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CHAGAS' DISEASE: DECREASED RESISTANCE TO *TRYPANOSOMA CRUZI* ACQUIRED INFECTION IN OFFSPRING OF INFECTED MICE

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Abstract. The course of *Trypanosoma cruzi* infection was studied in an experimental model, using the offspring of mice that were chronically infected with *T. cruzi*. When infected two months after birth, a higher mortality rate in heavily parasitized mice occurred in these offspring than in controls born to uninfected mothers. The harmful maternal influence reached a maximum when offspring were exposed both to prenatal (placental) and postnatal (lactating) influences. It was a reversible phenomenon that led to a *T. cruzi*–specific failure of the offspring to control the acute phase of the infection. Such features are suggestive of a maternally-induced impairment of the immune response of the offspring.

The congenital transmission of *Trypanosoma cruzi* occurs in the offspring of 0.7–10.5% of seropositive mothers and has been particularly well studied.[1] However, little attention has been focused on the consequences of maternal infection on the course of infection with this parasite in those offspring that were not congenitally infected. The most severe acute form of the acquired disease, which leads to a high mortality rate, is frequently found in children between the ages of one and five.[2] In endemic areas, the possibility of such children being born to chronically infected mothers is high. Since various immunologic elements, such as antibodies, antigens, immune complexes, cytokines or sensitized cells, are capable of modulating the immune response and can be transferred from infected mothers to their offspring and can induce some degree of immunosuppression,[3–5] the possibility that maternal factors may also induce harmful effects on the offspring deserves consideration.

In order to study this aspect of mother-offspring relationships, experiments were conducted in a mouse model of chronic *T. cruzi* infection, using mice in which a congenital infection was not observed.[6, 7] We report that a higher mortality rate in heavily parasitized mice occurred in the offspring of chronically infected mice, which suggests a decrease in resistance to *T. cruzi* infection.

MATERIALS AND METHODS

Experimental protocol

In experiment A, two groups of offspring of inbred BALB/c mice were studied. Mice in the first group (I) were born to and nursed by mothers chronically infected with *T. cruzi*. Mice in the second group (II) were born to and nursed by uninfected mothers and served as controls. In experiment B, a complementary cross-nursing experiment was conducted to determine if maternal influence was occurring during gestation or during lactation. In addition to groups I and II, two other groups of offspring groups were studied. Mice in group III were born to infected mothers, but were nursed by uninfected ones. Mice in group IV were born to uninfected mothers, but were nursed by infected ones. The newborn mice used for the cross-nursing experiment (groups III and IV) were permuted after delivery. The nursing period lasted until one month after birth. These experimental groups are listed in Table 1.

To avoid the protection period due to passively transferred maternal *T. cruzi*–specific antibodies,[8, 9] offspring were kept for two or five months before being infected with *T. cruzi* parasites (experiments A and B) or by other parasites to study the specificity of the maternal influence (experiment B) (see below). The course of infection was monitored for each mouse.

TABLE 1
Experimental groups used in the study

Group	Born to infected mothers	Nursed by infected mothers
I	Yes	Yes
II	No	No
III	Yes	No
IV	No	Yes

Female BALB/c mice that were used as mothers (48 for experiment A and 111 for experiment B) were purchased from the Experimental Animal Breeding Facility of the University of Leuven (Leuven, Belgium). They were nulliparous and weighed 19.4 ± 1.9 g (mean ± SD). Some of them (24 mothers in group I of experiment A and 52 in groups I and III of experiment B) were infected with 10^2 parasites when they were 60 days old. Mating was allowed when the infected mice were 120 days old, i.e., on postinfection (pi) day 60 (during the chronic phase of the infection when blood parasites were cleared). All mice were maintained in the same animal care facility and received water ad libidum and a standard diet. The number of offspring used in each experiment is indicated in the Results.

Parasitic infections

All *T. cruzi* infections were induced by intraperitoneal injection of blood trypomastigotes of the Tehuantepec strain, as previously described.[7] In most mice (experiments A and B), an inoculum size of 100 parasites was chosen for its low mortality rate.[7] Some female offspring (groups I and II, experiment B) were infected with a higher inoculum size of 10^4 parasites (see Results). All offspring in experiment A were infected at the same time (two months of age), whereas offspring in experiment B were infected at different times (two and five months of age).

