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ACUTE CHAGASIC CARDIOPATHY PRODUCED BY A STRAIN OF *TRYPANOSOMA CRUZI* (TYPE I) IN AN EXPERIMENTAL MODEL

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SUMMARY

We have carried out a study of the tissular tropism of the strain Y of *T. cruzi* considering that the different strains of *T. cruzi* show a great instability in their pathogenic properties with the aim of proving that the classifications of his parasite based in its tissular tropism are not reliable given that these characteristics are subject to change as can be seen by comparing our results with those of other authors.

In our study, the Y. strain of *T. cruzi* shows a strong pancytotropic action, specially with marked cardiotropic aspects. The lack of affectation in the lymphatic ganglion and the small proportion of spleen lesions (8 %) as well as the absence of pseudocysts at this level is surprising in a strain which was described as eminently reticulotropic. Our data show that this strain produces cardiac pseudocysts without lesions of the parasitized muscle fiber.

The above mentioned data evidence that the biological behavior of a strain and specially its tissular tropism are susceptible to present variations with time.

RÉSUMÉ : **Cardiopathie chagasique aiguë dans un modèle expérimental produit par une souche de *T. cruzi* (type 1).**

Étude du tropisme tissulaire de la souche Y de T. cruzi et comparaison des résultats avec les données des autres auteurs.

La souche Y présente un grand pancytotropisme avec un cardiotropisme très marqué. L'absence de localisation ganglionnaire, le fait qu'il y a seulement 8 % de lésions spléniques, l'absence de pseudokystes à ce niveau sont inattendus, pour une souche qui a été décrite comme éminemment réticulotropique.

Nos données montrent que cette souche produit des pseudokystes dans le cœur sans lésions de la cellule parasitée. Les données mentionnées prouvent que le comportement biologique et spécialement son tropisme tissulaire peuvent changer avec le temps.

INTRODUCTION

The tissular affection by *T. cruzi* occurs *in vivo* following a chronological evolution (Vollerthun, 1980). The initial alteration affects the mononuclear phagocytic system (MFS) followed by the invasion of the remaining organs (Anselmi and Moleiro, 1974). However, the parasite can be seen in all the tissues although the most frequently affected one is the cardiac tissue (Fife, 1977).

The goal of this study was the investigation of serial sections of heart, brain, liver, spleen, skeletal muscle, lymphatic ganglion and colon of mice infected by *T. cruzi* (Y strain) which were sacrified at various intervals during the acute period of the infection in order to show that the tissular tropism of *T. cruzi* is not a reliable mean for its classification.

We have chosen the Y strain for it was first classified as belonging to the type I and being eminently reticulotropic (Andrade, 1974) and was later described as being able to produce pseudocysts in cardiac fibers as soon as the eleventh day post-infection (Andrade and Freitas, 1987), being therefore a good model to show variations in tissular tropism.

Some changes concerning the virulence and tissular tropism of *T. cruzi* were described in early investigations on this subject (Vianna, 1911). These changes were attributed both to host and parasite characteristics (Fife, 1977; Postan *et al.*, 1987).

Based upon these data, we attempted to perform a more detailed study about the tissular behavior of strains which were preserved in the laboratory during a long period of time to show that this tropism may become altered in comparison with the original description of the isolated strain.

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MATERIAL AND METHODS

1 — Experimentation animals

We have used sets of mice *Mus musculus* of the Swiss (OF_1 IOPS Caw) strain, which were maintained inbred in the animalarium of the School of Medicine of the University Autónoma of Madrid under standard environmental conditions.

2 — Inoculum

The mice were inoculated with trypomastigotes from the Y strain, isolated by Pereira das Freitas in 1950 in São Paulo, which was obtained from the Faculty of Pharmacy of the University Complutense of Madrid in October 1987 and since then has been maintained in our laboratory by passes only in the above mentioned mice.

For our study we have used doses of 10^5 trypomastigotes intraperitoneally to inoculate sets of three mice.

In addition, a set of ten uninfected mice was used as a control set in order to discard non-specific inflammatory infiltrates.

3 — Histopathological study

The sets of three mice were sacrified at different intervals in the acute stage in the 7th, 12th, 17th, 20th and 30th day postinfection (p. i.) using ethyl ether.

