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RAT ADRENAL GLAND IN EXPERIMENTAL AMERICAN TRYPANOSOMIASIS: IMMUNOCYTOCHEMICAL STUDY OF TISSUE PARASITISM[1]

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SUMMARY

The rat adrenal gland is poorly parasitized during the experimental infection with Y strain of *Trypanosoma cruzi.* In both cortical and medullary regions, the parasitism peaked at day 10 and was characterized by the predominance of single amastigotes over nests containing 2 or more parasites. After day 10 of infection, the tissue parasitism dropped rapidly to become practically null at day 32 of infection. In cortical tissue, the amastigotes occurred mainly inside the endocrine cells. In the medulla, they found mainly in glial cells and non-identified stromal cells. In both cortex and medulla, inflammatory processes were found till day 20 of infection. Our data do not support the hypothesis that a corticoid rich environment would favor *T. cruzi* parasitism in adrenal medulla.

RÉSUMÉ : **La glande surrénale chez le rat au cours de la trypanosomiase américaine expérimentale : étude immunocytochimique du parasitisme tissulaire.**

La glande surrénale est peu parasitée au cours de l'infection expérimentale avec la souche Y de *Trypanosoma cruzi.* Dans le cortex comme dans la médullaire, le parasitisme atteint son maximum le 10e jour d'infection et est caractérisé par une prédominance d'amastigotes isolés sur les sites contenant deux ou plusieurs parasites. Après le 10e jour, le nombre de parasites dans la glande tombe rapidement et devient pratiquement nul après 32 jours d'infection. Dans le cortex, les parasites sont localisés principalement dans les cellules endocrines. Dans la médullaire, ils ont été trouvés principalement dans les cellules névrogliques et dans des cellules non identifiées du stroma. Des processus inflammatoires ont été observés jusqu'au 20e jour d'infection. Nos résultats ne favorisent pas l'hypothèse d'une augmentation du parasitisme par un environnement riche en corticoïdes.

The involvement of the adrenal glands in the human Chagas' disease has been studied in autopsic cases with cardiac and/or digestive complications. In 30 % of the patients examined, amastigote nests were found in muscle cells of the adrenal central vein, an incidence higher than that obtained for the myocardium and other tissues (Almeida *et al.,* 1981; Barbosa and Andrade, 1984). A corticoid rich environment has been thought as a possible reason for this preferential location (Almeida *et al.,* 1981) and a correlation between the adrenal central vein parasitism and chronic cardiac lesions was postulated (Teixeira *et al.,* 1986; Teixeira, 1988). On the other hand, the absence of parasites in adrenal cortical and medullary regions seems to be a rule in chronic chagasic patients (Vianna, 1911; Mayer, 1954; Teixeira, 1988). However, parasites were found in the adrenal cortex of an autopsic eight-month-old child who died during the acute phase of Chagas' disease (Crowell, 1923).

The involvement of the adrenal gland during the experimental disease has been neglected and only a few studies on the acute phase report the presence of amastigotes inside the mouse and rat glands (Taliaferro and Pizzi, 1955; Shoemaker and Hoffman, 1974; Melo and Brener, 1978). However, a careful localization of the parasites was not aimed.

The rat has been proved to be a useful experimental model for studying the involvement of the autonomic nervous system in Chagas' disease as reviewed elsewhere (Machado *et al.,* 1987; Camargos and Machado, 1988; Machado and Ribeiro, 1989). Using this rat model, we study now the time course of the parasitism and inflammatory infitrate in adrenal tissues, especially in the medulla, using

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a specific and sensitive immunocytochemical method for parasite identification.

MATERIAL AND METHODS

Holtzman rats of both sexes aged 27-30 days were inoculated intraperitoneally, with 0.15 ml of mouse blood containing about 300,000 trypomastigotes of *Trypanosoma cruzi,* Y strain. Littermates were kept as control. Infection was confirmed by fresh blood examination at days 6 and 10 after inoculation. Fifteen control and 25 infected animals were killed under chloral hydrate anesthesia (300 mg/kg of body weight) at day 6, 10, 13, 20 and 32 of infection. The right adrenal was fixed in 25 % Bouin fluid (20 hours) and 10 % formalin (4 hours) in sequence, embedded in paraplast and serially sectioned at 5 μm.

Tissue parasitism was studied at every 5th section containing both cortex and medulla after staining with a peroxidase-antiperoxidase method (PAP) followed by 0.25 % haematoxylin (Sternberger, 1986). Rabbit antiserum against *T. cruzi,* CL strain, was used at 1 : 1,000 dilution (Barbosa, 1985). The areas occupied by cortical and medullary tissues were planimetrically determined in each section used for *T. cruzi* amastigote counting. The drawings for planimetry were obtained in the screen of a Zeiss Ultraphot II microscope at a magnification of × 54.5. After parasit counting, some sections were restained with haematoxylin and eosin for a better identification of the parasitized cell.

