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SUSCEPTIBILITY OF INBRED MICE TO TRYPANOSOMA CRUZI STRAIN Y

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SUMMARY

Inbred mice infected i.p. with 100 parasites of **T. cruzi** Strain Y varied in their resistance to infection from highly susceptible C_3He/J to resistant (CBA x C_{57} BL/10) F_1. CBA was found susceptible and C_{57} BL/10 moderatly resistant. F_1 mice are recommended as a good model for experimental Chagas'disease since an acute and chronic phase are well characterized in these mice.

INTRODUCTION

Chagas' disease (CHAGAS, 1909) [3] is a serious health problem in many Latin American countries (WHO, [10]). Different clinical manifestations of the disease have been described, for instance, heart involvement is very common in Brazil but unusual in Argentina and Chile where digestive tract manifestation are more common (REZENDE [8]). Brazilian Chagas' disease comprises heart, aesophagus and colon "megas", isolated or combined in the same patient (KOBERLE [5]).

Those geographical differences have been thought to be due to different strains of **Trypanosoma cruzi** and/or differences in the genetic background of south-American populations as well as influences due to the general state of health of patients since sometimes multiple parasitic diseases overlap in the same subject.

Since previous work of PIZZI et al.[7] inbred mice have been shown to differ in their resistance to **T. cruzi.** That resistance is not linked to the major histocompatibility locus since mice sharing the same H_2 locus manifested different susceptibility (TRISCHMAN et al. [9]).

A continuity of resistance from highly susceptible C_3He/J to resistant Balb/C and $C_{57}B1/10$, possibly related to a single or multiple genetic component, were demonstrated by TRISCHMAN et al. [9] using Brazil strain of **T. cruzi.**

On the other hand variations in susceptibility related to different strains of **T. cruzi** were described in $C_{57}BL/10$ since this mouse strain was found more susceptible to Tulahen than to Brazil strain. Moreover, higher inoculum led to higher parasitaemias and increased mortality (TRISCHMAN et al. [9]).

The Brazilian strain Y isolated from a human case of Chagas Disease (PEREIRA DA SILVA & NUSSENZWEIG [6]) has been largely used in experimental Chagas'disease and has been shown highly virulent to outbred Swiss mice (BRENER [2]). Usually a large inoculum (10^4-10^5) has been currently used to infect mice i.p. On the other hand as little as 100 parasites are enough to infect A/Sn mice that are highly susceptible to strain Y (Mota-personal communication).

In order to verify the course of infection in C_3H, CBA, $C_{57}BL/10$ and (CBA x $C_{57}BL/10$) F_1 mice were infected i.p. with 100 parasites and parasitaemia scored daily.

The results showed that mice differed in their susceptibility to strain Y and only (CBA x $C_{57}BL/10$) F_1 were found resistant over a large

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dose of parasites used to infect mice. Those F_1 mice are recommended as a good model to study experimental Chagas'disease since an acute and a chronic phase are well characterized.

MATERIAL AND METHODS

1. **Animals** — C_3He/J, CBA, $C_{57}BL/10$ and $(CBAxC_{57}BL/10)F_1$ female and male mice bred and kept in clean conditions in our animals facilities were used throughout. Mice were 3 months old when infected.

2. **Trypanosome — Trypanosoma (Schizotripanum) cruzi** strain Y (PEREIRA DA SILVA & NUSSENZWEIG [6]) obtained from Dr. Zigman Brener (Belo Horizonte, Brazil) in 1970, weekly passaged in outbred Swiss 55 mice were used. Swiss mice were infected i.p. with 10^5 parasites and bled 7 days later at the peak of parasitaemia (BRENER [2]).

3. **Infection of mice** — Infected blood harvested in heparin (10U/ml) from the axillary plexus was diluted in Eagle MEM-1% FCS. Mice were infected i.p. with 100 parasites in 0.2 ml of Eagle MEM serum free. Counts were made in a Neubauer haemocitometer.

4. **Parasitaemia** — Parasites in peripheral blood were scored daily in all mice using wet blood films as described by BRENER [2]. Mice were cut at the tail tip and 5 μl of blood harvested in a haemoglobin pipette after the first drop had been discharged. The sample was dispersed under a coverslip (22 x 22 mm) over slides. Parasites were counted in 100 microscopic fields (40 x magnification) and the results expressed per mm^3 of blood.

Mice were marked in the footpad so parasitaemia could be followed-up daily in every single mouse. Experiments were performed 4 times per each strain using at least 10 animals/group.

