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AMERICAN TRYPANOSOMIASIS (CHAGAS' DISEASE) IN CONVENTIONAL AND GERMFREE RATS AND MICE (1)

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SUMMARY

Germfree (GF) and conventional (CV) CFW (LOB) mice and Wistar and Sprague-Dawley rats were infected with **Trypanosoma cruzi.** The disease was more severe in the GF than in the CV animals as revealed by: (1) an earlier and more intense parasitemia; (2) a more precocious mortality; (3) a twice enlarged spleen: (4) a more intense cell and tissue parasitism; (5) visceral signs of cardiac failure.

KEY WORDS: Chagas' disease; Trypanosomiasis; Germfree rats; Germfree mice.

INTRODUCTION

The difference between germfree (GF) and conventional (CV) animals of the same species is not restricted to the absence of the normal flora in the former. To this characteristics, the lack of reaction of the host to germs and a series of direct effects of these germs on the host should be added (GORDON & PESTI, 1971).

GF animals, when infected with pathogens may exhibit higher or lower resistance to the infecting organism (GORDON & PESTI, 1971; PLEASANTS, 1974). GF mice are five times more resistant and show more intense mitogenic and immunogenic responses to bacterial endotoxins than their CV counterparts (KIYONO **et al.**, 1980). FINERTY **et al.** (1972) infected GF and CV mice with **Plasmodium herghei;** the rise in parasitemia was more precocious in CV animals even although antibodies could be detected earlier in GF mice which also had higher plasma immunoglobulin titers. On infection with **Eperythrozoon coccoides,** CV reacted more efficiently than GF mice (HYDE **et al.**, 1972). VIEIRA **et al.**, (1985) presented preliminary evidences that schistosomiasis mansoni was less severe in GF than in CV mice.

In the present work, the infection with **Trypanosoma cruzi** was studied in GF and CV mice and rats.

MATERIAL AND METHODS

Colombian and Y strains of **T. cruzi** were maintained in CV mice and in irradiated CV rats. To obtain the inocula, the animals were killed under ether anesthesia. Blood was collected from the axillary plexus of mice or directly from the

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heart of rats with syringes containing heparin. The evaluation of the number of trypomastigotes was done according to BRENER (1962). The adjustment of the number of parasites to the desired inocula was done by dilution with blood obtained from uninfected animals. The manipulations were performed in a laminar flow hood. The blood was transferred to sterile ampoules. A sample was seeded in thioglycollate medium and brain-heart broth for control of asepsis. The ampoules were then sealed in a flame and introduced into the isolator. Contamination was never detected in the control samples after incubation at 28°C and 37°C.

Breeding nuclei of GF (LOB) mice and GF Wistar rats were supplied by Dr. Morris Pollard, University of Notre Dame, Notre Dame, Indiana, USA. A breeding nucleus of GF Sprague-Dawley rats was obtained from Dr. Edward Balish, University of Wisconsin, Madison, Wisconsin, USA. Those animals are free of all demonstrable microbes. Mice, however, bear a leukemogenic virus acquired prenatally which remains latent unless activated by radiation (POLLARD, 1965).

The CV animals were derived from the GF colony. Rats and mice support very well the conventionalization.

GF rats and mice were maintained in flexible plastic isolators (TREXLER, 1959) and manipulated according to established procedures (PLEASANTS, 1974). Unless otherwise stated, the CV counterparts were also maintained in isolators and handled accordingly.

GF and CV 21 and 56 days old Wistar rats and 14 months old Sprague-Dawley rats were inoculated intraperitoneally (i. p.) with 10^5 trypomastigotes per gram of body weight of Colombian strain of **T. cruzi.** GF and CV three months old CFW (LOB) mice were inoculated i. p. with 8×10^4 trypomastigotes of Y strain of **T. cruzi.** GF and CV 21 days old CFW (LOB) mice were inoculated similarly with $1.0\text{-}1.3 \times 10^4$ trypomastigotes of Y strain of **T. cruzi.**

Blood trypomastigotes were counted daily, as described by BRENER (1962).