The inflammatory infiltrate was studied in sections stained with haematoxylin and eosin.

RESULTS

The PAP method allowed easy identification of *T. cruzi* amastigotes in adrenal tissues. Small nests and even single amastigotes were easily detected. In both cortical and medullary regions, single amastigotes by far outnumbered the nests containing 2 or more amastigotes at all periods of infection *(table I and II).* Regardless the adrenal region, the values for single amastigotes peaked at day 10 and a significant drop was already present at day 13 of infection. At day 32, they were hardly found.

In the cortex, amastigote nests were found to be more frequent at day 6, 10 and 13, and extremely rare at day 32 of infection *(table I).* In the medulla, the values were about the same from day 6 to day 20, but no nests were found at day 32 *(table II).* Most amastigote nests until day 13 an all nests at days 20 and 32 contained only 2 to 3 amastigotes *(tables III and IV).* Nests with several amastigotes (more than 10) were observed only at day 6 *(fig. 1 and 2).*

At each period of infection, no statistical differences was found between the values for cortical and medullary tissues regarding both single amastigotes and nests per unit of area.

In the cortex, most parasites were seen inside endocrine cells *(fig. 1)* but in the medulla, they were found mainly

TABLE I. — Trypanosoma cruzi *(Y strain) parasitism in the rat adrenal cortex: single amastigotes and amastigote nests/mm² of tissue at different periods of infection, with 5 rats in each group.*

Days after inoculation	*Cortical area (mm²)* *	*Single amastigotes/mm²*		*Amastigote nests/mm²*	
		Mean **	*Range*	*Mean* **	*Range*
6	3.94 ± 0.29	7.46 (*a*)	1.74-22.61	4.41 (*a*)	1.82-11.3
10	4.16 ± 0.74	15.13 (*b*)	9.61-20.80	1.80 (*a*)	0.84- 4.00
13	3.86 ± 0.81	5.04 (*c, a*)	3.71- 8.53	0.65 (*a*)	0.09- 1.27
20	4.66 ± 0.91	3.15 (*c*)	0.65- 6.78	0.26 (*b*)	0.13- 0.58
32	4.67 ± 0.80	0.11 (*d*)	0- 0.27	0.006 (*c*)	0-0.031

* Mean ± SD.

** In each column, mean values with different letters (*a, b, c* or *d*) are significantly different from each other ($P < 0.05$ or less by analysis of variance).

TABLE II. — Trypanosoma cruzi *(Y strain) parasitism in the rat adrenal medulla: single amastigotes and amastigote nests/mm² of tissue at different periods of infection, with 5 rats in each group.*

Days after inoculation	*Medullary area (mm²)* *	*Single amastigotes/mm²*		*Amastigote nests/mm²*	
		Mean **	*Range*	*Mean* **	*Range*
6	0.61 ± 0.16	3.80 (*a*)	0.84-11.50	1.26 (*a*)	0.16-3.99
10	0.49 ± 0.16	12.90 (*b*)	6.28-24.05	2.04 (*a*)	0.96-2.86
13	0.44 ± 0.08	2.54 (*c, a*)	1.08- 4.70	0.72 (*a*)	0.32-1.25
20	0.55 ± 0.22	1.73 (*c, a*)	0.35- 3.07	0.50 (*a*)	0.07-1.93
32	0.63 ± 0.19	0.11 (*d*)	0- 0.42	0	

* Mean ± SD.

** In each column, mean values with different letters (*a, b, c* and *d*) are significantly different from each other ($P < 0.05$ or less by analysis of variance).

TABLE III. — *Frequency of amastigote nests containing different numbers of parasites in the adrenal cortex of rats infected with* Trypanosoma cruzi. *The values are percentages and in parenthesis are the total nests counted in each class. Five animals in each period of infection.*

Days after inoculation	*Number of amastigotes/nest*				
	2-3	*4-5*	*6-9*	*10 or more*	*Total*
6	69.05 (886)	16.99 (218)	9.5 (120)	4.44 (57)	100 (1,283)
10	92.1 (556)	6.3 (38)	1.6 (10)	0	100 (604)
13	95.8 (229)	3.8 (9)	0.4 (1)	0	100 (239)
20	100.0 (48)	0	0	0	100 (48)
32	100.0 (2)	0	0	0	100 (2)