Heart, brain, liver, spleen, lymphatic ganglion, skeletal muscle and colon were separated and introduced in 10 % saline-formalin solution for a period of 15 days.

The organs were subjected to a macroscopical examination before cutting and including them in paraffin (Morales *et al.*, 1987). Then, sections of 5 μm were made being placed in slides and stained with H/E.

The sections were examined under the microscope and the presence of pseudocysts as well as the amount of the affected tissue were observed. In the study of the cardiac tissue we also noted the percentage of affectation due to each type of lesion in each cardiac cavity.

Due to the possible existence of non-specific cardiac lesions, ten non infected mice were sacrified at the same intervals using the same method of sacrifice and heart extraction. After being cut and fixed, the sections were stained with green-methyl-pironine in order to establish the existence of an activation of lymphocytes. This process makes visible the existence of a lymphocytic activation through the red staining of the cytoplasmic ribosomes of the lymphocytes indicating all the non-specific inflammatory processes in which *T. cruzi* cannot be implicated.

Finally, we also carried an ultrastructural study of various sections of cardiac tissues which were selectioned by the presence of pseudocysts through optical microscopy in order to establish the type of lesions produced at this lebel by a strain formerly described as reticulotropic.

RESULTS

Macroscopical examination

The macroscopical alterations were observed only at the cardiac level. The hearts of mice which were sacrified at 23, 26 and 28 days postinfection, showed the most important levels of alteration.

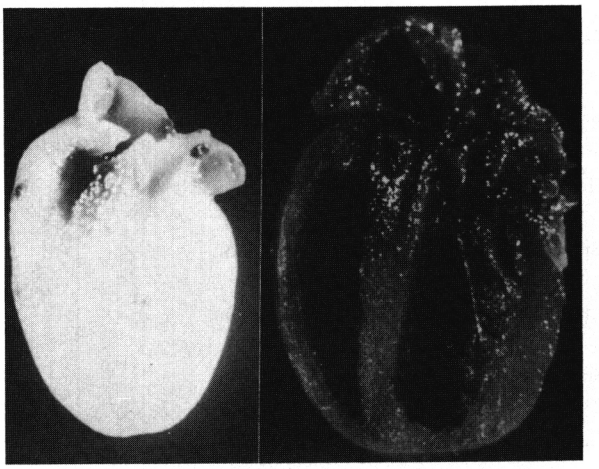

Fig. 1. — Macroscopic study. White mouse heart without infecting (left) and heart with acute chagasic miocarditis (right). Y strain. (X 5).

These hearts presented a homogeneous grayish coloration of the tissue. After cutting the four cardiac cavities, we found a dilatation and a slenderness of the free wall of both ventricular cavities, presenting a dilated miocardial pattern *(fig. 1)*.

Microscopical examination

Cardiac tissue

The existence of miocarditis was demonstrated dating from the beginning of the study in the 7th day postinfection. The inflammatory focuses were more important in mice which were sacrified between the 12th and the 22nd day, presenting an affected area of more than 50 % of the examined tissue *(table I)*. The inflammatory affectation in heart cavities was more important in the left ventricle (56 %) and less important in the right ventricle (40 %). In the right auricle the percentage of inflammation was of 30 % and in the left one of 20 % *(table II)*.

The percentage of mice inoculated with the Y strain showing miocarditis was of 67 % and half of them presented inflammatory areas covering more than 50 % of the examined tissue *(table III)*.

Cardiac pseudocysts were visualized during all the studied period until the 22nd day postinfection but in the 30th day they were not visible and the inflammatory lesions were also less important. The percentage of mice presenting pseudocysts at the miocardial level was of 70 % *(table IV)*. In 56 % of all the examined animals, the affected areas occupied more than 50 % of the examined tissue *(table III)*. The presence of the parasite was more frequent in the left ventricle in a percentage of 66 % of the examined sections. In a percentage of 60 % we found pseudocysts in the right ventricle, and in 20 % we also