The experiment was run until the death of the animals. In experiments with rats, some animals were killed under ether anesthesia. The animals were opened ventrally from the neck to the end of the abdomen and immersed in 4% formaldehyde solution. Fragments measuring 2-4mm were removed from the thymus, liver, spleen, lymphonodes, brain, and skeletal, cardiac, and smooth muscles. The fragments were fixed in 4% formaldehyde solution and processed for paraffin embedding. The sections were stained with hematoxylin-eosin. The slides were examined by only one person who did not have access to the codification of the slides, whose identification was done only after each report had already been written.

The control of isolator asepsis was done according to WAGNER (1959).

RESULTS

Mice

Figure 1 shows the evolution of the parasitemia and the cumulative mortality of six GF and four CV three months old male CFW mice infected with 8×10^4 blood forms of trypomastigotes of **T. cruzi,** Y strain. An earlier and more intense

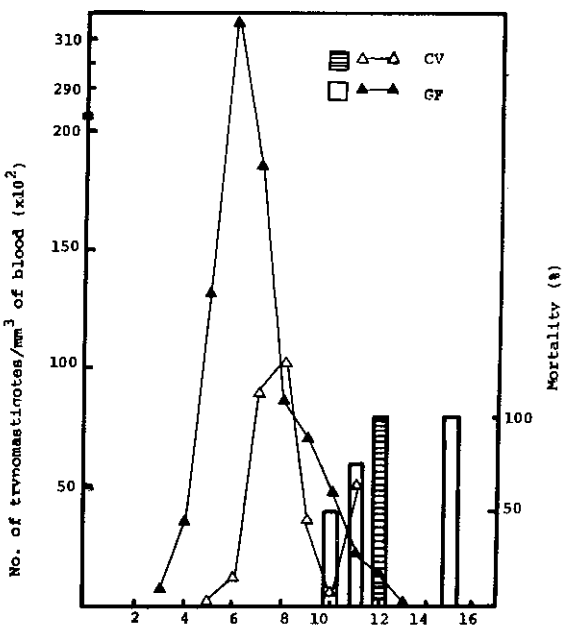

Fig. 1 — Parasitemia and cumulative mortality in groups of germfree (GF)[6] and conventional (CV)[4] three months old CFW mice inoculated with 8×10^4 blood forms of **Trypanosoma cruzi,** Y strain.

parasitemia was observed in the GF group. The mortality also was more precocious in the GF than in CV group, even though two GF animals survived up to the 15th day after infection. At the 12th day, all CV and 66% of the GF animals were dead.

The experiment was repeated with four male and five female GF and six male and twelve female CV, 21 days old mice infected with 1.0-1.3 x 10^4 trypomastigotes of Y strain of **T. cruzi.** Again, both parasitemia and mortality were more precocious in the GF group, although the peak of parasitemia was higher in CV mice. In this experiment, the CV animals were kept in the CV animal room. After the beginning of the experiment, there was a drop in the temperature that affected mostly the CV mice.

Rats

A preliminary experiment with one female and one male GF and two female CV 14 months old Sprague-Dawley rats was carried out. A higher parasitemia and a more precocious death were observed in GF group.

Figure 2 shows the parasitemia and the mortality of five GF and six CV Wistar male 21 days old rats weighing approximately 60g and inoculated with 6 x 10^6 trypomastigotes of Colombian strain of **T. cruzi.** The parasitemia was much higher in the GF group reaching 150.000 trypomastigotes/mm^3 of blood. The mortality was also more precocious in the GF group. At the 21st day, all GF had died whereas one CV lived up to the 47th day, when it was sacrificed.