The *T. cruzi* specificity of the maternal influence was studied by intraperitoneal inoculation with 10^5 *Plasmodium chabaudi*–infected erythrocytes or subcutaneous inoculation with 250 *Schistosoma mansoni* cercariae (low virulence Puerto Rican strain) into some of the mice in offspring groups I and II (experiment B).

Infection parameters

Mortality rates were regularly recorded for *T. cruzi, P. chabaudi,* and *S. mansoni* infections. In *T. cruzi*–infected offspring, parasitemia was determined from tail blood specimens every three days from day 0 to 60 pi, using the method of Brener.[10] To evaluate late-developing congenital infection, some randomly selected offspring from *T. cruzi*–infected mothers (experiment B) were killed one month after birth, i.e., without being submitted to experimental *T. cruzi* infection. The existence of blood parasites were investigated using a sensitive microhematocrit tube method.[7] Blood was inoculated into previously irradiated (700 rad) naive mice in which the parasitemia was monitored every three days until day 60 pi. We also investigated the presence of intracellular parasites (amastigotes) in tissue sections (skeletal muscle, diaphragm, heart, spleen, and liver) that had been previously fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin-safron.

For the determination of *P. chabaudi* infections, blood smears were performed every three days from day 3 to day 15 pi. Infected erythrocytes were counted after staining with Giemsa.

For each *S. mansoni*–infected animal killed on day 50 pi, the mean size of the granuloma reaction surrounding liver-trapped eggs was determined by measuring 30 individual granuloma in 5-μm sections that were embedded in paraffin and stained with hematoxylin-eosin-safron. Adult worms were collected by liver perfusion and counted.

Statistical analysis

The associations among the three categorical variables (lethality, sex, and offspring groups) were investigated using a log-linear model, and the statistically significant effects were assessed with a backward elimination procedure. Chi-square tests were used in analyses restricted to two categorical variables. The parasitemia was logarithmically transformed and subjected to an analysis of variance with lethality, sex, and offspring groups as grouping factors. The Mann-Whitney test was used for comparing parasitemia between two categories of a same variable. Offspring data are given as the arithmetic mean ± SEM.

RESULTS

Offspring data before experimental infection

The mean litter sizes and sex-ratios were similar ($P > 0.05$) for offspring from infected or

uninfected mice in both experiments A (litter size 4.00 ± 0.33 versus 5.25 ± 0.40, sex ratio 1.13 versus 0.91) and B (litter size 4.60 ± 0.33 versus 4.02 ± 0.46, sex ratio 1.10 versus 1.08).

When tested by either direct (microhematocrit test) or indirect (inoculation to irradiated animals) methods, blood parasites were not found in 14 randomized offspring mice (seven males and seven females in experiment B) that were killed one month after being born to infected mothers (group I). All histologic examinations for amastigotes showed negative results. These findings indicate the absence of a congenital *T. cruzi* infection in offspring born to mice infected by our experimental conditions.

Since fetal growth retardation was previously observed in mice similarly infected by *T. cruzi*,[7] offspring weights were recorded just before the experimental infection, at 60 days after birth (experiment B). The mean weights of the mice were similar ($P > 0.05$) in the different groups (ranges 25.2–26.5 g for male and 20.4–22.1 g for female offspring). Because a weight-related lower resistance could occur in mice that subsequently died after experimental infection (see below), the mean weights of dying and surviving mice were compared, but they were also similar ($P > 0.05$; unpublished data).

Mortality rates in T. cruzi*–infected offspring*

The cumulative mortality rates at the end of experiments A and B, and the number of mice used in the different offspring groups are indicated in Figure 1. Multivariate statistical analysis with a log-linear model showed significant interactions between offspring groups, sex, and lethality in 314 mice infected two months after birth by 10^2 parasites ($P < 0.01$) (experiment B). This confirms the following associations derived from two by two variables analyses. Indeed, male offspring born to chronically infected mothers (group I) displayed a significantly greater mortality than control offspring born to uninfected mothers (group II) (experiment A: 58.8% versus 38.3%; $P < 0.05$; experiment B: 70.7% versus 22.9%; $P < 0.001$).

