Int. J. Exp. Path. (1994), 75, 453–462

Trypanosoma cruzi: histopathology of endocrine system in immunocompromised mice

KATIA S. CALABRESE*, P. H. LAGRANGE† AND SYLVIO CELSO GONÇALVES DA COSTA*

**Laboratório de Imunomodulação, Departamento de Protozoologia, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil, and †Laboratoire Central de Microbiologie, Hôpital Saint-Louis, 1, avenue Claude Vellefaux 75475 Paris, Cedex 10, France*

Received for publication 3 August 1993
Accepted for publication 8 August 1994

Summary. Naturally immunocompromised athymic mice, neonatal mice and adult outbred OFI mice treated with the immunosuppressive agents cyclophosphamide (CY), dexamethasone (DM) and indomethacin (IM) were infected with trypomastigotes of *Trypanosoma cruzi* Y and CL strains. 10^4 parasites were used, except in the case of IM treatment, where mice received 10^3 trypomastigotes in one group and 10^5 in another. The course of parasitaemia, tissue distribution of amastigotes and time of mortality were compared with an infected thymus intact control group.

Neonate and indomethacin treated mice presented the same pattern of parasitaemia. Death occurred as early as 9–10 days after infection. A single dose of CY 200 mg/kg given 5 days after infection enhanced the parasitaemia and increased the number of parasites in the tissues. All groups were similar in terms of colonization of the endocrine system by parasites and the adrenals showed the highest density of amastigotes nests. The thyroid gland (analysed only in neonates) showed intense amastigote accumulation. Colonization of the ovary was observed with amastigotes in both the theca interna and in the stroma. The testes (also examined only in the neonate) showed that the interstitial cells, the tunica albuginea of the seminiferous tubules and the loose connective tissue were infected. Athymic nude mice showed the most intense parasite colonization of the islets of Langerhans.

Keywords: Trypanosoma cruzi, endocrine glands, immunocompromised mice, cyclophosphamide, athymic mice, corticosteroid, indomethacin

Pathological manifestations of the exocrine and endocrine system in Chagas' disease received little attention from either clinical or experimental investigators, despite the emphasis that was given initially by Carlos Chagas in his early description of the disease, having first proposed the name of parasitic thyroiditis for American trypanosomiasis (Chagas 1911), but confusion with the cumulative effect of endemic goitre unfortunately led to an underestimate of the importance of exocrine and endocrine pathologies in Chagas' disease.

Correspondence: Katia Calabrese, Laboratório de Imunomodulação, Departamento de Protozoologia, Instituto Oswaldo Cruz, FIOCRUZ. Av. Brasil, 4365, Manguinhos, 20045-900, Rio de Janeiro, Brasil.

However, some contributions can be found in the literature, for example the symmetrical enlargement of the parotid gland with sialorrhoea observed in chagasic patients by Chagas and Villela (1922) and confirmed by other authors (Mineiro 1958; Vieira *et al.* 1962), and alteration of glucose metabolism (Reis & Vichi 1965; Lomonaco *et al.* 1966). Using compromised models, colonization of the parotid gland has been described (Gonçalves da Costa *et al.* 1986b). Histopathological observations have demonstrated that in athymic (nude) mice, the islets of Langerhans are frequently and highly colonized, which may explain the inflammatory reaction associated with mononuclear infiltration observed in the conjunctive tissue around the acini and islets of Langerhans of the littermates in which the colonization of the pancreas is discrete (Gonçalves da Costa *et al.* 1984). Involvement of the gonads has received more attention recently. The early studies of Ferreira and Oliveira (1965) showed diminution of the semen volume and a modification of the innervation of testis that might explain the observed reduction or inhibition of spermatogenesis. Alencar *et al.* (1991) observed that 77% of mice injected intraperitoneally with the semen of mice previously infected with the Colombian strain of *T. cruzi* presented parasitaemia and myocarditis as well as a clear process of neuritis with neuronal destruction. They also showed that transmission may occur by mating, since when the instillation of semen was preceded by vaginal scarification, infection by the semen using this route occurred and was followed by severe myocarditis.

In this paper, we report that immunocompromised mice (nude, neonate, or adult mice treated by immunosuppressive drugs) did present enhanced colonization of the endocrine system by *T. cruzi.*

Materials and methods

Animals

OF1 specific pathogen free outbred male or female mice, adult thymus intact and newborn, as well as athymic mice were used. These animals were purchased from IFFA-CREDO (Domaine des Oncins, St Germain sur l'Arbresle, France) and then introduced in the Animal Facilities of the Oswaldo Cruz Institute in 1980 by one of us (SCGC).

Parasites

Two strains of *T. cruzi*, Y and CL, were used. The Y strain was originally isolated from a patient in the acute phase of the disease (Pereira da Silva & Nussensweig 1953), while the CL strain was from naturally infected *Triatoma infestans* bugs collected from a house in Rio Grande do Sul, Brazil (Brener & Chiari 1963). The parasites were routinely maintained by serial passage in mice at the Laboratory of Immunomodulation (Department of Protozoology), Oswaldo Cruz Institute. For experimental infection, blood was drawn by cardiac puncture, diluted in PBS and adjusted to a final desired concentration, and intraperitoneally inoculated into mice.