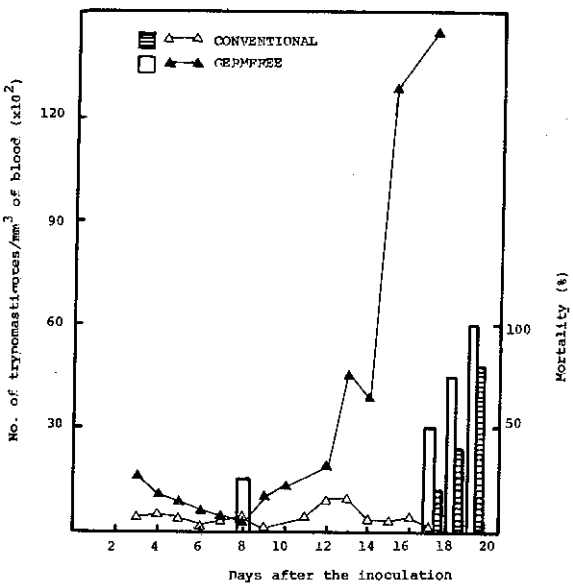

Fig. 2 — Parasitemia and cumulative mortality of germfree[5] and conventional[6] days old Wistar male rats weighing 60g, inoculated with $6x10^6$ blood forms of U strain of **Trypanosoma cruzi.**

The histopathological findings were similar in rats and mice from the above mentioned groups. Macroscopically: (1) the spleens from GF animals were twice the size of CV animals; (2) pronounced signs of congestive cardiac insufficiency (such as, ascites and generalized visceral congestion) in GF animals. Microscopically: (1) more intense cell and tissue parasitism in GF than in CV animals; (2) the parasitism was much more intense in the organs rich in cells of the mononuclear phagocytic system and in the parenchyma of liver, adrenals and muscles (cardiac, skeletal and smooth) of both GF and CV animals. In the central nervous system, there were sparse and discrete inflammatory lesions and cellular parasitism.

Figure 3 shows a sample of the histopathologic findings in hearts of GF and CV mice. The more intense agressiveness of the disease in GF animals is evident.

The experiment was repeated with four male and four female GF and three male and three female CV, 56 days old Wistar rats weighing approximately 110g. The animals were inoculated with 12 x 10^6 trypomastigotes of Colombian strain of **T. cruzi.** The parasitemia was more precocious and higher in the GF groups. All animals survived to the 71st day, when they were killed.

On histopathological examination, no difference between the GF and the CV animals could be detected. A discrete focal parasitism in the muscular and macrophagic phagocytic system could be visualized.

DISCUSSION

Mice are the animals most widely used as hosts in experimental Chagas' disease. They develop an acute and a chronic phase following infection with **T. cruzi.** Figure 1 shows that GF

mice had an earlier and higher parasitemia than their CV counterparts. These results may be explained by the fact that GF mice have a less developed lymphoid system when compared with CV mice, impairing the cell-mediated immune response (SZERI **et al.**, 1976). ROGERS & BALISH (1978) and GOODMAN **et al.** (1978) reported evidences for the "immunological immaturity" of GF animals. The rate of gamma-globulin synthesis is 50 times higher in CV than in GF mice (SELL & FAHEY, 1964). These data are also suggestive of a slower humoral immune response in GF than in CV mice. The histopathological data confirm that the disease is much more severe in the GF group (Fig. 3). The higher cell and tissue parasitism in GF mice may be explained by the smaller number of cells involved in immunological defense such as: lymphocytes (OLSON & WOSTMAN, 1966a) and neutrophils, monocytes, and eosinophils (OLSON & WOSTMANN, 1966b).

