THE AVIRULENCE OF THE CULTIVATED Y STRAIN OF TRYPANOSOMA CRUZI. IV — THE EFFECT OF IMMUNOSUPPRESSIVE AGENTS IN MICE.

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Antiproliferative drugs (Azathioprine and Methotrexate) were able to enhance a T. cruzi *virulent infection in mice, but failed to induce evident infection in animals previously vaccinated with the live PF strain of the same parasite.*

Vinblastine (Vbl) had a similar effect with the virulent Y strain, but promoted the appearance of positive blood cultures in animals vaccinated and treated previously with the drug. Negative results are obtained if the immunosupressive agent is used 15 days after the vaccine. The flagellates isolated from the vaccinated Vbl treated mice remained avirulent for baby mice, after i.p. inoculation.

The immunity induced by the PF strain is probably sterile i. e. does not imply in the persistence of a latent infection (premunition).

INTRODUCTION

Since we have described the avirulence of what we suppose to be a mutant (PF) of the "Y" strain of *Trypanosoma cruzi*, (Menezes [3, 4, 5]), we have tried to demonstrate through several tests that such avirulence was fixed and not due to environmental circumstances (Menezes [7, 8, 9]; Menezes & Albuquerque [10]).

The observation of primary immunedepression in experimental trypanosomiasis (Walker[14]; Camargo et al. [1]; Luckins[2]), viral (Squame et al.[12]; Zlotnik et al. [15]) and bacterial infections (Tripathy & Mackaness [13]) of animals treated with antiproliferative drugs, induced us to use such agents in mice vaccinated with *T. cruzi* PF strain.

With such experiments, we intended to produce further evidence of the avirulence of our *T. cruzi* live vaccine.

MATERIAL AND METHODS

1 — Sixty male albino mice with 10g of mean body weight were divided into six groups of ten animals each.

Four groups were treated subcutaneously with 0,02 mg of 4-amino-N^{10}-methyl pteroylglutamic acid sodium * (Mtx).

Three days later two of the four groups were vaccinated by subcutaneous route with 0.2 ml of a Saline suspension of *T. cruzi*, PF strain, cultivated for 8 days in liquid medium of Nöller — (PF/Mtx 1 &

* Methotrexate Sodium — Lederle Laboratories.

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2). The estimated number of parasites in the inoculum was 14 x 10^6/ml, 60% being mobile forms and almost 4%, metacyclic forms. One group received the same dose of the vaccine and became the control group (PF). One group of the Mtx treated animals was infected, intraperitoneally, with blood forms of *T. cruzi*, Y virulent strain (Y/Mtx), in a 5,000 parasites /g body weight ratio.

One more group was infected as the preceding one, and kept as control of the infection (Y).

Finally, the last group was only Mtx-treated, and maintained as control of the drug (Mtx).

All the animals of the different Mtx-treated groups received every 3 days, for a 15 day period, a subcutaneous Mtx injection of 0.02 mg.

During the subsequent 15 days they received, every 3 days, 0.05 mg of the same drug.

Parasitemia and mortality rates were recorded 8, 15 and 30 days after the Y inoculation and PF vaccination. The animals of the PF/Mtx-1 group were sacrificed by exsanguination (heart puncture): 5 after 15 days and the last surviving 3 mice, on the 30th day. In both cases all the blood drawn from the animals was cultivated in Warren's liquid medium for trypanosomes.

Fragments of several viscerae were fixed in 10% Formalin solution for histologic examination.

All the surviving animals of the PF/Mtx2 group were killed and the blood cultivated for trypanosomes, 30 days after the vaccination.

2 — Sixty albino male mice, with 10g of body weight, were divided into six groups of ten each.

Four groups received intraperitoneally 0,12 mg of azathioprine * (AZT). This treatment was pursued, daily, for 33 days.

The suspension of the drug was made in carboxy — methyl cellulose as recommended by Okumura & Decourt[11].

Two of the above groups (PF/AZT 1 and 2), after three daily injections of AZT, were vaccinated by subcutaneous route with 0.2 ml of a saline suspension of *T. cruzi*, PF strain, cultivated for 30 days in Packchanian medium.

The number of flagellates was about 2.5 x 10^6/ml with 50% of mobile forms and about 10% metacyclic parasites.

One AZT treated group was infected with virulent *T. cruzi*, Y strain, (Y AZT) as in the previous experiment and the other AZT group was kept as control of the drug (AZT).

One more group was infected on the same day with the same dose and strain as the Y/AZT group and remained as the control of the virulent strain (Y).

The last group was injected sub-cutaneously with 0.2 ml of the PF strain saline suspension and kept as control of the vaccine (PF).

Four animals of the PF/AZT 1 group were sacrificed by exsanguination after a blood search for parasites, 8 days after vaccination.

The blood was cultivated in liquid medium for trypanosomes.

Five more of the same groupe were killed, after blood search for parasites, 15 days following the vaccination.

All the blood removed by heart puncture was cultivated in Warren medium.

Five of the nine surviving mice of the PF/AZT2 group were bled to death on the 30th day of vaccination and the blood cultivated for trypanosomes.

The four remaining animals were submited to xenodiagnosis until total exsanguination and death (5 *R. prolixus* nymphs for each mice).

Fragments of organs and tissues were saved from all animals for histologic examination.

3 — Seventy male albino mice with 10g of mean body weight were divided into seven groups of ten each.

Four groups received intraperitoneally 0.01mg of Vinblastine ** (Vbl).

The treatment was continued by injections of 0.02 mg, 0.03 mg and 0,06 mg, in the second, third and fourth week respectively.

Two of the Vbl-treated groups, three days after the first dose, were vaccinated with 0.1 ml of a saline suspension of *T.*

* Imuran — Lbs Burroughs Wellcome do Brasil S/A.
** Velbian — Elli Lilly do Brasil Ltda.

cruzi, PF strain, cultivated for 27 days in Packchanian medium (PF/Vbl 1 and 2).

The vaccine had approximately 2 x 10^7 parasites/ml with 40% mobile forms and about 4.5% metacyclic forms.