Drugs

Cyclophosphamide (Cy) was purchased from Pravaz Division, Abbott Laboratories, Brazil; dexamethasone (Decadron), from Prodome Quimica & Farmaceutica Ltda, Brazil. The drugs were diluted in pyrogen free saline and filtered through 0.22 μm Millipore before injection. Indomethacin, purchased from Sigma Chemical Co., St Louis, MO, was dissolved in buffer 0.1 M H_2PO_4–NaOH, pH 6·2, and mixed with 0.119 M $NaHCO_3$ and membrane filtered (0.22 μm Millipore) before use.

Experimental schedule

CY treatment. Twenty-four adult OF1 female mice were divided into 2 groups: (A) treated with a single injection intraperitoneally (i.p.) of CY, at a dose of 200 mg/kg body weight on day +5 of infection; (B) untreated (control group).

Corticosteroid treatment. Thirty-six adult OF1 female mice were divided into 3 groups: (C) treated daily i.p. with dexamethasone at a concentration of 0.3 mg/kg of body weight during infection; (D) treated daily i.p. with dexamethasone at a concentration of 10 mg/kg of body weight during infection; (E) untreated (control group).

Indomethacin treatment. Twenty-four female and 24 male adult OF1 mice were divided into 2 groups each: (F) 12 female and 12 male treated i.p. daily with a dose of 1.12×10^{-5} M of the drug in 0.1 ml of the solvent during the course of the experiment; (G) the same number of mice received only the solvent. Before infection, each group was divided into two other groups, H and I. Toxic effects were previously observed with doses higher than that used in the present work.

All groups were infected i.p. with a low dose of 10^4 trypomastigotes of *T. cruzi* Y strain, except groups H and I which received 10^3 or 10^5 trypomastigotes respectively of *T. cruzi.*

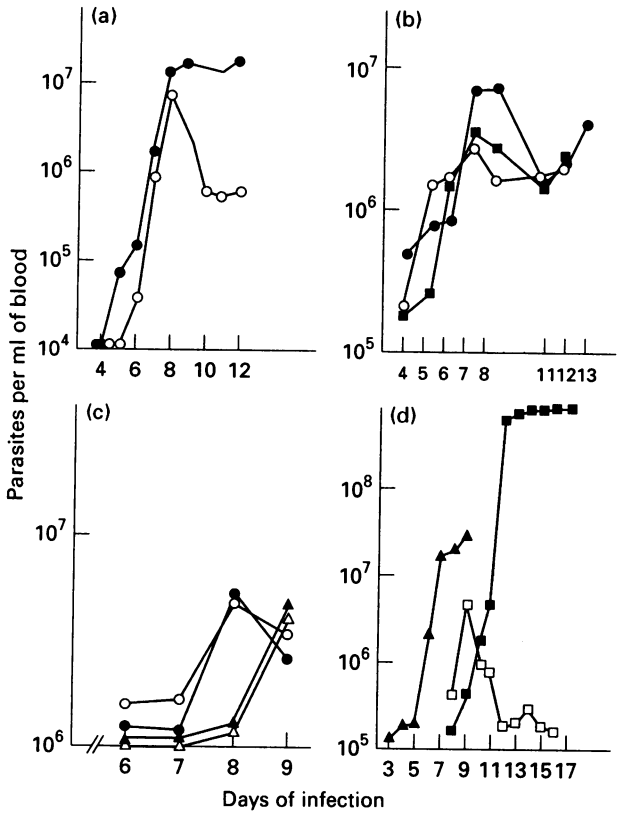

Figure 1. Parasitaemia of different groups of mice infected IP with 10^4 trypomastigotes of *T. cruzi* Y strain, except in the case of indomethacin in which groups the doses were 10^3 and 10^5. a, ●, Mice treated with CY (200 mg/kg) on day +5 after infection; ○, control group. b, Mice treated with dexamethasone ○, 0.3 mg/kg; ●, 10 mg/kg and ■, control mice. c, ▲, Mice treated with indomethacin 1.12×10^{-5} M, infected with 10^3 forms of the parasite; ○, mice treated with indomethacin 1.12×10^{-5} M, infected with 10^5 forms of the parasite; △, control group of 10^3; ●, control group of 10^5. d, ■, nude mice Nu/Nu; □, littermate; ▲, neonate. Calculated mean of parasitaemia including 6 mice per group. In the indomethacin treated group, parasite counts in peripheral blood were not recorded after the first death.

Athymic and intact mice. J, Twelve adult nude female mice (Nu/Nu); K, 12 adult female littermates (Nu/+); L, 12 neonates. Each of the following groups (J, K and L) has been divided into two sub-groups in which the Y and CL strains of *T. cruzi* were used.

