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HISTOPATHOLOGY OF THE HEART AND MUSCLES IN MICE IMMUNIZED AGAINST *TRYPANOSOMA CRUZI*

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SUMMARY

Among mice inoculated with high numbers of living *Trypanosoma cruzi*, Y strain, cultivated for 3 days in presence of Actinomycin D, survival was 100%, no parasitemia could be detected and no parasites were found in tissues.

When challenged with virulent blood forms of the same strain, all mice thus treated survived, while all controls died within 13 days. Parasitism was much lower in the immunized than in the control mice, but the acute inflammatory reaction was much more severe in the first group. This reaction frequently subsided, only a few discrete lesions being found in later examinations.

INTRODUCTION

Several procedures have been tried to induce resistance to infection with virulent *T. cruzi* in animals. Those that succeeded were based on inoculations of living trypanosomes from the following sources:

1) Blood forms of a virulent strain inoculated in small dosis (HAUSCHKA et al.[6]). 2) Culture forms of avirulent strain (HAUSCHKA et al.[6]). 3) Culture forms of a virulent strain attenuated by prolonged growth in artificial media (PIZZI & PRAGER [9]). 4) Blood forms originally virulent but attenuated by drugs (COLLIER [3]). 5) Virulent strains followed by treatment with a suppressive drug (BROWING et al.[2], HAUSCHKA et al.[6], PIZZI et al.[10]).

All these methods produce a mild infection with low mortality, the surviving animals becoming highly resistant to infection with virulent strains. On the other hand, no protection was conferred by injecting parasites killed by various means (MUNIZ et al.[7], HAUSCHKA et al.[6]). These results indicate that when a strong immunity was achieved it was the consequence of a previous infection. The search for "strong immunity without a previous infection" became the most important goal in this field.

During our studies on protein and nucleic acids synthesis by *T. cruzi* we have found that Actinomycin D renders the flagellates unviable and devoided of multiplication capacity (FERNANDES et al.[4]). The living but irreversibly inhibited flagellates do retain, however, the ability to induce resistance in mice in spite of the apparent loss of their infectivity as indicated by the negative re-

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sults of a throughout search for parasites in animals inoculated with Actinomycin D treated cultures and their low parasitemia and long survival following lethal dosis of virulent blood trypanosomes (FERNANDES et al.[5]).

The present paper is an extension of the above mentioned work in order to include pathology studies. The marked and constant lesions produced in the heart, skeletal and visceral muscles by infection with blood forms of the Y strain are well known (OKUMURA et al.[8]) and we proceeded to investigate if the picture would be modified in mice which had previously received the Actinomycin D treated cultures.

METHODS

To cultures containing 1.5×10^7 flagellates per ml, Actinomycin D was added and maintained for 3 days. The medium and the culturing conditions were as described by FERNANDES & CASTELLANI [4]. The final concentration of Actinomycin D was 1 μg per ml of medium. The culture was then centrifuged, the sediment washed in saline and resuspended in a volume of saline calculated to contain in 0.2 ml the number of flagellates to be injected in each mouse.

Each of 20 young adult mice weighting around 20 g received 0.2 ml of the flagellate suspension intraperitoneally once a week, during 3 weeks, the number of flagellates increasing from 10^6 in the last inoculation to 10^7 and 3×10^8 in the 2 subsequent ones. Eighteen days after the last dose, 10 mice of this group were injected intraperitoneally with 200,000 virulent blood forms of the parasite Y strain (SILVA et al.[11]). At the same time a control group of 10 clean mice of the same weight received a similar inoculum.

Twelve days after the virulent inoculation parasitemia was recorded for all animals as previously described (FERNANDES et al.[5]). On the next day the only surviving and very ill animal of the control (non protected) group and one animal taken at random from each of the other two groups were killed for the pathology studies.

On the 87th and 238th days after the last immunizing inoculation, one mouse was taken at random from each of the immunized groups, having or not received the virulent inoculation, and sacrificed.

Hearts of the killed animals were opened longitudinally to permit examination of the four cavities. Colons were opened longitudinally, washed and twisted in spiral so that the whole lenght of the organ could be sectioned. Fixation was in 10% neutral formalin solution for 6 days. Twenty sections 6 micra thick were obtained from each organ and stained by haematoxylin-eosin.

RESULTS

After the inoculations with Actinomycin D-treated flagellates, no blood parasites were detected either by careful direct examination or through xenodiagnosis. Also, no intracellular parasites or lesions were found in mice killed for tissue examination (Table I and Fig. 1).

Fig. 1 — Mouse heart ventricle 13 days after inoculation with 3×10^8 forms of *T. cruzi* cultivated during 3 days in a medium containing Actinomycin D (see methods). Observe the absence of parasitism as well as the normal aspect of the organ. H.E. 80×.