Curiously, female offspring showed lower mortality rates without any difference between groups I and II (experiment A: 22.2% versus 19.7%; experiment B: 15.5% versus 11.5%; $P > 0.05$). To test the resistance to infection of female offspring, other female mice from experiment B were infected with a higher inoculum dose of 10,000 parasites instead of the dose of 100 that was previously used. As a result, a 4.4-fold increase in mortality occurred in the group I compared with the controls (group II) (31.2% versus 7.1%).

Survival times in infected offspring

Most mice died between the third and the sixth week of infection, i.e., during the acute phase. The mean survival times were similar, regardless of the group or the experiment (range 26.2–29.2 days pi; $P > 0.05$).

Trypanosoma cruzi *parasitemia in infected offspring*

The study of individual parasitemia kinetics showed that most of mice died when they reached their highest parasitemia (unpublished data). Representative kinetic data of mean parasitemia of each offspring group (including dead and surviving mice) are shown in Figure 2. The maximum mean parasitemia on day 28 pi was significantly higher in male mice of group I (mean + SEM 3.31 × 10^6 parasites/ml, mean − SEM 1.74 × 10^6 parasites/ml) than in the controls (group II) (mean + SEM 1.20 × 10^6 parasites/ml, mean − SEM 0.83 × 10^6 parasites/ml) ($P < 0.01$). Although parasitemias in female mice were lower than in male mice, the maximum mean parasitemia in female group I (mean + SEM 0.66 × 10^6 parasites/ml, mean − SEM 0.38 × 10^6 parasites/ml) on day 24 pi was also significantly higher than in the female control group II (mean + SEM 0.23 × 10^6 parasites/ml, mean − SEM 0.17 × 10^6 parasites/ml) ($P < 0.01$).

When comparisons were made between dead and living mice (Table 2), the maximum mean parasitemias were significantly higher in dead mice than in surviving mice of both sexes, and analysis of variance indicated significant effects of both sex and lethality on maximum parasitemia (experiment A: $P < 0.03$, experiment B: $P < 0.001$). The maximum mean parasitemias of dead or surviving mice were similar ($P > 0.05$) in both groups I and II, indicating that the numerous heavily parasitized dead mice of group I contributed to increase the global mean parasitemia previously mentioned in this group, as compared with control group II.