Parasitaemia, mortality and histopathology

Individual parasitaemia was measured in six mice from each group at different time intervals after inoculation by a standard microscopic procedure developed by Pizzi (1957). The time course of mortality was evaluated by recording deaths every day among these mice, until 30 days after infection. The rest of the mice were used for histopathological studies. Autopsies were performed 8, 12 and 15 days after infection (two mice per time point) except in neonates that usually die earlier; this group were autopsied 9 days after infection. Various organs, including pancreas, kidneys and adrenals, testes, ovaries and neck sections, were fixed in Millonig's fluid. After dehydration, all tissues were cut into semi-thin sections (5 μm) and were stained with haematoxylin–eosin.

Distribution of parasites in the glandular system

The number of pseudo-cysts in different endocrine glands was determined by examining 50 random microscopic fields (at ×400) in each section. The mean number of pseudocysts was determined by examining five sections from each organ or tissue and using three mice from each experimental group.

Results

Time course of parasitaemia and mortality in different immunosuppressed mice

Cyclophosphamide treated mice receiving a single dose of 200 mg/kg i.p. showed absence of control of parasitaemia 5 days after infection (Figure 1a). The highest level of parasitaemia (more than 10^8 parasites/mm^3 of blood) was observed (Figure 1d) in athymic nude mice (Nu/Nu), their littermates being able to control parasitaemia after the 9th day. In the other immunocompromised mice, the peak levels of parasitaemia were always around 10^7 parasites per mm^3 of blood. However, the kinetics of parasitaemia and the day of occurrence of death varied among groups. Occurrence and rate of parasitaemia were identical in control and dexamethasone treated mice (Figure 1b); the occurrence and the peak level of parasitaemia were sooner and higher respectively in mice treated with 10 mg/kg b.i.d. as compared to those treated with 0.3 mg/kg b.i.d. or to the control. No difference in the kinetics was observed when mice were pretreated with indomethacin. As shown in Figure 1c, similar peak levels of parasitaemia were observed when mice received 10^3 or 10^5 trypomastigotes, but they occurred one day earlier in the 10^5 infected mice. In neonates (Figure 1d), increase of parasitaemia occurred sooner and reached 10^7/mm^3 on day 7, and increased further 2 days before death. All neonates died on the ninth day after infection (Table 1). In nude mice, the mean time of death was slightly (11.8

Table 1. Mean mortality time (MMT) and the ratio between survivors and total mice in different groups of immunocompromised mice: naturally immunocompromised mice, or mice treated with different immunosuppressor drugs and infected with 10^4 trypomastigotes of *T. cruzi* Y strain except for the protocols receiving indomethacin, in which two groups were assayed with a challenge of 10^3 or 10^5 trypomastigostes

			Control	
Group	Survival	MMT	Survival	MMT
Indomethacin (10^3)	(0/5)	12.6 ± 1.9	(2/5)	23.4 ± 2.7
Indomethacin (10^5)	(0/6)	12.7 ± 1.3	(7/7)	15.7 ± 0.9
CY (200 mg/kg)	(0/5)	13.0 ± 0.5	(1/5)	19.4 ± 2.9
Nude mice	(0/5)	11.8 ± 1.5	(0/5)	18.6 ± 1.9
Newborn	(0/6)	9.7 ± 0.3		
Dexamethasone			(2/6)	49.5 ± 3.3
0.3 mg/kg	(3/6)	20.3 ± 4.6		
10 mg/kg	(0/6)	43.5 ± 0.2		

days) later, but earlier than in the littermates (15 days). Such differences in mortality rate and time of death were also observed in the other immunosuppressed groups of mice, except in a group of mice treated with 0.3 mg/kg b.i.d. dexamethasome amongst which 50% of mice survived (as compared to 40% in control) and the mean time of death was more delayed (20.3 *vs* 49.5 days) in treated group as compared to control.

Histopathology

The intensity of tissular invasiveness of the glands by *T. cruzi* in different groups of immunosuppressed mice is shown in Figure 2. The histopathologic description of the glands was made by comparing all immunocompromised mice, except for the thyroid gland which was studied only in neonates. In athymic mice the nude, Y and CL strains showed the same pattern (Figure 2).

Thyroid gland. In contrast with adult infected normal mice, the colonization of the thyroid gland was easily seen in newborn mice. Amastigotes were observed in the parafollicular and follicular cells. In the connective tissue, many disperse amastigotes and pseudocysts were observed. Figure 3 shows amastigotes located in the colloid zone, and in the conjunctive tissue.

Adrenals. All groups of immunocompromised mice exhibited the adrenal cortical regions colonized by *T. cruzi* at an unexpected level. Histopathological studies of the adrenals of nude mice showed that in the cortex the colonization of the fasciculata region is higher than of the glomerular (Figure 4). The colonization of the medulla is less frequent.

The islets of Langerhans. When comparing different models of immunocompromised mice, athymic nude mice showed the most consistent colonization of the islets of Langerhans (Figure 5). The littermates showed a mononuclear inflammatory reaction without any parasite nests. Both groups of mice were examined on day 13 after infection.