TABLE I. — *Histopathological study of* T. cruzi *(Y. Strain).*

Postinfection days		*7*	*12*	*17*	*22*	*30*
Parasitaemia	$\overline{X}$	4,16	10,33	56,50	3,25	0,16
	DT	7,80	11,29	104,31	3,94	0,40
Heart	Inflammation	+	+ + +	+ + +	+ + +	+
	Pseudocysts	+ + +	+ + +	+ + +	+ + +	–
Brain	Inflammation	–	–	–	+	+
	Pseudocysts	–	–	+ + +	–	+
Liver	Inflammation	+	+	+	+	+
	Pseudocysts	+	+ +	–	+	–
Spleen	Inflammation	–	–	–	–	–
	Pseudocysts	–	–	–	–	–
Ganglion	Inflammation	–	–	–	–	–
	Pseudocysts	–	–	–	–	–
Skeletal muscle	Inflammation	–	+	+	+	+
	Pseudocysts	–	+	+	–	–
Colon	Inflammation	–	–	–	+	+
	Pseudocysts	–	–	+	–	–

+ < 25 % of the affected area; + + between 25 and 50 %; + + + > 50 % of the affected area.

TABLE II. — T. cruzi *(Y Strain): percentage of mice presenting lessions in the distinct cardiac cavities.*

Cardiac cavities	*Inflammation*	*Pseudocysts*
Right auricle	30	20
Left auricle	20	16
Right ventricle	40	60
Left ventricle	56	66

TABLE III. — T. cruzi *(Y. Strain): percentage of mice presenting inflammatory processes and pseudocysts in conection with the affected tissular superficy.*

	Inflammation			*Pseudocysts*		
Tissues	+	+ +	+ + +	+	+ +	+ + +
Heart	16	16	35	12	3	56
Brain	38	–	–	15	–	15
Liver	92	–	–	21	10	–
Spleen	8	–	–	–	–	–
Limphatic ganglion	–	–	–	–	–	–
Skeletal muscle	54	–	–	23	–	–
Colon	31	–	–	8	–	–

+ < 25 % of the affected tissular area; + + : between 25 % and 50 % of the affected tissular area; + + + > 50 % of the affected tissular area.

TABLE IV. — T. cruzi *(Y. Strain): comparative percentage of mice presenting alterations in the distinct studied tissues.*

Tissues	*Inflammation*	*Pseudocysts*
Heart	66,75	70,75
Brain	38,46	30,76
Liver	92,30	30,76
Spleen	7,69	—
Limphatic ganglion	–	–
Skeletal muscle	53,84	23,07
Colon	30,76	7,69

observed lesions at the level of the intracardiac-conduction system as well as intracardiac calcification and necrosis in 18 % of the examined mice.

We haven't been able to establish any correlation between the level of the parasitaemia and the number of the observed pseudocysts.

Brain tissue

Lymphocytary Infiltration focuses were observed at the end of the acute period of infection, although in all the cases their extension represented less than 25 % of the examined tissue *(table III)*.

Brain pseudocysts were observed in 30 % of mice, all of them at the 17th day p. i.

Liver tissue

Inflammatory focuses were observed during all the acute stage of infection, although the affected areas occupied less than 25 % of the examined tissue *(table I)*. These lesions were observed in 92 % of the examined mice *(table IV)*. Close to the multifocal infiltrates of round cells, we observed in many cases multifocal necrosis focuses. A percentage of 9 % of the mice showed granulomas and other 9 % a hyperplasy of the Kuppfer's cells.

Pseudocysts were present in 31 % of the studied animals occupying areas of less than 50 % in all the cases *(table III)*.

Spleen tissue

The inflammatory lesions were present in 8 % of the studied mice occupying areas of less than 25 % of the examined tissue *(table III)*. The pseudocystic lesions were absent in this tissue.

Lymphatic ganglion tissue

We didn't find any inflammatory lesions nor pseudocysts at this level.

Skeletal muscle tissue

The inflammatory lesions appeared in the observed tissues dating from the 12th day p. i. being present until the end of the acute stage *(table I)*. They were obvious in more than 54 % of the studied mice. In all the cases their extension was less than 25 % of the examined tissue *(table III)*. In 10 % of the cases granulomas were evident in the striated musculature.

Together with the miositis, we also observed inflammatory infiltrations and pseudocysts in the perimuscular adipose tissue.