TABLE IV. — *Frequency of amastigote nests containing different numbers of parasites in the adrenal medulla of rats infected with* Trypanosoma cruzi. *The values are percentages and in parenthesis are the total nests counted in each class. Five animals in each period of infection.*

Days after inoculation	*Number of amastigotes/nest*				
	2-3	*4-5*	*6-9*	*10 or more*	*Total*
6	66.7 (32)	14.6 (7)	10.4 (5)	8.3 (4)	100 (48)
10	89.7 (70)	7.7 (6)	2.6 (2)	0	100 (78)
13	90.4 (19)	4.8 (1)	4.8 (1)	0	100 (21)
20	100.0 (7)	0	0	0	100 (7)
32	0	0	0	0	0

in glial cells around neuronal perikaria *(fig. 3)*, and in non-identified cells laying in the connective stroma. These cells were most probably Schwann cells or macrophages *(fig. 2 and 3)*. Some amastigotes were also found inside the medullary reticular layer cells. Parasitism of chromaffin cells was rarely observed *(fig. 2)* and sometimes dubious since the amastigotes could well be inside pheochromocyte satellite cells. Medullary neurons *(fig. 3)* were never seen parasitized. In the rat, the adrenal vein is a thin walled vessel even in its course through the cortical tissue and adrenal capsule. In this vein, only few smooth muscle cells were identified, especially along its cortical route. Only one amastigote nest was found in the wall of the medullary central vein (one animal at day 6 of infection presenting the highest tissue parasitism) *(fig. 4)*. Single amastigote and small nests were also observed close to the wall of the central

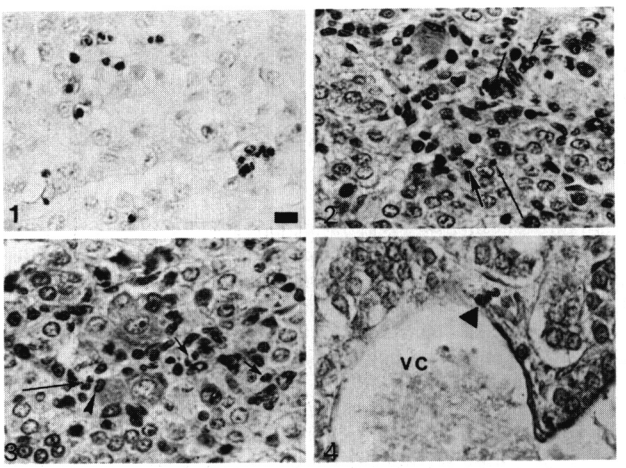

PLATE I *(figs. 1-4)*. — Peroxidase-antiperoxidase (PAP) immunostaining of amastigotes in adrenal sections of *T. cruzi*-infected rat killed 6 days after inoculation. *Fig. 1:* single amastigotes, nests containing 2 amastigotes and one nest with several amastigotes in the reticular layer. PAP plus 0.25 % haematoxylin. *Fig. 2:* amastigote nest in stromal cells of the adrenal medulla (short arrows) and single amastigote probably inside a chromafin cell (long arrow). A nest with 2 amastigotes (white arrow) are probably in a stromal cell. PAP plus haematoxylin and eosin. *Fig. 3* nest with 3 amastigotes inside a neuron satellite cell (long arrow); the nucleus of this glial cells is indicated (arrow head). Other nests with 2-3 amastigotes are seen in stromal cells (short arrows). *Fig. 4:* three amastigotes are seen in the wall of the adrenal central vein (CV) nearby two elongated nuclei (triangle). Bar = 10 μm (for all figures).

vein at its capsular segment at day 6 (3 animals) and 13 (1 animal) of infection. The adrenal capsule was excluded from our quantitative studies although presenting single amastigotes and nests in all infected animals till day 13.

At days 10 and 13 of infection in contrast with control animals *(fig. 5)*, all glands of *T. cruzi*-infected animals presented some inflammatory cellular exudate in the capsule, cortex and medulla as discrete, diffuse or focal accumulation of neutrophils and mononuclear cells *(fig. 6, 7, 8 and 9)*. Only very discrete inflammatory processes were found in 2 and 3 infected animals, respectively at days 6 and 20 of infection but at day 32 no abnormality was found *(fig. 10)*.