One Vbl group was injected with 5,000 parasites/g body weight, of blood forms of the Y virulent *T. cruzi* (Y/Vbl). The last Vbl group was kept as control of the drug (Vbl).

One group of non-treated animals was infected by the same virulent strain with an identical dose (Y).

One group received only the vaccine (PF) as the PF/Vbl group, and finally, the last non-treated non-vaccinated 10 mice were kept as normal control (N), receiving weekly intraperitoneal injections of 0.2 ml saline solution.

Search for parasites in the peripheral blood was done 8, 15 and 30 days after the vaccination or infection in the animals of the groups PF/Vbl2; PF; Y and Y/Vbl.

The mice of the PF/Vbl 1 group also had parasite blood search 8 and 15 days after the use of the vaccine.

Five of these animals were sacrificed by heart puncture 8 days following the vaccination, and the blood drawn was cultivated in Warren medium.

The other five mice were killed in the same way 7 days later, i. e., 15 days after vaccination. All the PF/Vbl2 surviving animals on the 30th day of vaccination were bled and the blood was cultivated for trypanosomes.

The experiment was considered finished 30 days after the vaccination and infection, and all surviving animals were killed and had fragments of heart, liver, spleen, esophagus, lymph nodes and central nervous system saved for histologic examination.

4 — Seventy baby mice were divided into seven groups of ten each: the group PF/Vbl 1 was vaccinated subcutaneously with 0.2 ml of a PF vaccine containing 2.7 x 10^7 parasites ml, with almost 80% mobile forms and about 3% metacyclic forms.

Fifteen days later the animals were treated intraperitoneally, with weekly injections of Vinblastine (Vbl) in successive doses of 0.05 mg and 0.1 mg.

Five days after the first Vbl injection a blood search for trypanosomes was made and after this, four mice of the group were bled to death and the blood cultivated in liquid medium.

Six days after the second Vbl injection the procedure was repeated in the remaining mice.

A similar group (PF/Vbl2) was vaccinated as the PF/Vbl 1 and treated 15 days later, intraperitoneally, with weekly injections of Vbl in successive doses of 0.02 mg, 0.05 mg, 0.07 mg and 0.1 mg, per mouse.

The surviving animals were killed by exsanguination on the 3rd day after the fourth and last Vbl injection.

The total blood was cultivated for trypanosomes. Each animal of the Y group was infected with 2,500 (250/g) virulent blood forms of *T. cruzi* Y strain.

Parasitemias were performed 8, 15, 20 and 30 days after the infection.

The Y/Vbl group was infected as the Y group and treated 15 days later with successive weekly intraperitoneal injection of 0.05 mg and 0.1 mg of Vbl.

Blood searches for parasites were done as for the preceding group.

Finally the last group of the normal (N) animals received weekly intraperitoneal injections of saline solution (0.2 ml).

All blood cultures were examined after 30 days of cultivation and when negative at the usual microscopy, the medium was centrifuged and the sediment examined for trypanosomes.

The xenodiagnosis were equally examined at the 30th day.

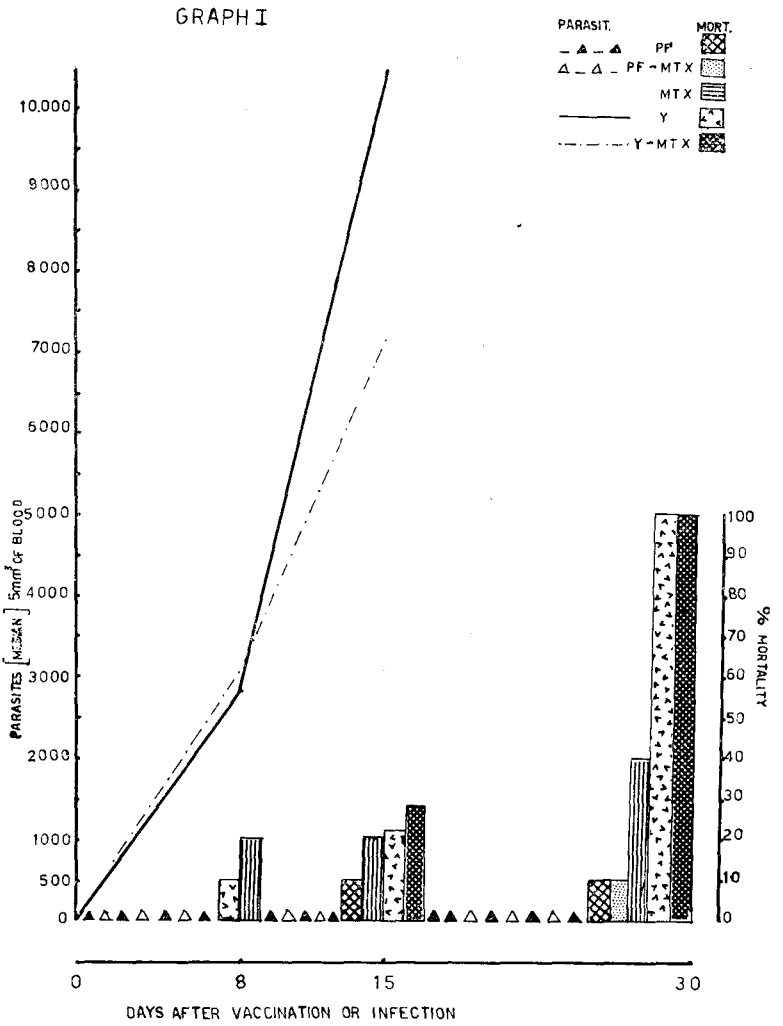
The fragments of organs and tissues saved for histologic examination were fixed in 10% Formalin solution, imbedded in paraffine and the sections stained by hematoxylin-eosin.

RESULTS

1 — As shown by Table 2 and Graph I the parasitemia peak was attained in groups Y and Y/Mtx, 15 days after infection, with the greatest parasitemia in the control Y group.

The mortality rate was slightly higher among the Y/Mtx animals, at the end of the 15th day, but was the same (100%) in both groups at the end of the experiment (30th day).