RESULTS

1. **Parasitaemia** in C_3He/J and CBA MICE — C_3H mice were found highly susceptible to the infection. Although this strain showed the longest pre-patent period (7 days) they developed an increasing parasitaemia from the 9th day up to 5000 parasites/mm^3 at the 11th day after infection.

Mice usually controlled their parasitaemia by day 12 but again parasitaemia increased from the 13th day onwards killing 93.3% of animals around day 15 (Fig. 1, Table I).

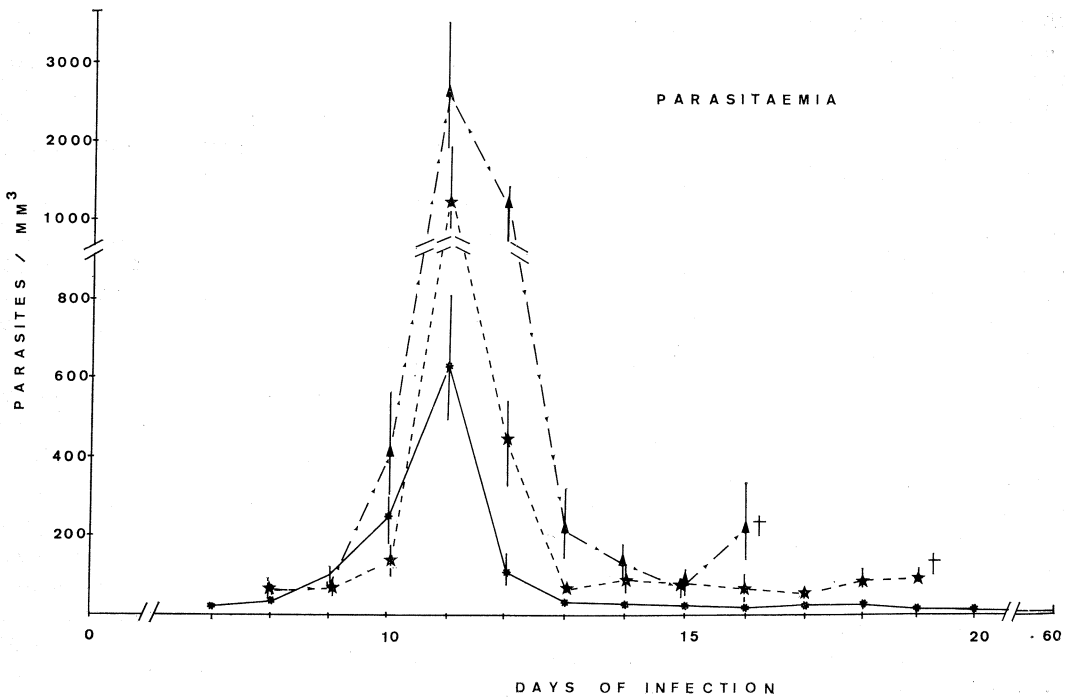

Fig. 1 — Title: Parasitaemia of C_3H, CBA and $C_{57}B1/10$ mice infected with **T. cruzi.**
Legend: Mice were infected i.p. with 100 trypomastigotes. ▲—▲—▲ C_3H; ★- - - -★ CBA; *——* $C_{57}B1/10$.

T A B L E I

Mortality of inbred mouse strains infected with **T. cruzi**[a]

Strains	Mortality	
	20 days	60 days
C_3H	23/25[b] (92%)	25/25 (100%)
CBA	20/33 (66.6%)	33/33 (100%)
$C_{57}BL/10$	10/61 (16.4%)	20/61 (32.8%)
(CBA x $C_{57}BL/10$) F_1	4/109 (3.7%)	4/109 (3.7%)

a — Mice were infected i.p. with 100 trypomastigotes
b — Number dead per number infected

C_3H showed a regular pattern of infection with a small SD of the mean at the peak, although larger SD were found on other days. As low as 10 parasites were able to infected and kill C_3H mice. The pattern of the infection was similar to that of 100 parasites but a higher variation was verified since some mice peaked on day 12 or 13 after infection (data not shown).

CBA mice were highly susceptible as well to strain Y. Mice had a pre-patent period of 5 days and developed from then a high parasitaemia. Usually mice controlled their parasitaemia around day 13 but a second wave killed as many as 90% of mice around day 15. Seldom mice survived infection longer than 20 days (Fig. 1, Table I). Although C_3H and CBA are highly susceptible to the infection, there are some differences between them.