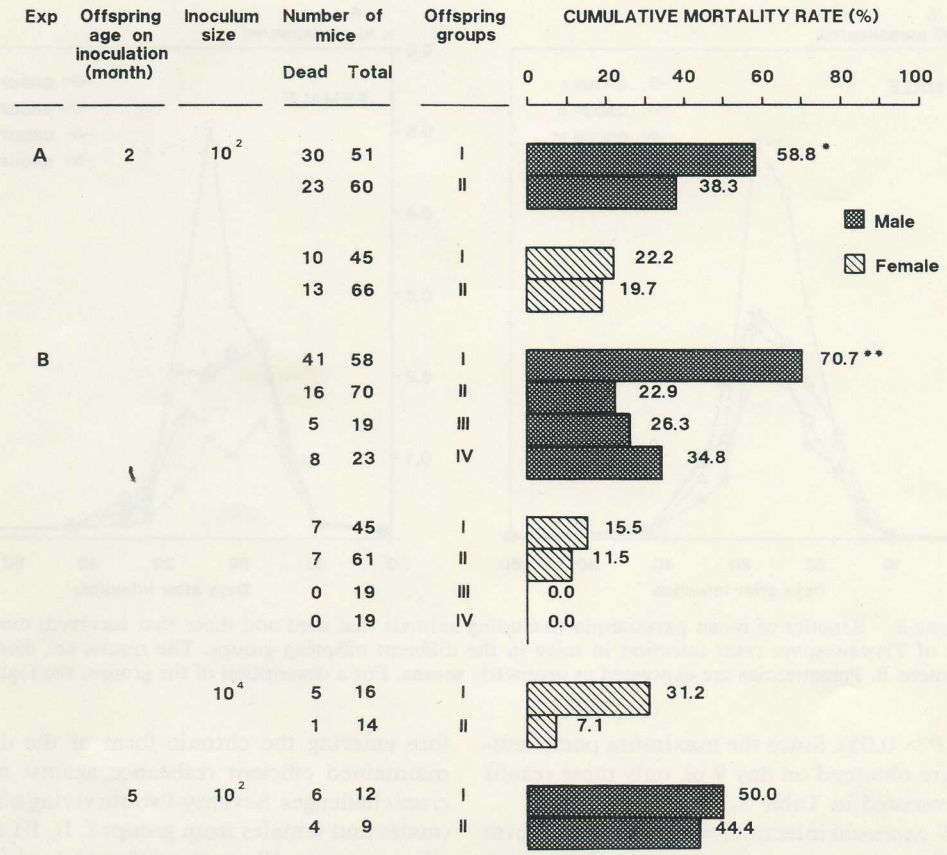

FIGURE 1. Cumulative mortality rates in the various *Trypanosoma cruzi*–infected offspring groups. Groups I and II correspond to offspring born to and nursed by infected or uninfected mice, respectively. Group III includes offspring of infected mice that were nursed by uninfected mice, and group IV includes offspring from uninfected mice that were nursed by infected mice. Results are expressed according to parasite inoculum size and mouse age at inoculation. * $\chi^2 = 4.7$, $P < 0.05$, group I versus group II; ** $\chi^2 = 9.4$, $P < 0.001$, group I versus group II. Exp = experiment.

Prenatal or postnatal occurrence of the maternal influence

In the cross-nursing experiment (Figure 1), the cumulative mortality rates of infected male offspring of groups III and IV were similar to that of control offspring (group II) ($P > 0.05$), but were significantly lower than in the offspring of group I (group III versus group I: $\chi^2 = 11.8$, $P < 0.001$; group IV versus group I: $\chi^2 = 8.8$, $P < 0.01$). As indicated in Figure 2, the maximum mean parasitemias of groups III and IV were similar to that of control group II, but were significantly lower than that of group I.

Duration of the maternal influence on offspring

All previous results were obtained with offspring infected two months after birth. To determine the duration of the maternal influence, male mice of offspring groups I and II of experiment B were infected with *T. cruzi* and maintained in the animal care facility until they were 5 months old. Mortality rates (group I 50.0% and group II 44.4%) (Figure 1) and the mean maximum parasitemia (group I mean + SEM 8.32×10^6 parasites/ml, mean − SEM 5.49×10^6 parasites/ml; group II mean + SEM 6.01×10^6 parasites/ml; mean − SEM 3.29×10^6 parasites/ml) were similar in both groups ($P > 0.05$).

Specificity of the maternal influence on offspring

As shown in Table 3, offspring of groups I and II (experiment B) infected by *P. chabaudi* had a similar rate of cumulative mortality on day 30 pi, and a similar percentage of infected red blood