There were no significant differences, from the parasitological point of view, between the two Y groups, but very impressive



contrast as seen histologically. The number of pseudocysts in the heart of the Y/Mtx mice was higher than in the Y animals, and the inflammatory reaction was scanty as compared with that of this latter group (Figs. 1A e 2C).

The immunodepressive activity of the drug could be documented by the great atrophy of the spleen and lymph nodes.

All the vaccinated PF and PF/Mtx mice had negative blood cultures for trypanosomes (Table 1). The mortality rate in both PF groups were 10% and 20% respectively, less than in the control Mtx (40%).

The histologic alterations in the two vaccinated groups were similar to those already described in PF vaccinated animals (Menezes[5]).

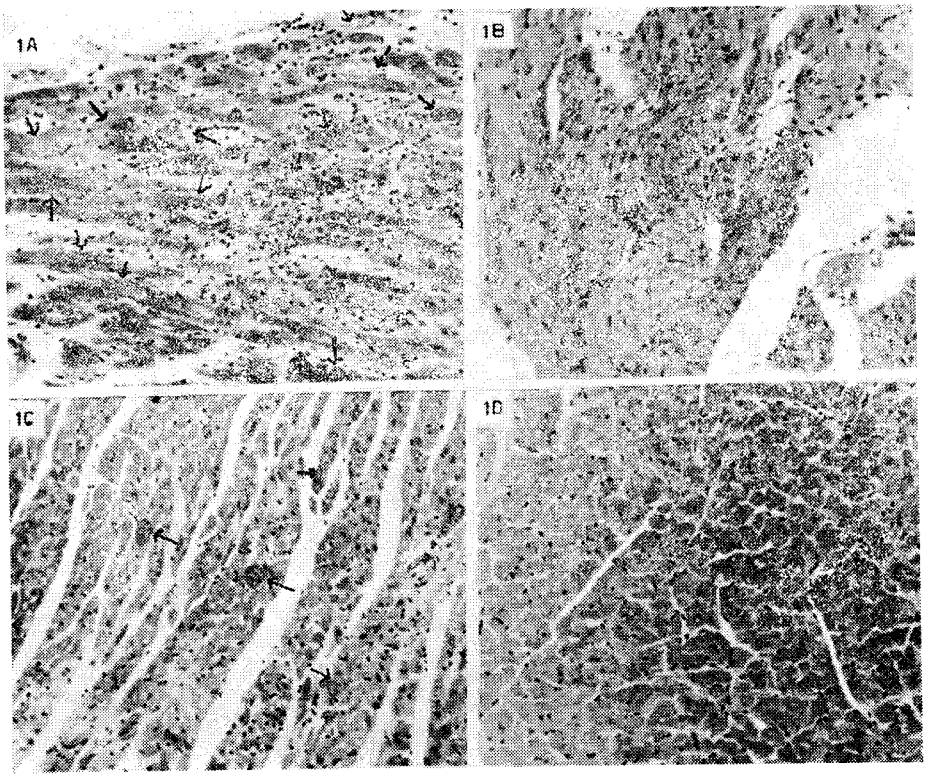
Mice of the Mtx group presented heart and liver lesions similar to those seen in the vaccinated animals, but less frequently.

2 — Tables 3 and 4 and Graph II give us a summary of what occurred with the AZT treated animals.

The parasitemia and mortality rates were much higher in the Y/AZT group than in the Y mice at the 8th day of infection.

The AZT and PF/AZT animals suffered the same mortality rate while no animal in the PF group died.

Parasitemias and blood cultures were negative in both PF-AZT (1 and 2) and negative parasitemias were seen in the PF control mice.


1A
1B
1C
1D

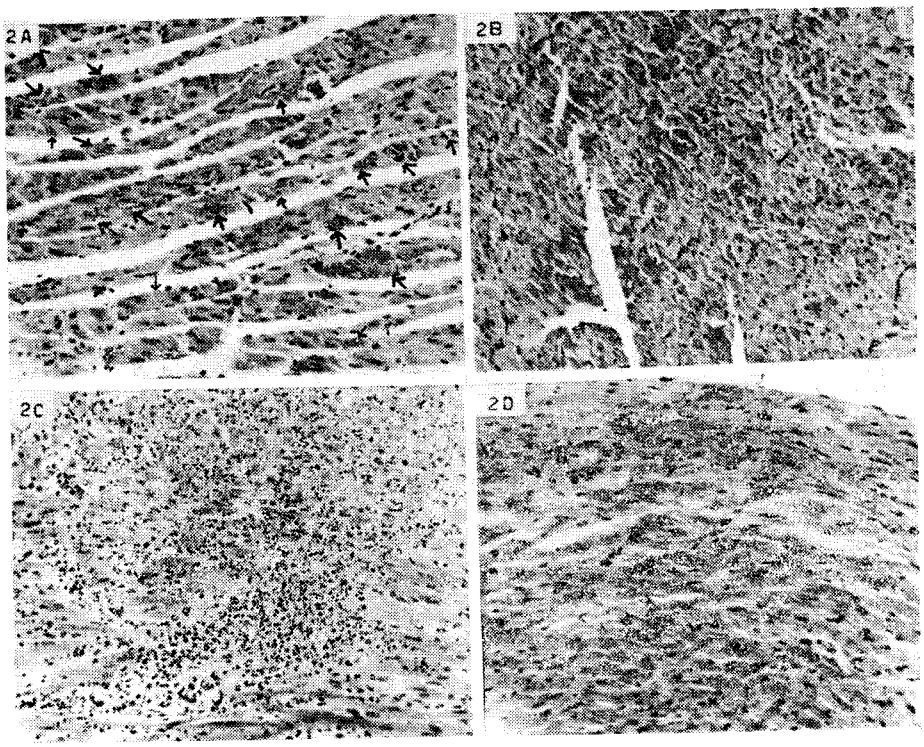

2A
2B
2C
2D

FIGURE 1

A — Myocardium — Mouse FR 60/9 — Y/MTx
Died 17 days after infection. Orig. 100 x.

B — Idem. — Mouse FR 60/7 — PF/MTx2
Killed 30 d.a. vaccination. Orig. 100 x.