Testis. A high density of parasites was also observed in the testis. Interstitial cells and the tunica albuginea of the seminiferous tubules (Figure 6), as well as the loose connective tissue, were largely infected by *T. cruzi*.

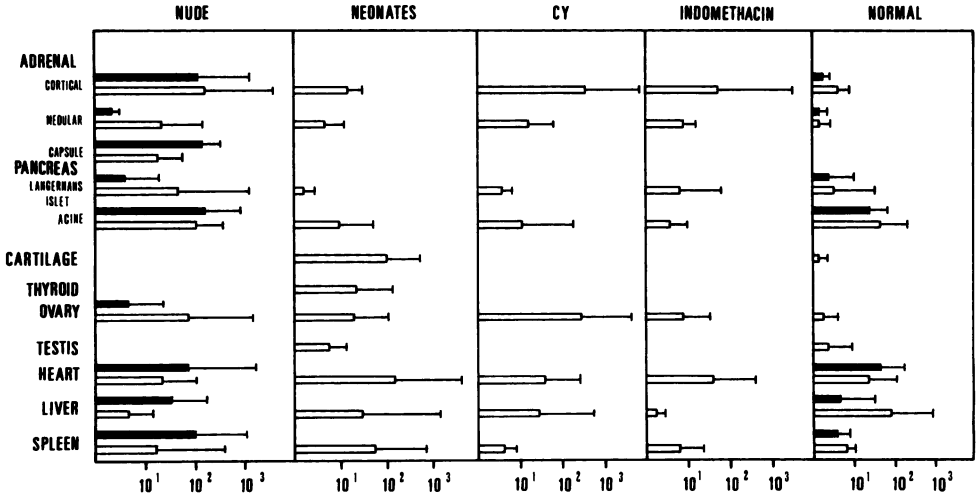

Figure 2. Density of amastigotes in the glandular system in various immunocompromised mice. In the group of athymic mice and normal mice, the □, Y and ■, CL *T. cruzi* strains were compared.

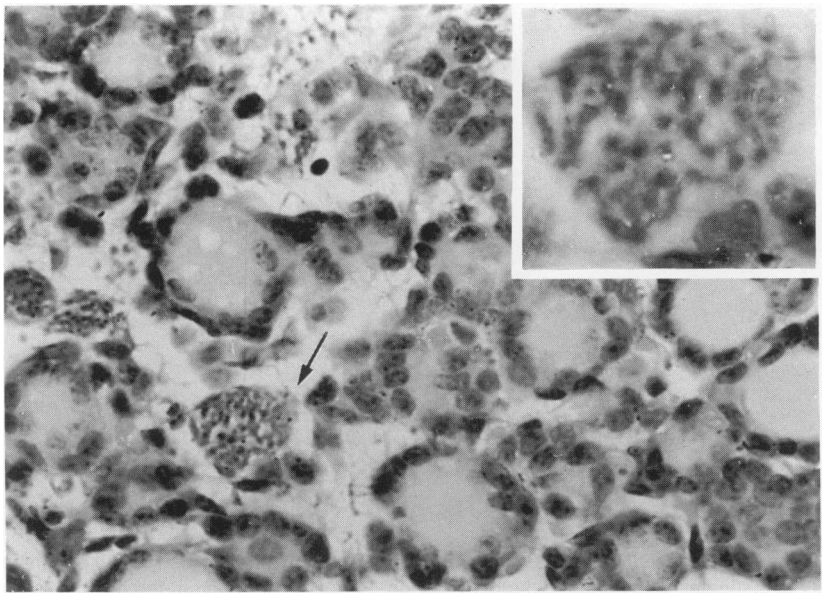

Figure 3. Histopathology of neonate OF1 mice infected with 10^4 trypomastigotes of the Y strain of *T. cruzi*, showing the colonization of thyroid gland. Amastigotes can be observed probably in the colloid zone (arrows). Haematoxylin and eosin staining. ×400. Inset shows high-power view of a nest. ×1000.