Thus, CBA mice had a lower parasitaemia (2000/mm³ parasites at the peak) and although the pattern was similar in several experiments i.e. two successive waves of parasites in peripheral blood, the peak of the first wave varied from day 9 to 11. Conversely C_3H had the same behaviour in several experiments. No differences were noticed between female or male C_3H and CBA mice.

2. **Parasitaemia in** $C_{57}BL/10$ — $C_{57}BL/10$ mice were found moderatly resistant to the infection since 30% of animals died. Parasitaemia developed after a pre-patent period of 6 days reaching a peak of 300 parasites/mm³ on day 11. From this point onwards animals cleared their peripheral blood and kept very low numbers of circulating parasites. Mortality (25-30%) was verified from the day 20 and beared no relation with the parasitaemia (Fig. 1, Table I).

Survivors were kept as long as 6 months apparently with no signs of disease. No differences between sexes were noticed.

3. **Parasitaemia** in (CBA x $C_{57}BL/10$) F_1 — Female and male (CBA x $C_{57}BL/10$) F_1 mice were found resistant to the infection with 100 parasites of the strain Y. Mice had a pre-patent period of 6 days then developed an increasing parasitaemia up to the 11th day. From the peak onwards animals usually cleared parasites from the peripheral blood. This pattern was found quite regular with no changes in several experiments (Fig. 2, Table II).

Higher inocula (10^3, 10^4 and 10^5 parasites) showed for 10^3 parasites a shorter pre-patent period (4 days) and a peak reached sooner (9th day) but still with the same pattern and 100% survival (Fig. 2, Table II).

Conversely, 10^4 parasites had also a shorter pre-patent period and a higher peak but killed as many as 50% of animals on day 20. Survivors were apparently healthy (Fig. 2, Table II).

On the other hand 10^5 parasites killed all mice around day 7 after infection. Death was not related to parasitaemia, since parasites increased only up to 600/mm³ on day 7.

DISCUSSION

Our results showed that mice differed in their susceptibility to **T. cruzi** strain Y. C_3H and CBA mice were found highly susceptible although with different patterns in their parasitaemia. Coincidently these strains peaked all on day 11 although with different pre-patent periods and parasites numbers at the peak. C_3H developed the highest parasitaemia (5000 parasites/mm³). CBA with a lower number of parasites at the peak developed usually a second wave which killed the animals.

There was not any direct relationship between parasites numbers in peripheral blood and death as was shown by TRISCHMAN et al.[9] using Brazil strain.

Although CBA mice did not show always the same peaking day as far as parasitaemia was concerned, the pattern was the same in different experiments. Those differences may be related to Y intra-strain variations (ARAUJO & NEVES[1]) unrevealed in another inbred mouse strain. These strain Y variations are possible to occur since we did not use cloned parasites.

TABLE II

Mortality of (CBA x C_{57}BL/10) F_1 mice, infected with **T. cruzi**[a]

Number of infecting Trypomastigotes	Mortality	
	20 days	60 days
10^3	1/15[b] (6.6%)	1/15 (6.6%)
10^4	10/20 (50%)	16/20 (80%)
10^5	10/10 (100%)	—

a — Mice were infected i.p.
b — Number dead per number infected

On the other hand C_{57}BL/10 has been described as resistant to **T. cruzi** strain Y (COSTA[4]) C_{57}BL/10 was described resistant as well to Brazil strain with a large inoculum (10^4) (TRISCHMAN et al.[9]). Our results showed a limited resistance since 25-30% of animals died around day 20 of infection with very low parasitaemia.

Conversely, (CBA x C_{57}BL/10) F_1 mice were found highly resistant since they developed a low parasitaemia, cleared the parasites from peripheral blood and kept a low or undetected