C — Idem. — Mouse FR 62/9 — Y/AZT
Died 12 d.a.i. Orig. 100 x.

D — Idem. — Mouse FR 62/8 — PF/AZT
Killed 15 d.a.v. Orig. 100 x.

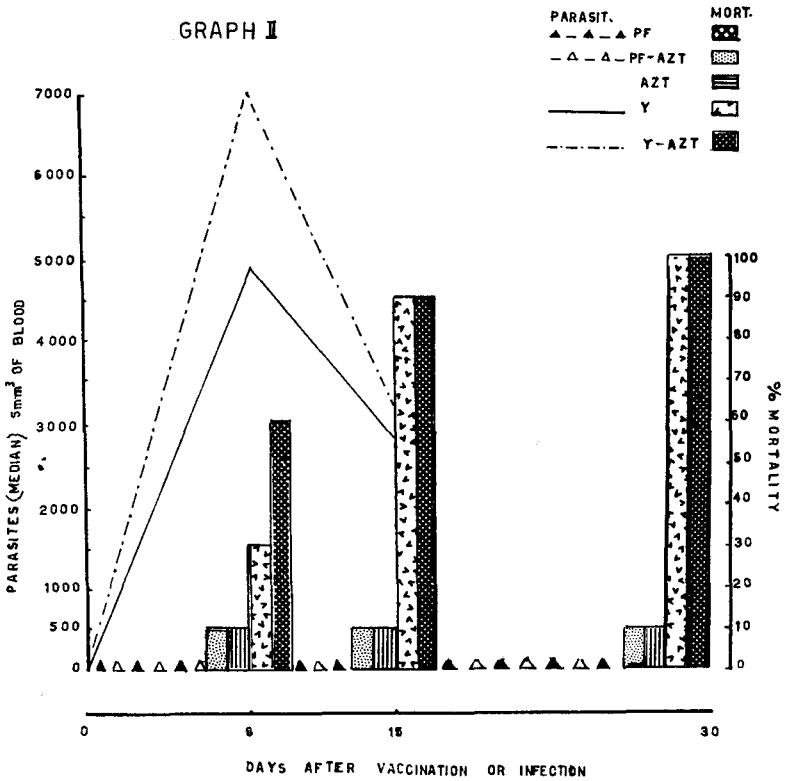
FIGURE 2

A — Myocardium — Mouse FR 64/1 — Y/Vbi
Died 14 d.a.i. Orig. 100 x.

B — Idem. Mouse FR 64/3 — PF/Vbl-1
Killed 8 d.a.v. Orig. 100 x.
Blood culture POSITIVE.

C — Idem. Mouse FR 62/3 — Y
Died 17 d.a.i. Orig. 100 x.

D — Idem. — Mouse FR 62/1 — PF —
Killed 30 d.a.v. Orig. 100 x.



The histologic lesion of the heart and liver of the Y/AZT animals (Fig 1 C) were less conspicuous than those seen in the Y mice, but we must point out that 60% of the animals of that group died before the 9th day of infection, i. e. when the histologic lesions in general are not yet very impressive.

The animals of the Y/AZT group that died after this period presented lesions quite similar to those of the Y control group with almost the same age.

The lymphoid organs of all AZT-treated mice presented a pronounced atrophy, morphological evidence of the immune-suppressive effect of the drug.

3 — The experimental design of this experiment is shown in Tables 5 and 6 and Graph III.

Animals treated simultaneously with Vbl and vaccine had positive blood cultures 8 and 30 days later, but the mortality rate was only 10%, the same as the control Vbl mice.

Histological examinations of the heart (Fig. 2 B), lung, liver, spleen, lymph nodes, esophagus and central nervous system of those animals presented no evidence of infection.

A very important observation in the positive vaccinated cases was that the parasites isolated from blood cultures, when inoculated in baby mice, were incapable of promoting infection even when the inoculations were successive as shown in Table 7.

No vaccinated animals died during the 30 days period while the mortality rate was 100% among all infected mice (Tables 5 and 6).

The frequency of early mortality and parasitemia was higher in the Y/Vbl than in any other group (Table 6. Graph III).

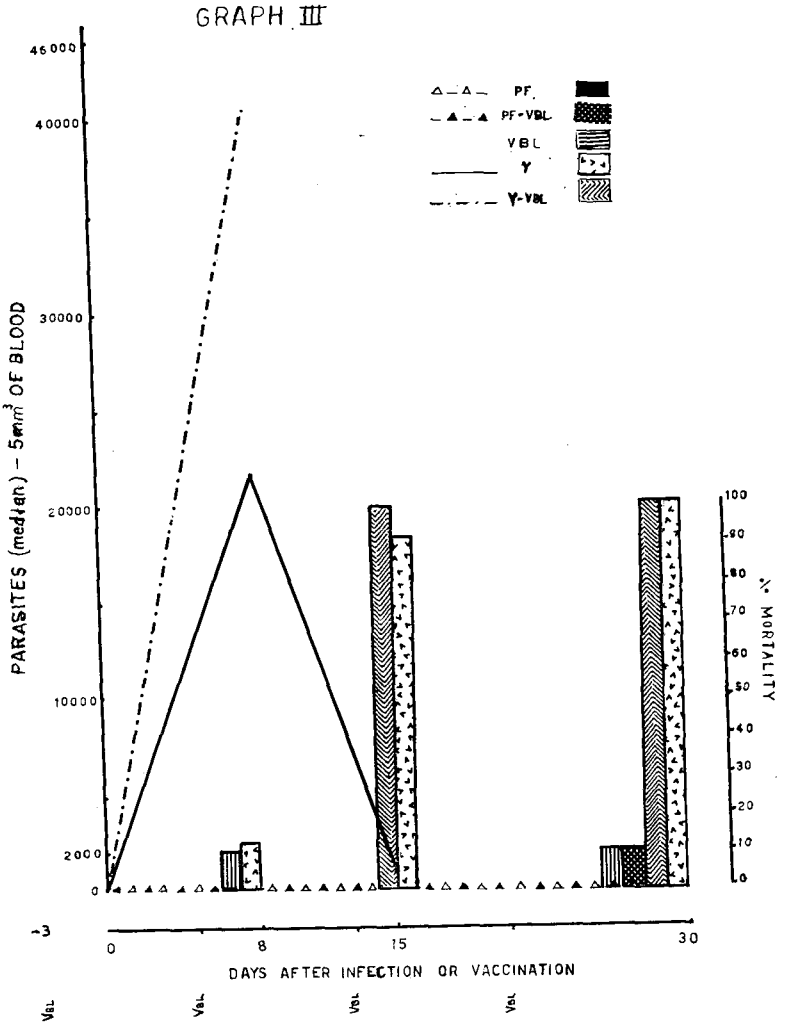
4 — As shown by Table 9 and Graph IV, the animals vaccinated and treated 15 days later with high doses of Vbl had negative parasitemias and blood cultures, 5, 13, 21 and 29 days after the first drug injection.