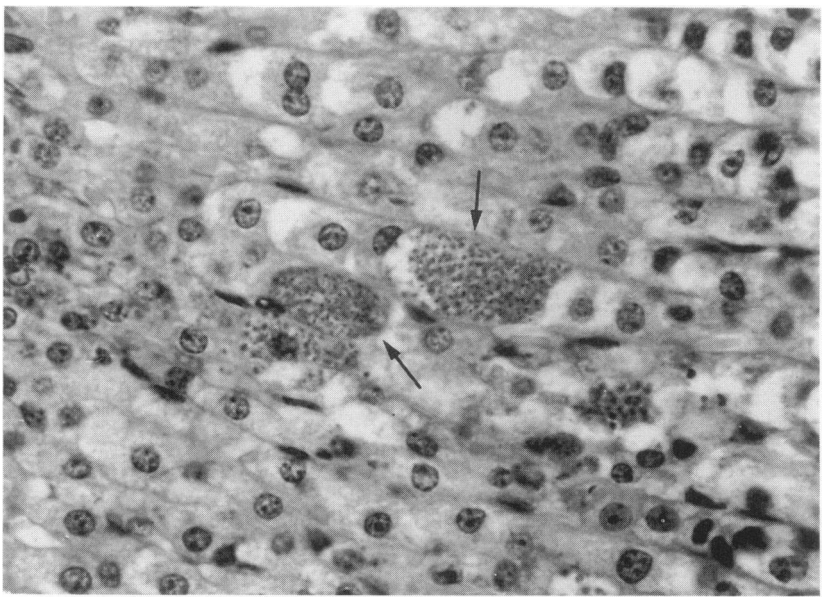

Figure 4. Histopathology of the colonization of nude mice adrenals infected with 10^4 trypomastigotes of Y strain of *T. cruzi*. The fasciculata of the cortical zone can be observed with a great number of amastigotes (arrows). Haematoxylin and eosin staining. ×160.

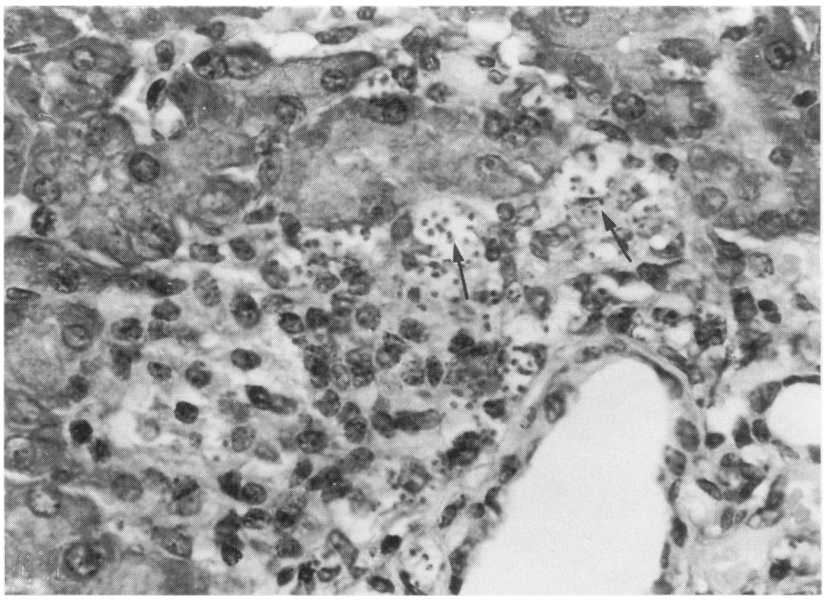

Figure 5. Histopathology of nude mice (Nu/Nu) infected with 10^4 of the *T. cruzi* Y strain. The section of pancreas shows the islet of Langerhans colonized by amastigotes without inflammatory reaction. Parasites are seen in the muscular structure of a blood vessel (arrows). Haematoxylin and eosin staining. ×160.

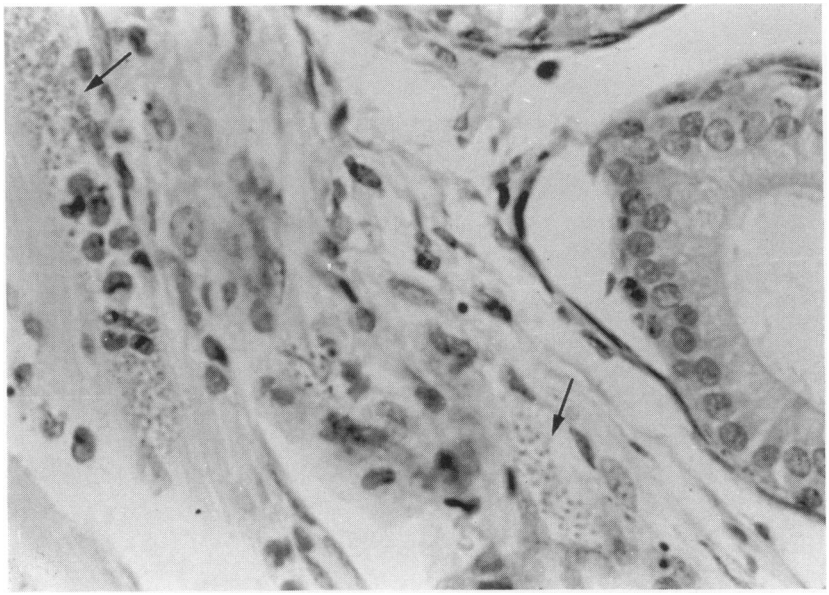

Figure 6. Newborn mice testis infected with 10^4 trypomastigotes of *T. cruzi* Y strain shows amastigotes in the interstitial cells and tunica albuginea of seminiferous tubule (arrows).