We could evidence the presence of pseudocysts in the skeletal musculature of 23 % of the examined mice *(table IV)*, occupying in all the cases areas of less than 25 % of the studied tissue *(table III)*.

Colon

31 % of the examined mice presented inflammatory focuses in the muscular layer of the intestine *(table IV)*. These focuses were visible dating from the 22nd day p. i. *(table I)* and in all the cases the affected area extended less than 25 % *(table III)*.

Pseudocysts were also present in 8 % of the studied mice being the affected area less than 25 % *(table III)*.

Study with the green methyl-pironine staining

This staining method was used in a control group of 10 non infected mice due to the presence in these mice

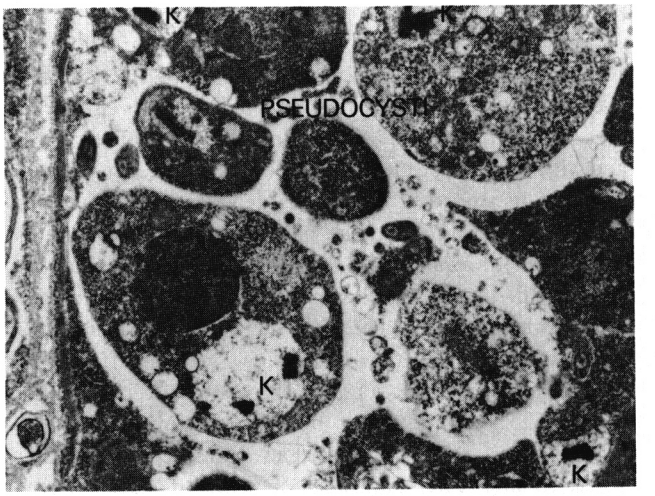

FIG. 2. — Transmission electron micrograph of cardiac tissue, with amastigote forms into the cardiac fibre. (X 8.200). K: kinetoplast. 1 μm.

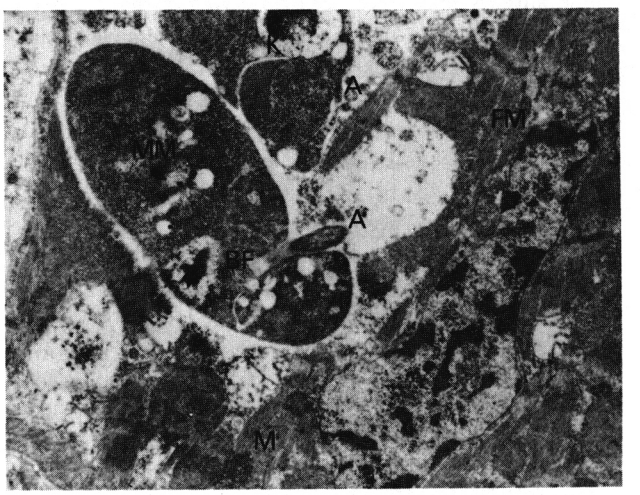

FIG. 3. — Transmission electron micrograph of miocardium, with a micromastigote (central zone) and amastigote form (top zone). (X 8.200). MM: micromastigote form; A : amastigote form; FM : muscular fibre; BF : blefaroplast; F : flagellum; M : mitochondria. 1 μm.

of inflammatory infiltrations of unknown origin. This method gave us the possibility of differentiating between a lymphocytic proliferation due to *T. cruzi* infection from a non specific one.

Ultrastructural study

This study has confirmed the presence of intracellular forms of *T. cruzi* without lesions in the cardiac fibers affected by the parasite *(fig. 2 and 3)*. The observed forms were only amastigotes which curiously showed from time to time a short free flagellum of 0,4 μm.

DISCUSSION

Changes in virulence and tissular tropism of *T. cruzi* have been described as early as 1911 by Vianna. It is well known that *T. cruzi* can modify its morphologic, histopathologic and virulence properties with time in order to be able to maintain its infectious properties depending upon factors both from the host and the parasite (Fife, 1977, Postan *et al.,* 1987). Morales *et al.* (1987) have given more importance to the host in the elaboration of chagasic pathology.