Fifty per cent of the PF/Vbl2 mice died (Table 9), but the control animals died in higher percentages (Table 11).

TABLE 1

Mice vaccinated and vaccinated and treated with Mtx

Group	Mouse	DAYS AFTER VACCINATION						Observation
		8		15		30		
		Parasites 5 mm³ blood	Blood Culture	Parasites 5 mm³ blood	Blood Culture	Parasites 5 mm³ blood	Blood Culture	
PF — Mtx (1)	1	0		0	(—)			Killed 15 pv
	2	0		0	(—)			Killed 15 pv
	3	0		x	x			Died 14 pv
	4	0		0	(—)			Killed 15 pv
	5	0		0	x	0	(—)	Killed 30 pv
	6	0		0	x	0	(—)	Killed 30 pv
	7	0		0	x	0	(—)	Killed 30 pv
	8	0		0	(—)	x	x	Killed 15 pv
	9	x		x	x	x	x	Died 3 pv *
	10	0		0	(—)	x	x	Killed 15 pv
PF — Mtx (2)	1	0		0		0	(—)	Killed 30 pv
	2	0		0		0	(—)	Killed 30 pv
	3	0		0		x	x	Died 26 pv
	4	0		0		0	(—)	Killed 30 pv
	5	0		0		0	(—)	Killed 30 pv
	6	0		0		0	(—)	Killed 30 pv
	7	0		0		0	(—)	Killed 30 pv
	8	0		0		0	(—)	Killed 30 pv
	9	0		0		x	x	Died 19 pv
	10	0		0		0	(—)	Killed 30 pv
MEDIAN		0		0		0		* Excluded
Mortality		0%		0%		20%		
PF	1	0		0		0		Killed 30 pv
	2	0		0		0		Killed 30 pv
	3	0		0		0		Killed 30 pv
	4	0		0		0		Killed 30 pv
	5	0		x		x		Died 10 pv
	6	0		0		0		Killed 30 pv
	7	0		0		0		Killed 30 pv
	8	0		0		0		Killed 30 pv
	9	0		0		0		Killed 30 pv
	10	0		0		0		Killed 30 pv
MEDIAN		0		0		0		
Mortality		0%		10%		10%		



No animals died in the vaccinated (PF) and normal (N) group, during the days of the experiment (Tables 9, 11).

The degree of parasitemia and the mortality rate in the Y/Vbl group were still, higher in general, than in the Y group (Table 10).

COMMENTS AND CONCLUSIONS

With the aim of demonstrating that the PF strain is a mutant of the original Y strain that has lost its virulence for animals, we tried several known parasitologic methods recommended to increase the virulence of low-virulent trypanosome strains (Menezes[7, 8, 9, 10]).

Another way to approach the problem was simply to try to impair the immune response of laboratory animals through well-known immunosuppressive agents.

The capacity of these drugs to enhance trypanosome infections has been demonstrated by Walker[14] in *Trypanosome rhodesiense*, Luckins[2] in *T. brucei* and *T. congolense*, and by Camargo et al.[1] in *T. cruzi*.

Prednisolone in very high dosages was not able to induce infection in mice vaccinated with the live PF strain (Menezes[9]).

The occurrence of positive blood culture in animals treated with Vbl previous to the vaccination demonstrates that it is a more efficient immunosuppressive agent than the others already mentioned.

The sucessive negative inoculations of baby mice with the parasites isolated from the blood culture are, in our opinion, a