Ovary. As observed for other endocrine glands, histopathological studies of ovary showed a higher colonization rate by *T. cruzi* in different immunocompromised groups of mice. Mice treated with anti-inflammatory drugs, such as indomethacin, showed enhancement of tissue invasion. Figure 7a shows a secondary follicle with a great proliferation of amastigotes in the theca interna and stroma. Similar pictures were observed in CY-treated and nude mice (Figure 7b).

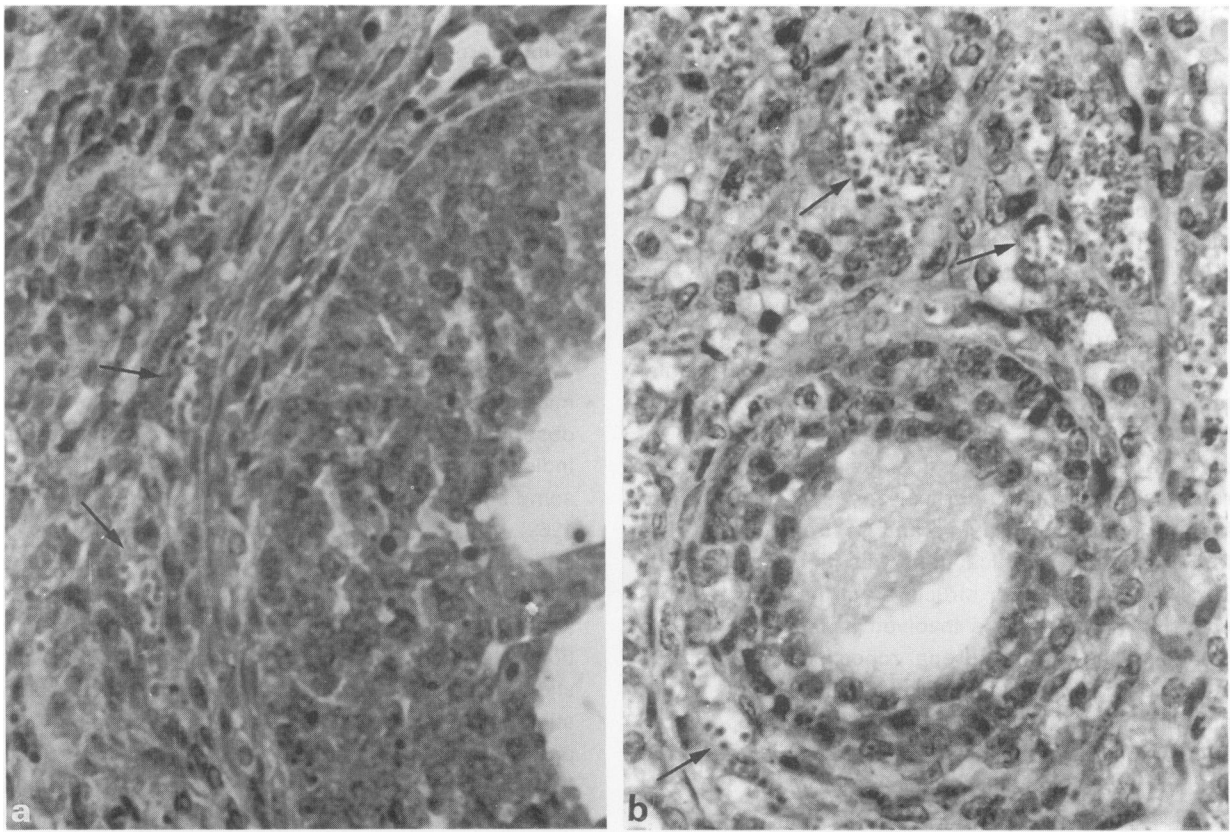

Figure 7. a, Histopathology of indomethacin adult OF1 treated mouse, infected with 10^4 trypomastigotes of *T. cruzi* Y strain shows the ovary in which a great number of amastigotes can be seen around an oocyte (arrows). ×1000. b, The ovary of nude mouse (Nu/Nu) with amastigotes around an oocyte and in the stroma (arrows). Haematoxylin and eosin staining. ×400.

Discussion

Trypanosoma cruzi trypomastigotes can invade and proliferate in almost all cell types of the vertebrate host. In a previous work it has been shown that the thymus, in which the parenchyma has rarely been found to be associated with infectious agents (Henry 1981), can be colonized by *T. cruzi* (Gonçalves da Costa *et al.* 1986b). It has been demonstrated that both macrophages and epithelial cells can be invaded by trypomastigotes (Gonçalves da Costa *et al.* 1991). It is accepted that the thymus acts as an endocrine gland whose hormones play a central role as mediators of T cell differentiation in the control of immune response (Low & Goldstein 1979). Concerning the *T. cruzi* invasiveness of the glandular system, the thymus is perhaps the most studied, since it has been suggested that it may be a target organ in experimental Chagas' disease (Savino 1990).

It has also been shown that cyclophosphamide induced immunosuppression is associated with the occurrence of nodular skin lesions, called schizotrypanids, in both man (Stolf *et al.* 1987) and in an experimental model (Gonçalves da Costa & Calabrese 1992) as well as with colonization of hepatocytes (Calabrese *et al.* 1991).

