained in white mice.

Trypanosome counts were made by the method described by BRENER [1] when parasitemias were low, and in the hemacytometer, after adequate dilution, when counts were sufficiently high.

The following experimental groups were used:

1) *Rats inoculated with* T. cruzi *after the end of an infection by* T. lewisi. Young rats, weighing 85 gm — 150 gm were inoculated with bloodstream forms of *T. lewisi*. After parasitemia had disappeared from the bloodstream, they were challenged with *T. cruzi*. Controls included rats inoculated with clean rat plasma instead of plasma infected with *T. lewisi*, and normal rats.

2) *Rats inoculated with* T. lewisi *one month after inoculation of* T. cruzi. About two months old rats, weighing between 100 gm — 170 gm were inoculated with bloodstream forms of *T. cruzi* obtained from infected mice or rats. One month later, when *T. cruzi* was no longer seen in their bloodstream, they were inoculated with *T. lewisi*.

This work was supported in part by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo

(1) Fellow of the FAPESP

(2) Instituto de Medicina Tropical de São Paulo, São Paulo, Brasil

Controls of uninfected rats, and rats previously inoculated with clean rat or mouse blood (instead of *T. cruzi* infected blood) were included.

3) *Mice inoculated with bloodstream forms of* T. lewisi, *and subsequently with* T. cruzi. A mixture of *T. lewisi* bloodstream forms obtained from rats on the 2nd and 5th day of infection was inoculated. This represented young and "adult" forms, since with our strain and under the experimental conditions used, parasitemia disappears between the 5th and 7th day and on the last two days of infection the trypomastigote population is monomorphic. Red blood cells were separated by differential centrifugation. Trypanosomes were inoculated in young white mice, having an average weight of 12 gm, either suspended in plasma or washed twice and suspended in physiological saline, with or without Freund's complete adjuvant. Inoculations were as indicated in individual experiments.

In all experiments animals were weighed, and an equal number of each weight group chosen at random for each experimental group.

RESULTS

Results are summarized in Tables I, II and III, and Fig. 1.

Table I summarizes results of experiments where young rats recovered from a parasitemia with *T. lewisi* were inoculated with *T. cruzi*. All rats previously inoculated with *T. lewisi* contracted *T. cruzi* infection. In one control *T. cruzi* was never seen in the bloodstream. Mortality of controls was lower than that of experimental animals: 42% of experimental animals died, as compared with 11% of controls and 16% of controls receiving normal rat plasma instead of rat plasma infected with *T. lewisi*.

Table II and Fig. 1 summarize results of experiments where rats were inoculated with *T. cruzi* and subsequently with *T. lewisi* after parasitemia was no longer patent. All control animals contracted *T. lewisi* infection, while only 74% of the rats previously infected with *T. cruzi* had a *T. lewisi* parasitemia. The mean duration of parasitemia and the maximum average parasitemia were also much lower in experimental animals than in controls — average duration of parasitemia was 2.9 days in experimental animals, 6.8 days in control animals, and 6.9 days in control animals inoculated previously with clean rat or mouse blood instead of *T. cruzi* infected blood; average maximum parasitemia was 486, 11.124 and 24.249/mm^3, respectively.

Figure 1 gives a clearer picture of average daily parasitemias in experimental animals and controls.

TABLE I

Rats recovered from *T. lewisi* infection, inoculated with *T. cruzi*

Group	no. of rats	"Immunizing" inoculations	*T. cruzi* infection		Mortality	
			Blood positive (*T. cruzi*)			
			no.	%	no.	%
Experimental	19	*T. lewisi* (infection)	19	100	8	42
Control	6	Normal rat plasma	6	100	1	16
Control	17	—	16	94	2	11

TABLE II

T. lewisi parasitemia in rats previously infected with *T. cruzi*

Group	no. of rats	"Immunizing" inoculations	*T. lewisi* infection			
			Blood positive for *T. lewisi*			
			no. of rats	%	Mean duration of parasitemia in days	Maxim. average parasitemia no./mm³
Experimental	43	*T. cruzi* (infection)	32	74	2.9	486
Control	25	Normal rat plasma	25	100	6.9	24.249
Control	40	—	40	100	6.8	11.124