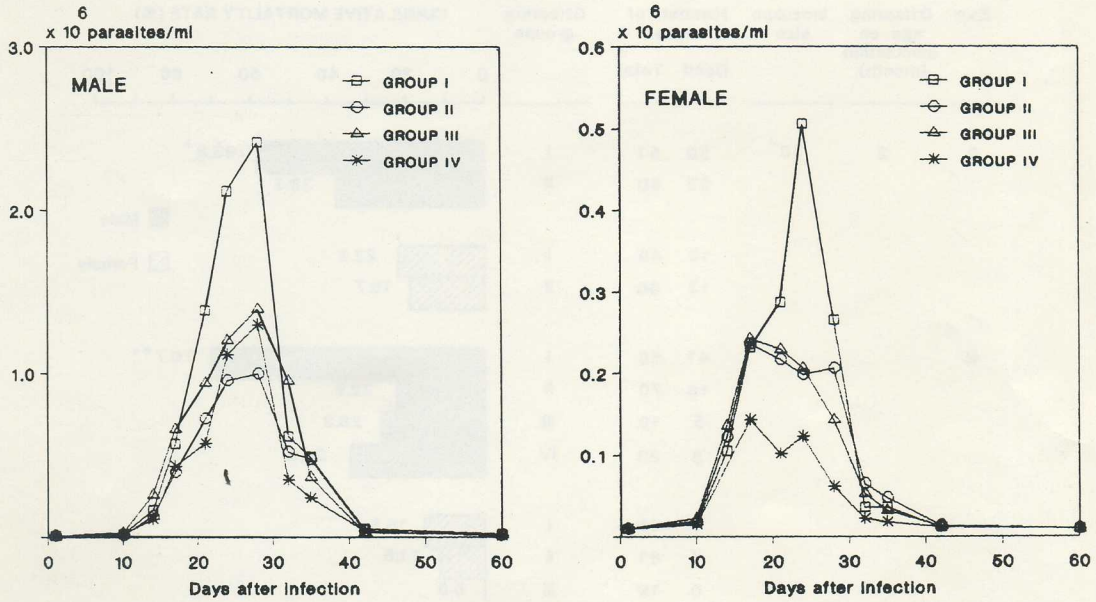

FIGURE 2. Kinetics of mean parasitemia (including animals that died and those that survived) during the course of *Trypanosoma cruzi* infection in mice in the different offspring groups. The results are those from experiment B. Parasitemias are expressed as geometric means. For a description of the groups, see Figure 1.

cells ($P > 0.05$). Since the maximum parasitemias were obtained on day 9 pi, only these results are presented in Table 3.

In *S. mansoni* infection, mortality rates, worm burdens, and mean liver egg granuloma sizes (which can be considered as a marker of the host response to this parasite, which does not multiply in the vertebrate host)[11] were also similar in groups I and II ($P > 0.05$) (experiment B).

T. cruzi *reinfection of surviving offspring*

Experiments were performed to determine if offspring surviving *T. cruzi* infection, and therefore entering the chronic form of the disease, maintained efficient resistance against new *T. cruzi* challenges. Seventy-two surviving offspring (males and females from groups I, II, III and IV of experiment B) were reinfected (one or two times) with 100, 1,000 or 10,000 *T. cruzi* parasites four to six months after the initial infection. Control mice inoculated only once with the same parasite doses always displayed positive parasitemia (range 0.11–11.5 × 10^6 parasites/ml) and their mortality rates were 44–83% for males and 0–10% for females. In contrast, all reinfected mice in all groups survived. In addition, when we attempted to identify blood parasites between days 7 and 35 pi, none were found.

TABLE 2

*Maximum mean parasitemias in offspring of infected (group I) or uninfected (group II) mothers**

Offspring		Maximum mean parasitemia (× 10^6 parasites/ml)	
Sex	Group	Dying mice	Surviving mice
M	I	6.40 (4.89–8.37)	0.99 (0.74–1.30)
	II	6.30 (4.80–8.30)	0.80 (0.70–0.90)
F	I	2.94 (1.34–6.40)	0.32 (0.24–0.41)
	II	0.49 (0.05–4.50)	0.23 (0.21–0.25)

* Results are from experiment B. Values in parentheses are the geometric means (mean − SEM; mean + SEM). The number of mice in each group is indicated in Figure 1. All differences between the parasitemias of dying and surviving mice were statistically significant by the Mann-Whitney test ($0.001 < P < 0.02$).

DISCUSSION

Our results clearly show that a higher mortality rate of heavily parasitized mice occurs in offspring of mice chronically infected with *T. cruzi*, which suggests a decreased resistance in response to infection with this parasite. As far as we know, the present study is the first to indicate a marked harmful influence of mothers chronically infected with *T. cruzi* on offspring that have not congenitally acquired the infection. Indeed, parasites were never found in the offspring studied before

TABLE 3

Effect of Plasmodium chabaudi *and* Schistosoma mansoni *infections on offspring of* Trypanosoma cruzi*–infected (group I) or control (group II) mice**

Offspring		*P. chabaudi*		*S. mansoni*		
Group	Sex	Mortality rates (%)	% of infected red blood cells	Mortality rates (%)	Worm burden	Size of egg granulomas (μm)
I	M	12/15 (80.0)	44.0 ± 1.5	3/14 (21.4)	67 ± 13	342 ± 13
II	M	10/10 (100)	44.8 ± 0.9	4/12 (33.3)	60 ± 13	361 ± 17
I	F	8/14 (57.1)	49.8 ± 2.2	3/13 (23.1)	50 ± 5	357 ± 12
II	F	5/11 (45.4)	55.0 ± 1.8	3/10 (30.0)	47 ± 8	336 ± 14