In experimental models several authors have observed that different *T. cruzi* strains present various tissue tropisms, e.g. for skeletal and heart muscles (Bice & Zeledon 1970; Melo & Brener 1978), pancreas and adrenals (Watkins 1966), brown adipose tissue (Shoemaker *et al.* 1970), mononuclear phagocytic system (Taliaferro & Pizzi 1955), nervous system (Amaral *et al.* 1975) and bladder (Bice & Zeledon 1970). It has also been shown that tissue tropism is highly affected in the immunocompromised host, as in the case of athymic nude mice (Gonçalves da Costa *et al.* 1984), corticosteroid treatment (Agosin *et al.* 1952; Rubio 1952) and non-hormonal anti-inflammatory drugs (Gonçalves da Costa *et al.* 1986a).

In the present report, investigations on endocrine glands were performed using various groups of immunocompromised mice infected with the two 'polar' strains of *T. cruzi*. In normal mice, the Y strain is known to have tropism for muscles, while the CL strain has mononuclear phagocytic system tropism. This tropism is lost in immunocompromised groups of mice. The first observation of the colonization of adrenals by *T. cruzi* amastigotes was made by Vianna (1911) who showed nests of parasites in the fasciculata zone in experimentally infected guinea-pigs. The inflammatory lesions observed in this experimental model were similar to those described by Carlos Chagas (1911) in man although Chagas did not observe amastigotes in the lesions. The present report shows that the cortical zones of adrenals are highly colonized in athymic and CY treated mice. The fasciculata of the cortical zone was more colonized by *T. cruzi* than the other structures of the adrenals. The underlying mechanisms involved in this difference in colonizing the various adrenal zones are not known. On the other hand, uncommon histopathological aspects appeared in the histopathology of immunosuppressed mice and humans infected with *T. cruzi*, such as (1) the colonization of hepatocytes (Calabrese *et al.* 1991); (2) skin lesions, showing a panniculitis with intense inflammatory involvement of the subcutaneous tissue and cell infiltration consisting predominantly of macrophages, lymphocytes, plasma cells and neutrophils, after heart transplantation in chagasic humans (Stolf *et al.* 1987); (3) exudative erythematous skin lesions without inflammatory cell reactions in nude mice (Gonçalves da Costa & Calabrese 1992). A great difference was observed between the thyroid glands of control mice and those of immunocompromised mice during the acute phase of experimental infection. In the earliest study of Penna de Azevedo (1936) it was shown that one out of five patients who died during the acute phase of Chagas' disease presented nests of amastigotes in the thyroid epithelium but without thyroiditis. In a second report, in which 26 patients in the chronic phase were studied, neither parasites nor thyroiditis was observed but, in both acute and chronic cases, endemic goitre was observed. The present report shows that thyroid glands of neonate and young mice are highly colonized by *T. cruzi* and that thyroiditis is frequent.

The consequences of glandular system failure on glucose metabolism have been reported (Vieira *et al.* 1962; Vieira & Meneghelli 1970) and colonization of adrenals as well as the islets of Langerhans may have some implication in the glycaemia. Changes in the α and β cells of the islets of Langerhans have been observed (Albuquerque *et al.* 1990).

Inhibition of spermatogenesis has been shown by some authors (Lamano Carvalho *et al.* 1990) and the histopathological studies reported here supply evidence that colonization by the parasites in the acute phase may be followed by immunopathological consequences resulting from the presence of soluble and particulate antigens of the parasite in the glandular structures. Binding of *T. cruzi* antigens on the surface of host cells has been demonstrated (Ribeiro dos Santos & Hudson 1980a,b; Abrahamsohn & Kloetzel 1980). In mice treated with corticosteroid a bimodal effect was observed upon the mononuclear phagocytic system indicating a depressed action of large doses and some beneficial effects of small doses (Vernon-Roberts 1969). It has been shown that cortisone induces an enhancement of *Trypanosoma* infection (Jarpa *et al.* 1951; Wolf *et al.* 1951; Nery-Guimarães & Lage 1970) and in Gram-negative or Gram-positive bacterial infections (Germuth 1956; Robinson 1956). The present report shows that a low dose (0.3 mg/kg) instigates a slight stimulation of the resistance against *T. cruzi* as compared to control or in mice treated with 10 mg/kg of dexamethasone. The effect of betamethasone administered in the early post acute phase of experimental Chagas' disease in mice did not aggravate the course of infection. Mortality rate however was higher in the treated group of mice and it was suggested to be a consequence of secondary infection (Abath *et al.* 1986). Bacteria associated with *T. cruzi* infection were also observed in mice treated with cyclophosphamide (Calabrese *et al.* 1991). There is now an increasing number of immunodeficiencies in clinical situations, in which Chagas' disease might be involved: immunosuppressive therapy used in heart transplantation of chagasic patients (Stolf *et al.* 1987) or kidney transplantation where the tissue is colonized by amastigotes (Chocair *et al.* 1981); inappropriate corticoid therapy at the start of the acute phase of Chagas' disease before a correct diagnosis (Nery-Guimarães *et al.* 1968); malnutrition in endemic zones, as suggested by Yaeger and Miller (1960), and acquired immunodeficiency syndrome (Del Castillo *et al.* 1990). It seems that the study of Chagas' disease in immunocompromised mice models has brought to light some situations that bear on clinical occurrences. As was emphasized by Stolf *et al.* (1987), experimental data concerning chronic infection in immunosuppressed animals are controversial. Further studies are being carried out in the laboratory to extend these studies in experimental models to the chronic phase.