TABLE III

Infection with *T. cruzi* in mice previously inoculated with bloodstream forms of *T. lewisi*

Group	no. of mice	"Immunizing" inoculations			*T. cruzi infection*	
		T. lewisi total no. per mouse	Suspension medium	no. of inoculations	no. days after last "immunizing" dosis	Average survival in days
Exper. 1a	12	18 x 10^6	irp	1	5	13.6
Control	4	—	—	—		11.2
Exper. 1b	12	18 x 10^6	irp	1	19	13
Control	7	—	—	—		11.5
Exp. 1c	8	18 x 10^6	irp	1	42	9.4
Control	12	—	—	—		9.5
Exper. 2a	8	16 x 10^7	s + Fa	8	6	15.6
Exper. 2b	9	16 x 10^7	irp + Fa	8	6	15.2
Exper. 2c	9	16 x 10^7	irp	8	6	16.1
Controls	7	—	nrp + Fa	8	6	15.2
	9	—	—	—		18.5
Exper. 3	16	25 x 10^7	s	8	5	13.2
Control	14	—	—	—		10.9

irp = infected rat plasma
nrp = normal rat plasma
Fa = Freunds' adjuvant
s = saline

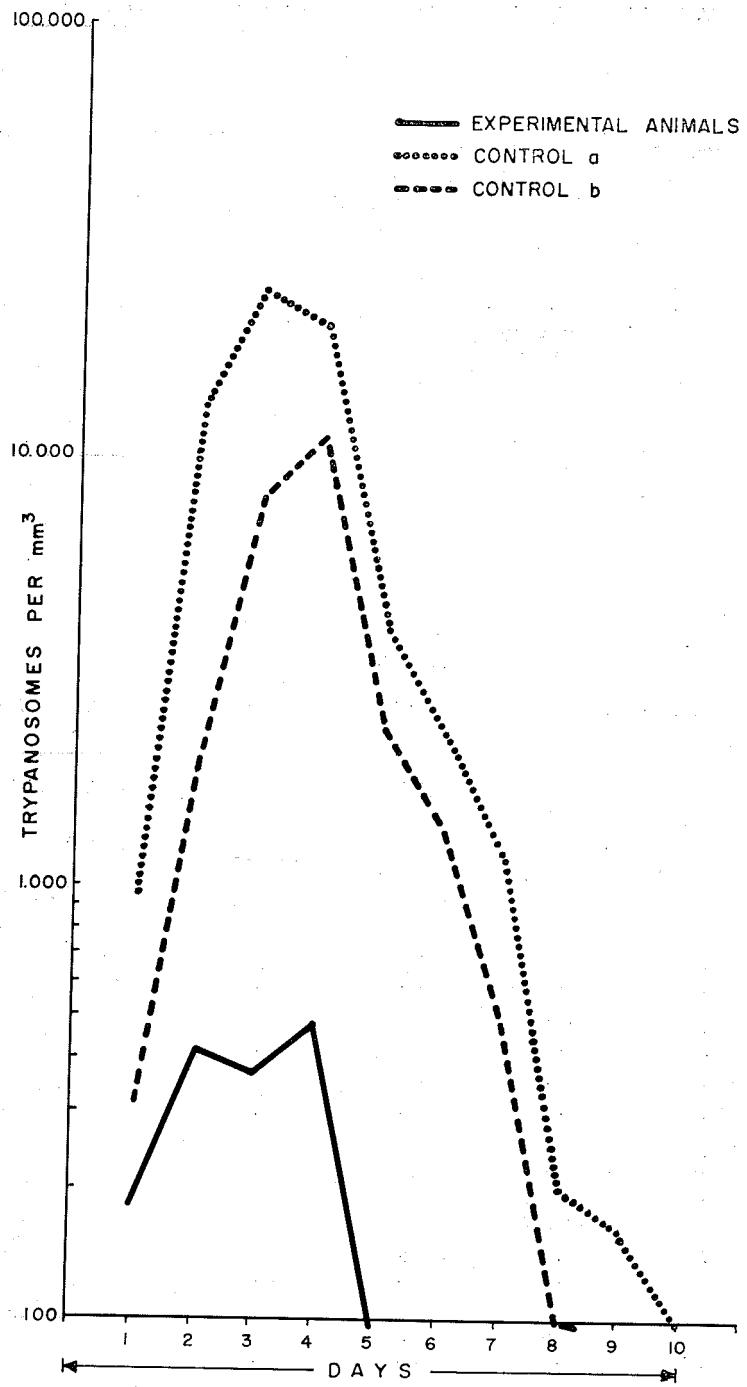

Fig. 1 — Daily average *T. lewisi* parasitemia of rats inoculated one month before with *T. cruzi*. Control a — rats previously inoculated with clean rat blood instead of *T. cruzi* infected blood. Control b — normal rats.

Table III indicates the mean survival of mice inoculated with *T. lewisi* bloodstream forms, and subsequently with *T. cruzi*. There was no appreciable difference between survival of inoculated animals, and controls.

In experiment 1, mice received one intraperitoneal inoculation of 18 x 10^6 *T. lewisi* with plasma of the donor rat. They were divided into 3 groups, being challenged with *T. cruzi* after 5, 19 and 42 days respectively. Only a slight increase of survival of "immunized" animals as compared with controls was noted in the first two groups, however the third group, challenged after 42 days, had no difference in survival between experimental and control animals. Parasitemias were comparable in experimental animals and controls.

In experiment 2, all animals received 8 doses of *T. lewisi*, subcutaneously, spaced 5 days apart, with a total number of 16 x 10^7 trypanosomes, being challenged 6 days after the last inoculation. The group which received *T. lewisi* infected plasma with Freund's adjuvant, had a mean survival of 15.2 days. A second group, inoculated with *T. lewisi* infected plasma without adjuvant had a mean survival of 16.1 days. The group which was immunized with washed trypanosomes suspended in saline, with Freund's adjuvant, had a mean survival of 15.6 days. There were two control groups, one inoculated with plasma of normal rats and Freund's adjuvant, having a mean survival of 15.2 days, the other of normal mice, with a mean survival slightly higher than that of experimental animals — 18.5 days.