* Two-month-old mice from experiment B received either 10^5 *P. chabaudi*–infected erythrocytes or 250 *S. mansoni* cercariae. The rates of infected red blood cells (day 9 postinfection), worm burden, and the size of egg granulomas (day 50 postinfection) are expressed as the mean ± SEM.

they received experimental infections. Such results confirm our own previous observations with 17-day-old fetuses obtained from similarly infected mice,[6, 7] and the findings of other studies that also failed to demonstrate congenital parasite transmission from chronically infected animals.[12–14]

The data obtained before experimental infection also confirm our previous observation that chronic *T. cruzi* infection has no role in modifying litter size[7] or the sex-ratio of the offspring. Thus, our results clearly indicate that chronic *T. cruzi* infection does not interfere with the reproductive capacity of the mice.

Male and female offspring are probably exposed to the same maternal influence, and the higher parasite dose necessary to kill female offspring could be related to the well-known higher natural resistance of female mice to *T. cruzi* infection.[15] Dying mice, which are found more frequently among the offspring of infected mothers (group I), had a reduced capacity to control their parasitemia, which was constantly higher than that in surviving offspring. The surviving mice in group I probably escaped or overcame the harmful maternal influence because their capacity to control infection was similar to that of the control offspring in group II born to uninfected mothers.

The decreased resistance became critical during the acute phase of the disease because offspring died when the parasitemia was highest. Although pathologic or EKG studies were not performed to estimate the maternal influence on chronic disease in the offspring, the surviving mice with chronic infections who were born to infected mothers benefited from a long-term protection against repeated *T. cruzi* challenges. This finding, which was also observed in the controls, indicates that their immune systems are functioning normally.

The harmful influence upon infection of the offspring reaches its maximum intensity (i.e., the highest offspring mortality rate) when prenatal (placental) and postnatal (lactation) exposures are combined. This was clearly indicated by the lower mortality rate observed in male offspring from groups III (born to infected mice) and IV (nursed by infected mice) compared with male offspring born to and nursed by infected mice (group I).

The decreased resistance of the offspring can be considered to be *T. cruzi* specific because infection courses with parasites unrelated to *T. cruzi,* such as *P. chabaudi* or *S. mansoni,* were shown to be similar in offspring from *T. cruzi*-infected mothers and in controls.

This maternal influence has been observed in two-month-old mice, i.e., after the protection period related to maternal antibodies.[8, 9] However, it is a reversible phenomenon, since it was no longer observed in five-month-old offspring. The higher mortality rate observed in older controls could be explained by the age-related natural modifications of host susceptibility to parasite infection, that has been previously described.[16] Indeed, the maternal influence on *T. cruzi* infection in offspring of infected mice can be considered as a biphasic and short-term phenomenon, with successive periods of postnatal protection and lower resistance, followed by the normal evolution of resistance in the adult stage. This period of decreased resistance in early life, during which an infection could have dramatic consequences, is lacking in offspring from uninfected mice.

The mechanism of such a harmful maternal influence is unknown. A possible explanation for the decreased resistance may be related to intra-

uterine growth retardation, which has been previously observed in the fetuses of infected mothers.[7] However, the weights of the offspring before experimental infection were found to be similar, regardless of the groups, indicating that the offspring had overcome their growth handicap. This latter data also rules out nutritional involvement, both during suckling (litter sizes were shown to be similar in infected and uninfected mothers) and in the post-weaning period, when all mice received a standard diet. Moreover, since our experimental protocol used inbred mice, the decreased resistance of mice offspring cannot be explained by genetic factors. The main features of the decreased resistance in the offspring, namely the higher mortality rate of heavily parasitized mice and its *T. cruzi* specificity, are suggestive of a maternally induced major impairment of the immune response in these offspring. This immunologic mechanism could be related to the transfer of immunomodulating agents from the mother to the young by both placental and suckling routes.

Extrapolation of such results obtained in mice to humans requires some caution. However, the possibility of a harmful maternal influence on the immune system of human offspring could also contribute to enhancing the severity of the acute phase of the Chagas' disease in young children.[2] Such a possibility must be kept in mind for further investigations on Chagas' disease in endemic areas.

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