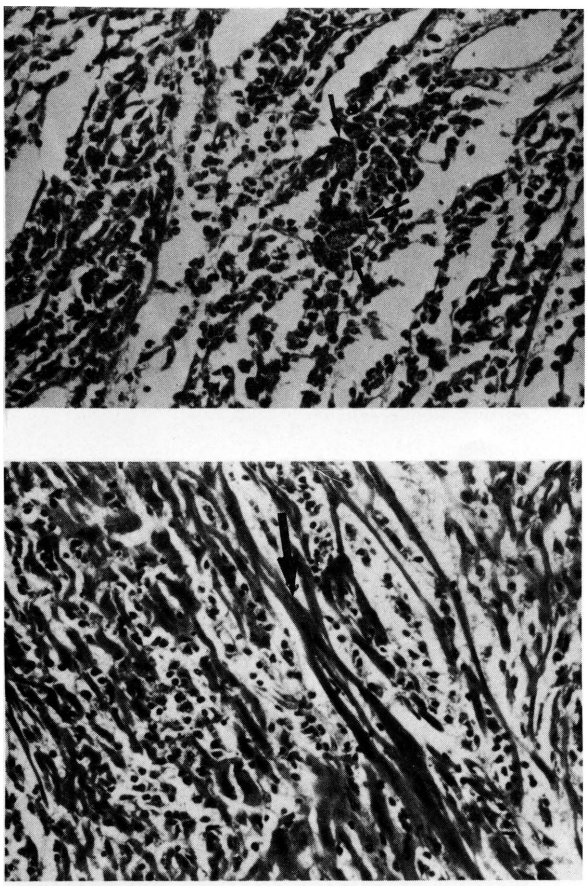

Fig. 3 — Histopathologic aspect of the heart of three months old germfree (A) and conventional (B) CFW mice. A: cellular parasitism (arrows) and intense edema dissociating the myocardial fibers. Mononuclear inflammatory cells are admixed with degenerated and dissociated fibers. B: cellular parasitism (arrow) and diffuse mononuclear inflammatory infiltration and interstitial edema. The less agressive inflammatory process in the myocardium of conventional mice can be observed (hematoxylin and eosin X320).

Moreover peritoneal macrophages from GF mice are possibly less activated than those obtained from CV animals. Macrophages from GF animals have smaller and more spherical nuclei, fewer mitochondria and a higher number of lysosome-like granules per unit volume of cytoplasm (WOODWARD, 1978). These differences may be possibly related to the lack of stimuli from intestinal flora (WOODWARD, 1978).

The higher parasitemia found in CV mice in the second experiment might be explained by the lower temperature of the room where the animals were housed. It is well known that there is a raise in parasitemia at lower temperatures (KOLODNY, 1940). Nevertheless, the death rate and the histopathological findings confirmed the results of the first experiment.

Figure 2 shows that, in rats, the levels of parasitemia were higher in the GF than in CV group. In 21 days old animals, the mortality was earlier and total in the GF group. All six GF and four out five CV rats died. The high mortality of young rats infected with **T. cruzi** confirms the results of KOLODNY (1940) and CULBERTSON & KESSLER (1942), who showed that the disease is more severe in younger animals. The morphological findings are compatible with the parasitological data, i. e., the disease was more severe in GF than in CV rats.

In older rats there was no mortality in either group of animals. The parasitemia, again, was more precocious and higher in the GF than in the CV rats. The histopathological findings were similar for both groups.

The results reported herein show that Chagas' disease is more severe in GF than in CV rats and mice. Further work will be carried out to elucidate the reason for the observed differences.

RESUMO

Tripanosomíase americana (doença de Chagas) em ratos e camundongos convencionais e isentos de germes

Camundongos CFW (LOB) e ratos Wistar e Sprague-Dawley isentos de germes (GF) e convencionais (CV) foram infectados com **Trypanosoma cruzi.** A doença foi mais grave nos animais GF do que nos CV, o que foi demonstrado por: (1) uma parasitemia mais precoce e mais intensa; (2) uma mortalidade mais precoce; (3) baço duas vezes maior; (4) um parasitismo celular e tissular mais intenso; (5) sinais viscerais de insuficiência cardíaca.

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