TABLE I

Protective effect of *Trypanosoma cruzi* preincubated with Actinomycin against a virulent strain. Results refer to examination made on day 3 after the last injection of the Actinomycin D treated flagellates and day 13 after the inoculation of the virulent strain

Inoculation	Concentration of the parasite in			Percentage of survivor***
	Blood*	Ventricle**	Auricle**	
Attenuated flagellates (control)	0	0	0	100
Attenuated flagellates + virulent strain	10 ± 6	25	18	100
Virulent strain	500 ± 60	417	488	0

* Blood parasites expressed as the number of parasite in 50 microscopic fields (×280) except when no parasite was found when 4,000 microscopic fields were examined.

** The parasitism in the ventricle and auricle are expressed as the number of *leishmania* nid in the whole microslide.

*** All survivors were killed 12 months after the inoculation of the virulent forms.

Following inoculation with virulent trypanosomes, both the protected and the control mice showed parasitemia which was, however, much lower in the 1st group. Table I shows these results as well as data on the surviving time. As for parasites and lesions in tissues for the 2 groups, results were as follows:

1) Mouse having received only the virulent flagellates (the only survivor of the control group on the 13th day).

Heart, striated and smooth muscles heavily parasitized, with leishmania nidi in every evolutive stage. When these nidi were intact there was no inflammatory reaction, but around ruptured nidi an intense, lymphohistiocytic infiltration was observed. These inflammatory processes were preferentially localized in the auricle, followed by the ventricle base and subepicardiac region of the right ventricle. In Table I and Fig. 2 the abundance of the myocardium parasitism can be appreciated.

2) Mice having received inoculations of the Actinomycin D-treated flagellates followed by challenge with the virulent forms: a) Animal killed on the 13th day after the virulent inoculation. The number of leishmania nidi was much lower than in the control mouse (Table I) but a much more intense inflammatory process was seen over the whole ventricle and even in the auricle

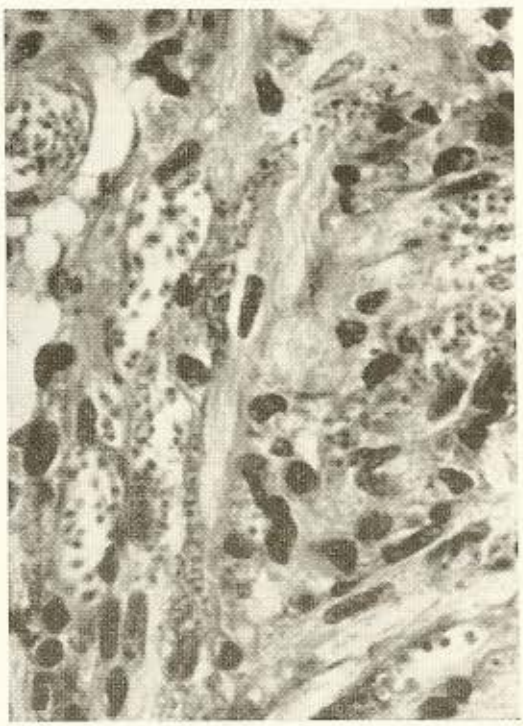

Fig. 2 — Mouse heart ventricle, 13 days after the inoculation of 2×10^5 virulent blood forms of *T. cruzi*. Observe the heavy intracellular parasitism without inflammatory reaction. H.E. 400×.

Fig. 3 — Heart ventricle of a mouse that was inoculated with 3×10^8 forms of *T. cruzi* cultivated in the presence of Actinomycin D, followed by a challenge dose of 2×10^5 virulent forms (see methods). The animal was killed 13 days after the inoculation of the virulent forms. H.E. 80×.

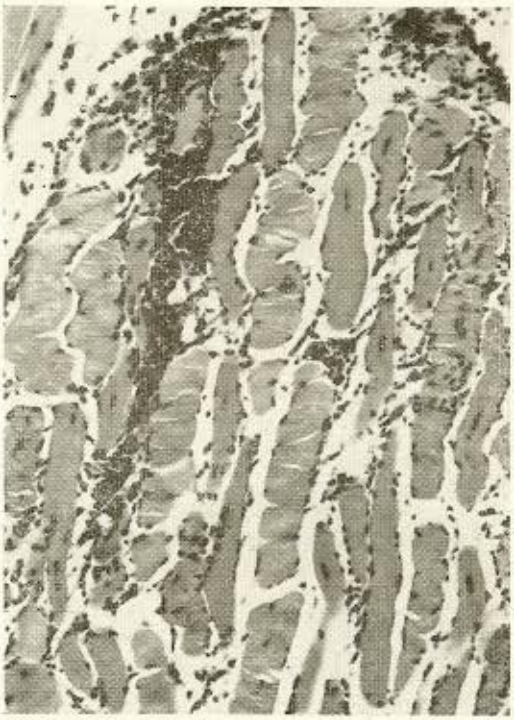

Fig. 4 — Striated muscle of the same mouse of Fig. 3. Observe the intense inflammation without parasites. H.E. 400×.