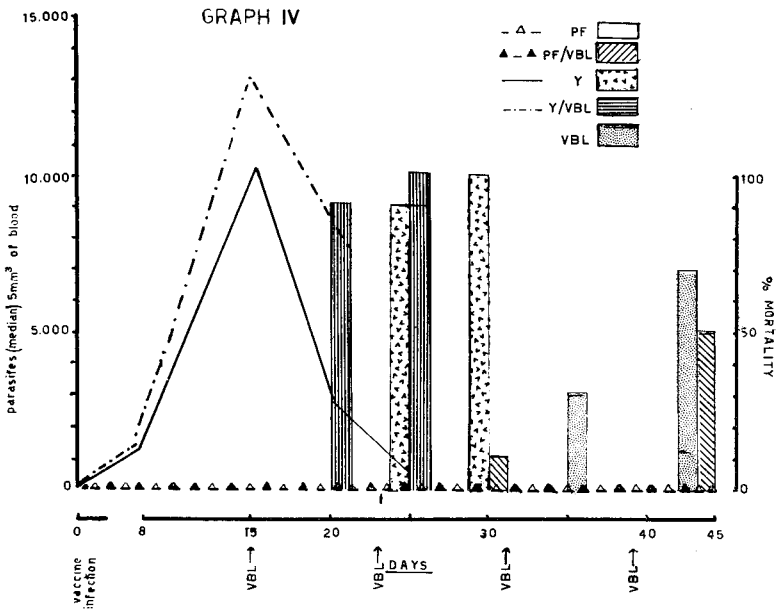


TABLE 2

Mice infected and infected and treated with Mtx

Group	Mouse	Number of parasites 5mm³ of blood — Days after infection: 8	15	30	Observation
Y	1	3.360	3.570		Died 17 p.i.
	2	2.275	x		Died 15 p.i.
	3	3.045	10.500		Died 17 p.i.
	4	x	x		Died 7 p.i.
	5	2.170	10.500		Died 16 p.i.
	6	x	x		Died 1 p.i. *
	7	2.730	3.675		Died 26 p.i.
	8	1.680	4.375		Died 19 p.i.
	9	4.900	17.500		Died 16 p.i.
	10	3.010	35.000		Died 17 p.i.
MEDIAN		2.870	10.500	—	* Excluded
Mortality		11%	22%	100%	
Y — Mtx	1	x	x		x Died 1 p.i. *
	2	3.045	4.025		Died 17 p.i.
	3	2.870	7.875		Died 20 p.i.
	4	3.780	7.175		Died 19 p.i.
	5	17.500	x		Died 15 p.i.
	6	2.590	x		Died 15 p.i.
	7	x	x		Died 1 p.i. *
	8	x	x		Died 4 p.i. *
	9	5.375	10.500		Died 17 p.i.
	10	2.445	4.375		Died 17 p.i.
MEDIAN		3.045	7.175	x	
Mortality		0%	28%	100%	* Excluded

very strong evidence of the avirulence of the PF strain.

The negative histologic findings reinforce this statement.

The Vbl acts upon the host but seems not to modify the parasite.

Our conviction of the PF avirulence becomes stronger when we analise the results of the last experiment.

When the immunosuppressive treatment is established after the immunologic induction period, the modified cells seem to be resistant to the action of the drug and able to destroy the parasites as demonstrated by the negative blood cultures.

Studies on a more efficient immunosuppressor — ALS is in course and will be reported later.

RESUMO

Drogas imunossupressoras (Azathioprina e Methotrexato) que se mostraram capazes de agravar infecções experimentais por Trypanosoma cruzi *virulento (cepa Y), em camundongos, não conseguiram favorecer o aparecimento de qualquer sinal de infecção em animais vacinados com a cepa avirulenta PF do mesmo tripanosoma.*

Vinblastine, quando dado 3 dias antes da vacina, não favoreceu parasitemias positivas nem mortalidade significativamente aumentada, mas induziu o aparecimento de hemoculturas positivas.

Os tripanosomas dessas culturas foram incapazes de infectar camundongos jovens. Quando o Vinblastine foi usado 15 dias após a vacinação dos animais, todos os testes foram negativos, inclusive as hemoculturas.

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TABLE 3

Mice vaccinated and vaccinated and treated with AZT

Group	Mouse	DAYS AFTER VACCINATION							Observation
		8		15		30			
		Parasites 5 mm³ blood	Blood Culture	Parasites 5 mm³ blood	Blood Culture	Parasites 5 mm³ blood	Blood Culture	Xeno	
PF — AZT (1)	1	0	x	0	(—)				Killed 15 pv
	2	0	(—)	x	x				Killed 8 pv
	3	0	x	0	(—)				Killed 15 pv
	4	0	x	0	(—)				Killed 15 pv
	5	0	(—)	x	x				Killed 8 pv
	6	x	x	x	x				Died 6 pv
	7	0	(—)	x	x				Killed 8 pv
	8	0	(—)	x	x				Killed 8 pv
	9	0	x	0	(—)				Killed 15 pv
	10	0	x	0	(—)				Killed 15 pv
PF — AZT (2)	1	0		0		0	x	(—)	Killed 30 pv
	2	0		0		0	(—)	x	Killed 30 pv
	3	0		0		0	x	(—)	Killed 30 pv
	4	0		x		x	x	x	Died 9 pv
	5	0		0		0	x	x	Killed 30 pv
	6	0		0		0	(—)	(—)	Killed 30 pv
	7	0		0		0	(—)	x	Killed 30 pv
	8	0		0		0	(—)	x	Killed 30 pv
	9	0		0		0	(—)	x	Killed 30 pv
	10	0		0		0	x	(—)	Killed 30 pv
MEDIAN		0		0		0			
Mortality		0%		10%		10%			
PF	1	0		0		0			Killed 30 pv
	2	0		0		0			Killed 30 pv
	3	0		0		0			Killed 30 pv
	4	0		0		0			Killed 30 pv
	5	0		0		0			Killed 30 pv
	6	0		0		0			Killed 30 pv
	7	0		0		0			Killed 30 pv
	9	0		0		0			Killed 30 pv
	9	0		0		0			Killed 30 pv
	10	0		0		0			Killed 30 pv
MEDIAN		0		0		0			
Mortality		0%		0%		0%			

TABLE 4

Mice infected and infected and treated with AZT

Group	Mouse	Number of parasites 5 mm³ of blood — Days after infection			Observation
		8	15	30	
Y	1	x	x	x	Died 7 p.i.
	2	x	x	x	Died 7 p.i.
	3	8.750	2.835	x	Died 17 p.i.
	4	10.500	x	x	Died 11 p.i.
	5	4.900	x	x	Died 12 p.i.
	6	4.375	x	x	Died 13 p.i.
	7	5.505	x	x	Died 11 p.i.
	8	4.025	x	x	Died 14 p.i.
	9	4.550	x	x	Died 11 p.i.
	10	x	x	x	Died 8 p.i.
MEDIAN		4.900	2.835	x	
Mortality		30%	90%	100%	
Y + AZT	1	x	x	x	Died 7 p.i.
	2	10.500	x	x	Died 12 p.i.
	3	x	x	x	Died 8 p.i.
	4	4.325	x	x	Died 10 p.i.
	5	5.250	3.220	x	Died 19 p.i.
	6	x	x	x	Died 8 p.i.
	7	x	x	x	Dierd 7 p.i.
	8	x	x	x	Died 6 p.i.
	9	8.750	x	x	Died 12 p.i.
	10	x	x	x	Died 6 p.i.
MEDIAN		7.000	3.220	x	
Mortality		60%	90%	100%	