In experiment 3, mice received a total of 25 x 10^7 washed bloodstream forms suspended in saline, in 8 doses, spaced 5 days apart, the first two into footpads, another two intramuscular, and the remainder intraperitoneal. The mean survival of experimental animals was slightly higher than that of controls, 13.2 days as compared with 10.9, however parasitemia of experimental animals and controls was comparable throughout.

DISCUSSION

Although rats are less susceptible to *T. cruzi* than mice, we believed that a cross-immunity between *T. lewisi* and *T. cruzi* would be easier demonstrable in these animals since on recovery from a *T. lewisi* infection their immunity against the homologous organism is complete [2]. Initially we inoculated very small rats with *T. lewisi*, so that they might be more susceptible to the *T. cruzi* challenge. However some of the rats were killed by the *T. lewisi* infection, and the subsequent groups of experiments were made with somewhat older rats. No protection whatsoever against *T. cruzi* was afforded to rats previously infected with *T. lewisi*: quite on the contrary, they seemed more susceptible than control animals.

In all rat experiments stained slides were examined, and no mixed *T. cruzi* — *T. lewisi* infections due to a relapse of the first infection, were observed.

On the other hand, rats infected with *T. cruzi* and subsequently with *T. lewisi* had definitely lower parasitemia levels, duration and incidence than control animals. Also in these experiments no mixed infections were observed.

In mice we could detect practically no protection against *T. cruzi*, after inoculation of bloodstream forms of *T. lewisi*.

In one experiment, where mice were inoculated with *T. cruzi* a few days after inoculation with *T. lewisi* infected plasma, the survival of experimental animals was only slightly longer than that of controls. When a longer interval between *T. lewisi* and *T. cruzi* inoculations was given, there was no difference whatsoever between control and experimental animals.