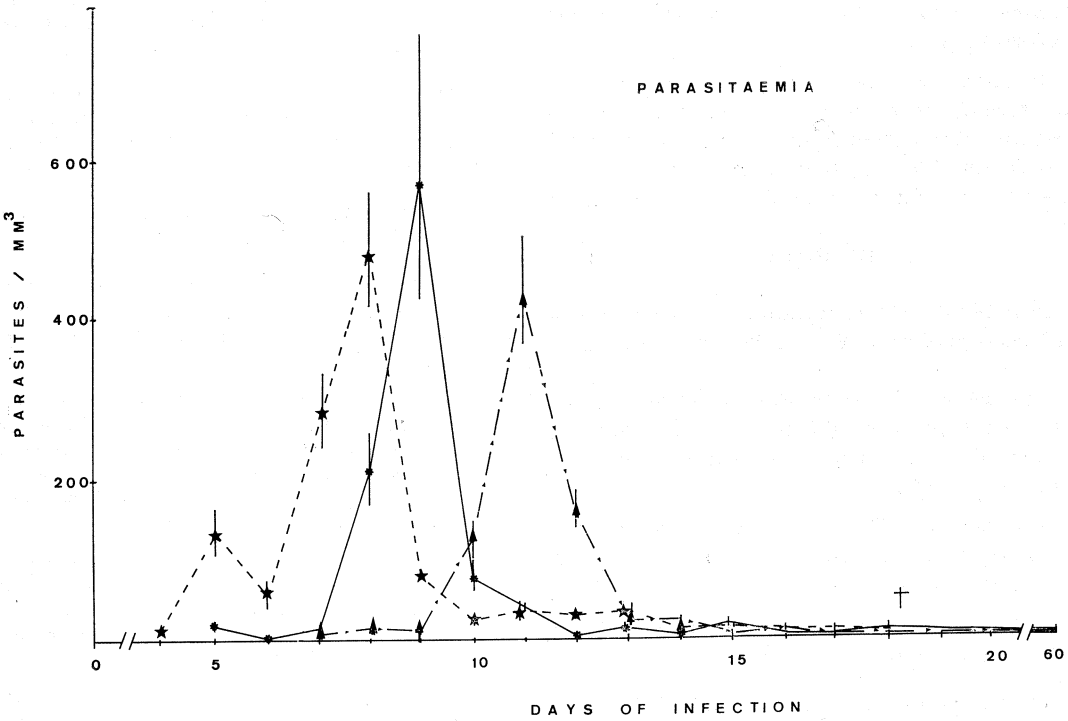

Fig. 2 — Title: Parasitaemia of (CBA x C_{57}B1/10) F_1 mice infected with **T. cruzi.**
Legend: Mice were infected i.p. with ▲—▲—▲ 10^2; *——*10^3 or ★- - - ★10^4 trypomastigotes.

parasitaemia for a long time been apparently healthy. We have mice infected for 10 months.

A large inoculum of 10^3 parasites showed a briefer pre-patent period and an earlier peak but mice still controlled the infection (Fig. 2). This behaviour of (CBA x C_{57}BL/10) F_1 towards infection with strain Y makes them a suitable model for experimental Chagas'disease since an acute and chronic phase are well characterized. Experiments are been carried out to study the behaviour of F_1 mice towards reinfection.

On the other hand higher inoculum of 10^4 parasites killed 50% of animals by 20 day of infection. As many as 10^5 parasites killed all mice by day 7 of infection.

Our results confirmed the existence of a continoum of resistance in inbred strains of mice towards infection with strain Y as was described for Brazil strain (TRISCHMAN et al.[9]) in 7 weeks old mice infected with 10^4 parasites.

Differences were found in relation to $C_{57}BL/10$, since this strain was found only moderately resistant.

Some differences related to sex were noticed in (CBA x $C_{57}BL/10$) F_1 mice since female mice usually showed a lower parasitaemia than male mice.

On the other hand we could not confirm the results of COSTA [4] since $C_{57}BL/10$ was not found as resistant as described. COSTA[4] described survival of these mice for months with inoculum of 500 parasites. Mice were susceptible only to 5.000 and 50.000 parasites.

Intra-strain variations may explain these differences and points to the necessity to clone **T. cruzi** strains.

In conclusion as low as 100 parasites of **T. cruzi** strain Y are enough to infect mice and to separate inbred strains of mice in susceptible or resistant. We feel that a small inoculum is closer to the natural infection than the larger ones.

RESUMO

Suscetibilidade de camundongos isogênicos à infecção pela cepa Y do Trypanosoma cruzi

Linhagens de camundongos isogênicos infectados intraperitonealmente com 100 parasitas da cepa Y do **T. cruzi** diferem quanto à resistência à infecção.

A linhagem C_3He/J é altamente suscetível à infecção contrariamente à (CBA x $C_{57}BL/10$) F_1 que é resistente.

A linhagem CBA mostrou-se suscetível e a $C_{57}BL/10$ moderadamente resistente.

Os camundongos (CBA x $C_{57}BL/10$) F_1 são recomendados como um bom modelo para estudos experimentais da doença de Chagas uma vez que nestes camundongos a fase aguda e a fase crônica estão bem caracterizadas.

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