Andrade in 1974 has described the Y strain as being eminently reticulotropic (type I) showing a cardiac affectation in the more advanced stage of the acute infection; these data were later confirmed by Abrahamson (1983) and Pereira *et al.* (1987). The cardiac damage may be attributed to interstitial parasitism inducing muscular fibers degeneration without direct parasitism of the fibers themselves. Later, Andrade and Freitas (1987) using the same host described this same strain as producing pseudocysts inside the cardiac fibers as early as in the 10th day p. i. These authors may have been observing a change in tissular tropism of the Y strain of *T. cruzi.* We have studied the tropism of our stock of *T. cruzi,* strain Y in the same host, the Swiss mice strain, that these authors to check any possible change in tissular tropism due to the parasite which could make less reliable the classifications based on tropism.

In our experience, we have met inflammation in all the studied organs except the lymphatic ganglions, being greater the number of animals with inflammatory affectation at the hepatic level (92 %) and the cardiac level (67 %). The main observed lesion in the acute chagasic affectation was the interstitial infiltrate, fundamentally mononuclear.

The phase of the greatest mortality coincides with that of highest degree of myocarditis, being this one a sufficient cause to justify the death of the infected mice. We have demonstrated the existence of direct parasitism in cardiac fibers at as an early date as the 7th day p. i. being the areas of pseudocystic affectation greater than 50 % of the examined tissue in 71 % of the studied mice. The cardiac tissue shows the greatest number of pseudocysts of all the studied tissues. We emphasize the evidence of change in the biological behavior which occurs in this strain compared with Andrade's data (1974).

Although the most intense and frequent lesions in the chagasic myocarditis were previously described in the auricles (Fife, 1977; Andrade, 1985; Andrade and Freitas, 1987; Morales and Milei, 1987), in our study the major inflammatory as well as pseudocystic affectation were observed in the left ventricle.

The coexistence of amastigote nests with inflammatory infiltration was considered by Molina *et al.* (1987) as an argument supporting the role of the parasite in the elaboration of local inflammation, but in our case these two types of lesions coexisted in the same period of time, but not in the same area, therefore the independence of parasite and inflammatory infiltrates argues against a direct role of the parasite in these lesions.

We have not found any correlation between the parasitaemia and the number of cardiac pseudocysts in contrast with the report of Bice and Zeledon (1970) who found a complete correlation between them.

Many authors have mentioned the presence of pseudocysts of *T. cruzi* and various degenerative processes in muscular fibers (Anselmi and Moleiro, 1974; Andrade, 1976; Prata, 1976; Fife, 1977). The ultrastructural study demonstrated the presence of intracellular pseudocysts inside the muscular fibers without presence of observable lesions in them. This situation was also mentioned by Andrade and Freitas (1987) although other authors have mentioned the presence of pseudocysts of *T. cruzi* coupled with various degenerative processes in muscular fibers (Anselmi and Moleiro, 1974; Andrade, 1976; Prata, 1976; Fife, 1977). In our study, the pseudocysts also showed the presence of micromastigotes with a free flagellum of 0.4 μm as described by Dvorak 1976 and Fife 1977.

Since we have used in our experiments the same strain of rodent host previously used by the mentioned studies, and considering very improbable that this host had suffered modifications because of its standard stabulating conditions and that it has been maintained inbred, we consider that the changes observed in tissular tropism of the Y strain cannot be attributed to the host, and hence are most probably due to a change in the intrinsic characteristics of the parasite. This possibility has already been accepted by other authors (Viana, 1911; Fife, 1977; Postan *et al.,* 1987). Thus, similarity of the results of histopathologic studies of a given *T. cruzi* strain is achievable only when the periods of time between the studies are not very long.

The described changes in tissular tropism can be explained by a change in the genetic characteristics of the Y strain. These changes may have led to different properties among the Y strains maintained in different laboratories. Since the Y strain has not being cloned in these studies, it is possible that the Y strain is composed by a mixture of genotypes, whose composition is susceptible of variations with time, being the differences reported the result of different prevalences of some of them in the different studies.

The findings reported here prove that histopathologic studies can yield different results with the course of time, probably due to variations in the genetic properties of the strains. This means that these studies should be carried out on several experimental clones of defined genetic characteristics rather than on whole strains to be able to separate the possible contributions of genetic variation to these changes and reach a more exact knowledge of the biologic behavior of *T. cruzi.*

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