DISCUSSION

Trypanosoma cruzi amastigotes could be found in adrenal tissues at all periods of infection, with a clear peak around day 10 followed by a rapid fall. By comparing the present results with those previously obtained in the heart of *T. cruzi*-infected rats (Machado and Ribeiro, 1989), the adrenal gland can be regarded as poorly parasitized. In *T. cruzi*-infected animals (Tulahuen and Brasil strains), the adrenal cortex was shown to be only slightly parasitized

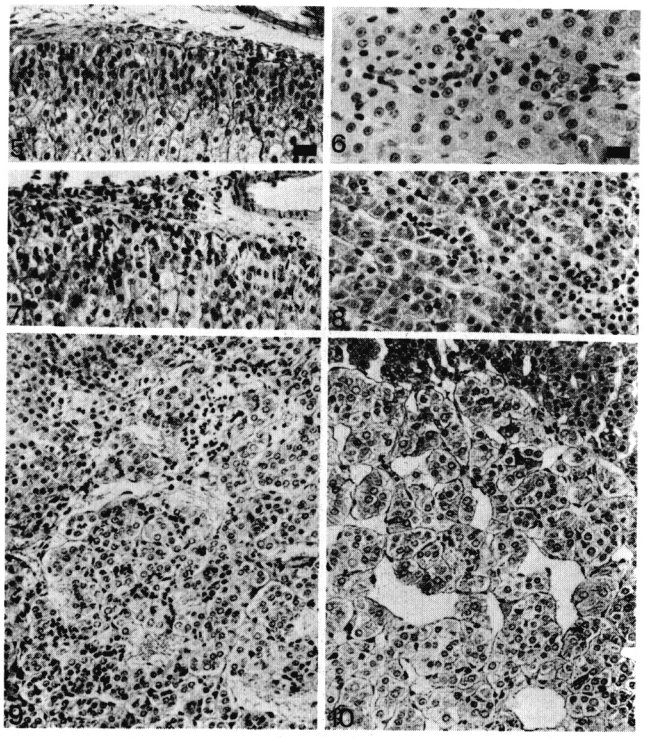

PLATE II *(figs. 5-10).* — Sections of adrenal glands of control *(fig. 5)* and *T. cruzi* infected rats killed 13 days *(fig. 6-9)* and 32 days after inoculation. Haematoxylin and eosin. *Fig. 5:* normal aspect of the adrenal capsule and cortical tissue. *Fig. 6:* focal inflammatory exudate in the capsule. *Fig. 7 and 8:* focal and discrete inflammatory infiltration in the glomerular and reticular layers respectively. *Fig. 9:* inflammatory processes in the medullary region. *Fig. 10:* normal aspects of the adrenal medulla found at day 32 of infection. Bar = 24 μm (for all figures, except for *fig. 6,* where it is 15 μm).

in rats and not parasitized at all in hamsters and guinea-pig (Shoemaker and Hoffman, 1974). In contrast the adrenal cortex of C_3H mice was heavily parasitized suggesting the presence of some attractive factor to the *T. cruzi* (Shoemaker and Hoffman, 1974). No comparative study had been undertaken on the adrenal medulla.

Chromaffin cells are embryological and functionally related to sympathetic neurons. Neurons, especially the parasympathetic ones, can be destroyed during the acute infection by *T. cruzi,* but they are seldom parasitized (Köberle, 1970; Tafuri, 1979). In the sympathetic neurons of *T. cruzi*-infected rats no parasitism or degenerative lesions were found (Camargos and Machado, 1988). The chromaffin cells are now shown to be also rarely parasitized.

According to our present results the *T. cruzi* parasitism in adrenal tissues is characterized by very small nests since single amastigotes profiles predominate even at the peak of parasitism. The identification of these single amastigotes was possible only in PAP-stained sections. Thus the complete absence of parasites in tissues of chronic chagasic patients or infected animals stained by routine histological methods should be taken with reserve as pointed out elsewhere (Barbosa *et al.,* 1986).

In rats, the adrenal medulla environment apparently is not suitable for growth of amastigotes as suggested for chronic patients (Almeida *et al.,* 1981). In fact, the few amastigotes found inside the various medullary cell types remained single or constituted tiny nests which number fell rapidly to become null at day 32 of infection.

Both cortical and medullary cell types were shown to be not a good target for *T. cruzi* penetration. Several evidences are pointing to the importance of specific surface receptors in mammal cells for ligands of the parasite membrane (Zingales and Colli, 1985; Schenkman *et al.,* 1988). The interaction of parasite molecules with these host cell surface receptors would be responsible for tissue tropism. The surface peculiarities of the longitudinal muscle coat of human adrenal central vein should be regarded as a possible factor to explain the high *T. cruzi* parasitism of this vessel in chronic chagasic patients. It is interesting to remember that smooth muscle heterogeneity occurs in relation to *T. cruzi* tropism since in mice the muscle of the bladder is much more parasitized than those of the stomach and intestines (Bice and Zeledon, 1970; Melo and Brener, 1978).

Anyway, the high corticoid level present in the medullary region could not be an enough explanation for the high parasitism of the human adrenal central vein.

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