TABLE 5

Mice vaccinated and vaccinated and treated previously with Vbl

Group	Mouse	DAYS AFTER VACCINATION						Observation
		8		15		30		
		Parasites 5 mm³ blood	Blood Culture	Parasites 5 mm³ blood	Blood Culture	Parasites 5 mm³ blood	Blood Culture	
PF — Vbl (1)	1	0	—					Killed 8 pv
	2	0	—					Killed 8 pv
	3	0	+(*)					Killed 8 pv
	4	0	—					Killed 8 pv
	5	0	—					Killed 8 pv
	6	0		0	—			Killed 15 pv
	7	0		0	—			Killed 15 pv
	8	0		0	—			Killed 15 pv
	9	0		0	—			Killed 15 pv
	10	0		0	—			Killed 15 pv
PF + Vbl (2)	1	0		0		0	—	Killed 30 pv
	2	0		0		0	—	Killed 30 pv
	3	0		0		0	+(**)	Killed 30 pv
	4	0		0		x	x	Killed 30 pv
	5	0		0		0	—	Died 24 pv
	6	0		0		0	+(**)	Killed 30 pv
	7	0		0		0	+(**)	Killed 30 pv
	8	0		0		0	+(**)	Killed 30 pv
	9	0		0		0	+(**)	Killed 30 pv
	10	0		0		0	—	Killed 30 pv
MEDIAN Mortality		0 0%		0 0%		0 10%		
PF	1	0		0		0		Killed 30 pv
	2	0		0		0		Killed 30 pv
	3	0		0		0		Killed 30 pv
	4	0		0		0		Killed 30 pv
	5	0		0		0		Killed 30 pv
	6	0		0		0		Killed 30 pv
	7	0		0		0		Killed 30 pv
	8	0		0		0		Killed 30 pv
	9	0		0		0		Killed 30 pv
	10	0		0		0		Killed 30 pv
MEDIAN Mortality		0 0%		0 0%		0 0%		

(*) (**) — See Table 7

TABLE 6

Mice infected and infected and treated with Vbl

Group	Mouse	Number of parasites 5 mm³ of blood — Days after infection			Observation
		30	15	8	
Y	1	28.000	x	x	Died 12 p.i.
	2	22.400	x	x	Died 15 p.i.
	3	x	x	x	Died 6 p.i.
	4	21.700	x	x	Died 13 p.i.
	5	x	x	x	Died 1 p.i. *
	6	18.900	x	x	Died 13 p.i.
	7	21.000	1.050	x	Died 16 p.i.
	8	44.800	x	x	Died 9 p.i.
	9	x	x	x	Died 1 p.i. *
	10	18.200	x	x	Died 13 p.i.
MEDIAN Mortality		21.700 12%	1.050 90%	x 100%	* Excluded
Y — Vbl	1	46.900	x	x	Died 14 p.i.
	2	44.800	x	x	Died 13 p.i.
	3	35.700	x	x	Died 13 p.i.
	4	43.400	x	x	Died 14 p.i.
	5	44.800	x	x	Died 14 p.i.
	6	35.700	x	x	Died 14 p.i.
	7	52.500	x	x	Died 12 p.i.
	8	48.300	x	x	Died 14 p.i.
	9	44.800	x	x	Died 14 p.i.
	10	46.900	x	x	Died 13 p.i.
MEDIAN Motality		44.800 0%	x 100%	x 100%	

TABLE 7

Mice inoculated with the positive blood cultures from the animals described in the Table 5

Group	Mouse Blood (*) Culture Positive	1st Inoculation		Material Inoculated	2nd Inoculation		Material Inoculated	3rd Inoculation			
		Mouse	Parasit. 8th p. inoc.		Mouse	Parasit. 8th p. inoc.		Mouse	Parasitemia		
									30th p.i.	15th p.i.	8th p.i.
PF—Vbl (1)	3	1	—	Centrifuged Blood Serum (Pool)	1	—					
		2	—		2	—					
		3	—		3	—					
		4	—		4	—					
PF — Vbl (2)	3	1	—	Centrifuged Blood Serum (Pool)	1	—	Pool of Total Citrated Blood	1	—	—	—
	5	2	—		2	—		2	—	—	—
	6	3	—		3	—		3	—	—	—
	7	4	—		4	—		4	—	—	—
	8	5	—		5	—		5	—	—	—
	9	6	—		6	—		6	—	—	—
	Pool of(**) 12 tubes	7	—		7	—		7	—	—	—
		8	—		8	—		8	—	—	—
		9	—					9	—	—	—
		10	—					10	—	—	—

*) To each was inoculated about 70.000 parasites

**) Age of the cultures = 30 days.

TABLE 8

Mice controls of the vaccine (PF) and Vinblastin (Vbl).

Group	Mouse	DAY AFTER VACCINATION				Observation
		8	15	30	—	
		Parasites 5 mm^3 blood	Parasites 5 mm^3 blood	Parasites 5 mm^3 blood	—	
PF	1	0	0	0		Killed 30 dpv
	2	0	0	0		Killed 30 dpv
	3	0	0	0		Killed 30 dpv
	4	0	0	0		Killed 30 dpv
	5	0	0	0		Killed 30 dpv
	6	0	0	0		Killed 30 dpv
	7	0	0	0		Killed 30 dpv
	8	0	0	0		Killed 30 dpv
	9	0	0	0		Killed 30 dpv
	10	0	0	0		Killed 30 dpv
MEDIAN Mortality		0 0%	0 0%	0 0%		
		WEEKLY Vbl INJECTIONS				
		0,01 mg	0,02 mg	0,03 mg	0,06 mg	
Vbl	1	alive	alive	alive	alive	Killed 30th day
	2	alive	died	x	x	Died 8th day
	3	"	alive	alive	alive	Killed 30th day
	5	"	"	"	"	Killed 30th day
	4	"	"	"	"	Killed 30th day
	6	"	"	"	"	Killed 30th day
	7	"	"	"	"	Killed 30th day
	8	"	"	"	"	Killed 30th day
	9	"	"	"	"	Killed 30th day
	10	"	"	"	"	Killed 30th day
Mortality		0%	10%	10%	10%	