(Fig. 3). Skeletal muscles showed few parasites but an intense myositis (Fig. 4). The colon had few leishmania nidi and mild myositis. The Auerbach plexuses showed inflammation. b) Animal killed on the 69th day after the virulent inoculation (on the 87th day after the last immunizing inoculation). There was a remarkable change from the previous picture: the intracellular parasitism was conspicuously down, since in all sections of the ventricle only one leishmania nidus and a discrete interstitial inflammation were found (Fig. 5). The inflammatory process was chiefly located in the subepicardiac and subendocardiac regions of the right ventricle, mainly close to its base. The skeletal muscle presented a discrete inflammation. There was partial lesion of the neurons of the Auerbach plexus and an inflammatory process of the muscular layer of the colon. c) Animal killed on the 220th day after the virulent inoculation (on the 238th day after the last immunizing inoculation). The colon and skeletal muscle appeared normal. A discrete inflammatory process (lymph-histiocytic type and perivascular) and some elongated cells around a muscular necrotic area were found in the ventricle (Fig. 6).

DISCUSSION

The high degree of immunity developed by mice submitted to our process of protection was demonstrated by the hypersensitivity of their tissues to *T. cruzi* materials, i.e., by the intensity of inflammatory reactions, followed by inflammatory oedema and fiber dissociation in spite of a low intracellular parasitism. The tissue reaction seemed even more marked on the 69th day after the virulent inoculation, a picture similar to the immune reaction described by BRITO [1].

Fig. 5 — Heart ventricle of a mouse on the 69th day after the challenge dose of virulent forms of *T. cruzi*. The animal had been protected by previous inoculation of Actinomycin treated flagellates as described under Fig. 3. Note the inflammatory reaction without intracellular parasites. H.E. 80×.

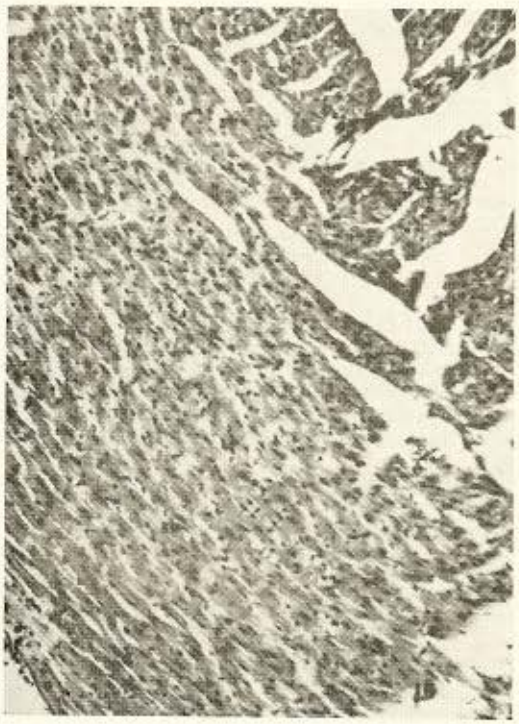

Fig. 6 — As described under Fig. 5, with the exception that the animal was killed 220 days after inoculation of the virulent forms. H.E. 80×.

However, all the inflammatory reactions subsided and, after 7 months, the organs presented a normal structure, except for a very discrete juxtavascular inflammatory infiltrate. A probable process of hypersensitivity as described by OKUMURA et al.[8] has been found once, accompanying a lesion of the pulmonary artery.

RESUMO

Histopatologia do coração e músculos em camundongos imunizados contra o Trypanosoma cruzi

Lotes de camundongos foram inoculados com elevado número de *T. cruzi* da cepa Y, flagelados vivos que tinham sido cultivados por 3 dias em presença de Actinomicina D. Observamos nestas condições sobrevivência de 100% e ausência de parasitas no sangue e tecidos dos animais inoculados.

Quando êstes animais recebiam uma inoculação de formas sanguícolas virulentas, ocorria 100% de sobrevivência enquanto morriam todos os seus contrôles, isto é, todos os animais que receberam como inóculo apenas os flagelados das formas sanguícolas virulentas (não imunizados). Observamos também parasitismo muito menor entre os animais imunizados que nos contrôles, mas a reação inflamatória era mais intensa entre os animais imunizados. Esta reação quase desaparece, permanecendo apenas insignificantes lesões observadas em exames posteriores.

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