In another experiment, where the immunizing scheme of GARCIA & MÜHLPFORDT [3] was followed, using a higher number of trypanosomes. the average survival of "immunized" animals was only slightly higher than that of controls, parasitemia of controls and experimental animals being comparable.

No protection seems to be afforded by the inoculation of several doses of *T. lewisi* with Freund's adjuvant, with or without plasma of the infected rats. In fact in some experiments the "immunization" procedure seems to have rendered mice slightly more susceptible to death by *T. cruzi*.

The mechanism of immunity in trypanosomiasis is not well known. Both humoral and tissue factors seem to be involved [2, 5, 6, 7, 9].

The reason for the difference between cross-protection afforded by a previous infection with *T. cruzi* against the lack of cross-protection with *T. lewisi* in rats might have many explanations.

In *T. cruzi* infections in the rat, after the acute stage, where parasitemia is evident, parasites become very scanty and are generally only demonstrable by xenodiagnosis. They continue alive and multiplying in tissues for a long time. Immunity in this case is due to premunition [9], and this could also be effective against the heterologous species, *T. lewisi*.

Another approach would be the susceptibility of the trypanosomes themselves, *T. lewisi* perhaps being more susceptible to unspecific factors than *T. cruzi*.

RESUMO

Imunidade protetora cruzada entre Trypanosoma cruzi *e* T. lewisi

Ratos jovens que se recuperaram de uma infecção com *T. lewisi* não tiveram resistência ao *T. cruzi*.

Por outro lado, ratos infetados com *T. cruzi* eram muito menos suscetíveis a uma infecção com *T. lewisi* do que os contrôles.

Camundongos inoculados com doses múltiplas de formas sanguícolas de *T. lewisi* pràticamente não apresentaram resistência ao *T. cruzi*.

REFERENCES

1. BRENER, Z. — *Contribuição ao estudo da terapêutica experimental da doença de Chagas. Tese.* Belo Horizonte, 1961.

2. CORRADETTI, A. — Acquired Sterile Immunity in Experimental Protozoal Infections. *In "Immunity to Protozoa"*. P. C. C. GARNHAM, A. E. PIERCE & I. ROITT, editors. Oxford, Blackwell Scientific Publications, 1963, 359 pp.

3. GARCIA, W. & MÜHLPFORDT, H. — Infección de *Trypanosoma cruzi* en ratones despues de su tratamiento con *Trypanosoma lewisi*. *Rev. Inst. Med. trop. São Paulo* **11**: 13-18, 1969.

4. GARCIA, W.; OELERICH, S. & MÜHLPFORDT, H. — Relaciones inmunológicas entre *Trypanosoma cruzi* y *Trypanosoma lewisi*. *Rev. Inst. Med. trop. São Paulo* **11**:67-70, 1969.

5. KLOETZEL, J. & DEANE, M. P. — Adherence of sensitized trypanosomes to peritoneal cells. *Rev. Inst. Med. trop. São Paulo* **12**:383-387, 1970.

6. PIZZI, T.; RUBIO, M. & KIERIM, F. — Inmunologia de la enfermedad de Chagas. *Bol. Chil. Parasit.* **9**:35-47, 1954.

7. PIZZI, T. & RUBIO, M. — Aspectos celulares de la inmunidad en la enfermedad de Chagas. *Bol. Chil. Parasit.* **10**:4-9, 1955.

8. SILVA, L. H. P. & NUSSENZWEIG, V. — Sôbre uma cêpa de *Trypanosoma cruzi* altamente virulenta para o camundongo branco. *Folia Clin. Biol.* **20**:191-208, 1953.

9. TALIAFERRO, W. H. — Cellular and Humoral Factors in Immunity to Protozoa. *In "Immunity to Protozoa"* — P. C. C. GARNHAM, A. E. PIERCE & I. ROITT, editors. Oxford, Blackwell Scientific Publications, 1963, 359 pp.

Recebido para publicação em 20/7/1970.