TABLE 9

Mice vaccinated and vaccinated and treated 15 days later with Vbl

GROUP	MOUSE	DAYS AFTER 1st Vbl INJECTION								OBSERVATION
		5		13		21		29		
		Parasites 5mm³ Blood	Blood Culture	Parasites 5mm³ Blood	Blood Culture	Parasites 5mm³ Blood	Blood Culture	Parasites 5mm³ Blood	Blood Culture	
PF — Vbl (1)	1	—	—							Killed 5 d.p. 1st Vbl
	2	—	—							Killed 5 d.p. 1st Vbl
	3	—	—							Killed 5 d.p. 1st Vbl
	4	—	—							Killed 5 d.p. 1st Vbl
	5	x	x							Died 11 d.p.v.
	6	—	—							Killed 5 d.p. 1st Vbl
	7	—		x	x					Died 5 d.p. 2nd Vbl
	8	—		—	—					Killed 6 d.p. 2nd Vbl
	9	—		—	—					Killed 6 d.p. 2nd Vbl
	10	—		—	—					Killed 6 d.p. 2nd Vbl
PF — Vbl (2)	1	—		—		—		x	x	Died 3 d.p. 4th Vbl
	2	—		—		—		—	—	Killed 5 d.p. 4th Vbl
	3	—		x		x		x	x	Died 3 d.p. 3rd Vbl
	4	—		—		x		x	x	Died 2 d.p. 3rd Vbl
	5	—		—		—		—	—	Killed 5 d.p. 4th Vbl
	6	—		—		—		—	—	Killed 5 d.p. 4th Vbl
	7	—		—		—		x	x	Died 4 d.p. 4th Vbl
	8	—		—		x		x	x	Died 8 d.p. 2nd Vbl
	9	—		—		—		—	—	Killed 5 d.p. 4th Vbl
	10	—		—		—		—	—	Killed 5 d.p. 4th Vbl
MEDIAN Mortality		0 0%		0 10%		0 30%		0 50%		
		DAYS AFTER VACCINATION								
		8		15		30		45		
PF	1									Killed 45 d.p.v.
	2	—		—		—		—		Killed 45 d.p.v.
	3	—		—		—		—		Killed 45 d.p.v.
	4	—		—		—		—		Killed 45 d.p.v.
	5	—		—		—		—		Killed 45 d.p.v.
	6	—		—		—		—		Killed 45 d.p.v.
	7	—		—		—		—		Killed 45 d.p.v.
	8	—		—		—		—		Killed 45 d.p.v.
MEDIAN Mortality		0 0%		0 0%		0 0%		0 0%		

TABLE 10

Mice infected with virulent y strain and infected with the same strain and treated 15 days later with Vbl

GROUP	MOUSE	PARASITES 5mm³ BLOOD				OBSERVATIONS
		Before Vbl		After Vbl		
		8 days	15 days	5 days	10 days	
Y — Vbl	1	560	12.600	8.680	x	Died 5 days post 2nd Vbl
	2	1.120	7.980	x	x	Died 3 d.p. 1st Vbl
	3	2.380	15.680	x	x	Died 4 d.p. 1st Vbl
	4	2.030	14.490	x	x	Died 4 d.p. 1st Vbl
	5	595	10.500	x	x	Died 4 d.p. 1st Vbl
	6	3.780	27.020	x	x	Died 3 d.p. 1st Vbl
	7	490	10.220	x	x	Died 3 d.p. 1st Vbl
	8	1.575	13.440	x	x	Died 3 d.p. 1st Vbl
	9	1.260	6.580	x	x	Died 5 d.p. 1st Vbl
	10	2.590	16.800	x	x	Died 4 d.p. 1st Vbl
MEDIAN Mortality		1.417 0%	13.020 0%	8.680 90%	x 100%	
		DAYS AFTER INFECTION				
		8	15	20	30	
Y	1	2.590	18.200	x	x	Died 17 days post infec.
	2	1.680	4.760	4.760	x	Died 23 d.p.i.
	3	1.890	6.720	6.720	x	Died 25 d.p.i.
	4	3.570	5.740	x	x	Died 20 d.p.i.
	5	1.330	7.840	2.800	560	Died 30 d.p.i.
	6	4.135	15.260	x	x	Died 18 d.p.i.
	7	1.785	8.260	x	x	Died 20 d.p.i.
	8	1.680	12.320	x	x	Died 20 d.p.i.
	9	910	14.980	x	x	Died 19 d.p.i.
	10	630	13.160	x	x	Died 20 d.p.i.
MEDIAN Mortality		1.737 0%	10.290 0%	2.800 70%	560 100%	

TABLE 11

Animals controls of the experiments described in the Tables 7 and 8.

GROUP	MOUSE	DAYS AFTER 1st INJECTION Vbl				OBSERVATION
		5	13	21	29	
Vbl	1	alive	alive	alive	dead	Died 2 days post 4th Vbl
	2	"	"	"	alive	Killed 5 d.p. 4th Vbl
	3	"	"	"	dead	Died 2 d.p. 4th Vbl
	4	"	"	dead	x	Died 5 d.p. 2nd Vbl
	5	"	"	alive	dead	Died 4 d.p. 4th Vbl
	6	"	"	"	alive	Killed 5 d.p. 4th Vbl
	7	"	"	"	dead	Died 2 d.p. 3rd Vbl
	8	"	"	"	"	Died 4 d.p. 3rd Vbl
	9	"	"	"	"	Died ; 3 d.p. 4th Vbl
	10	"	"	"	"	Killed 5 d.p. 4th Vbl
Mortality		0%	0%	10%	70%	
		DAYS AFTER 1st SALINE SOL. INJECTION				
N	1	alive	alive	alive	alive	Killed 5 days post 4th inj.
	2	"	"	"	"	Killed 5 days post 4th inj.
	3	"	"	"	"	Killed 5 days post 4th inj.
	4	"	"	"	"	Killed 5 days post 4th inj.
	5	"	"	"	"	Killed 5 days post 4th inj.
	6	"	"	"	"	Killed 5 days post 4th inj.
	7	"	"	"	"	Killed 5 days post 4th inj.
	8	"	"	"	"	Killed 5 days post 4th inj.
	9	"	"	"	"	Killed 5 days post 4th inj.
	10	"	"	"	"	Killed 5 days post 4th inj.
Mortality		0%	0%	0%	0%	

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