Acknowledgements

This work was financially supported by FIOCRUZ and by a grant from a Scientific Cooperation Program Brazil–France (CNPq-INSERM no. 910320/92-6).

We wish to thank Dr Maria Nazareth de Meirelles, head of the Department of Cellular Biology and Ultrastructure, for allowing the use of the photomicroscope. We gratefully acknowledge the excellent technical assistance of Ruy Quintella and we are indebted to Mr Genilton Vieira and Miss Heloisa Maria Nogueira Diniz for photographic assistance.

References

Abath F.G.G., Gomes Y.M., Coutinho E.M., Montenegro S.M.L., Belo M.E.B. & Carvalho A.B. (1986) Effects of betamethasone of the course of experimental infection with *Trypanosoma cruzi*. *Rev. Soc. Bras. Med. Trop.* **19,** 161–164.

Abrahamsohn, I.A. & Kloetzel, J.K. (1980) Presence of *Trypanosoma cruzi* antigens on the surface of both infected and uninfected cells in tissue culture. *Parasitology* **80,** 147–152.

Agosin, M., Christen, R., Jarpa, A., Atias, J.A. & Neghme, A. (1952) Cortisona y enfermidad de Chagas experimental. *Rev. Med. Chile* **80,** 34–38.

Albuquerque, S., Ribeiro, R.D., Lopes, R.A., Lamano Carvalho, T.L. & Paula-Lopes, O.V. (1990) Tissue tropism of different *Trypanosoma cruzi* strains. IX-Alterations in A and B Langerhans islets cells produced by the slender and broad forms of the Bolivia strain. *Mem. Inst. Oswaldo Cruz* **85 (Suppl. I),** 8.

Alencar, A.A., Brito, C.M.M. & Azevedo, B.A. (1991) Estudos sobre a infectividade do sêmen de camundongos inoculados com *Trypanosoma cruzi*. Comprometimento do aparelho genital masculino na doença de Chagas experimental. Alteração dos plexos nervosos com destruiçao neuronal. *Rev. Bras. Neurol.* **27,** 51–56.

Amaral, C.F.S., Tafuri, W.L. & Brener, Z. (1975) Freqüência do parasitismo encefálico em camundongos infectados experimentalmente com diferentes cepas do *T. cruzi*. *Rev. Soc. Bras. Med. Trop.* **9,** 243–246.

Bice, D.E. & Zeledon, R. (1970) Comparison of infectivity of strains of *Trypanosoma cruzi* (Chagas, 1909). *J. Parasitol.* **56,** 663–670.

Brener, Z & Chiari, E. (1963) Variaçes morfológicas observadas em diferentes amostras do *Trypanosoma cruzi*. *Rev. Inst. Med. Trop. (São Paulo)* **5,** 220–224.

Calabrese, K.S., Bauer P.G., Lagrange, P.H. & Gonçalves da Costa, S.C. (1991) *Trypanosoma cruzi* infection in immunosuppressed mice. *Immunol Lett.* **31,** 91–96.

Chagas C. (1911) Molestia de Carlos Chagas ou thyreoidite parasitaria. *Rev. Med. São Paulo* **14,** 337–356.

Chagas, C & Villela, E. (1922) Forma cardíaca da tripanosomiase americana. *Mem. Inst. Oswaldo Cruz* **14,** 5–61.

Chocair, P.R., Sabbaga, E., Amato Neto, V., Shiroma, M. & Góes, G.M. (1981) Transplante de rim: noval modalidade de transmissão da doença de Chagas. *Rev. Inst. Med. Trop. São Paulo* **23,** 280–282.

Del Castillo, M., Mendoza, G., Oviedo, J., Bianco, R.P.P., Anselmo, A.E. & Silva, M. (1990) AIDS and Chagas' disease with central nervous system tumor-like lesion. *Am. J. Med.* **88,** 693–694.

Ferreira, A. & Oliveira, J.S.M. (1965) Volume do sêmen obtido por electroejaculação de ratos chagásicos (inoculados experimentalmente). *Rev. Inst. Med. Trop. São Paulo* **7,** 127–130.

Germuth, F.G. (1956) The role of adrenocortical steroids in infection, immunity and hypersensitivity. *Pharmacol. Rev.* **8,** 1–24.

Gonçalves da Costa, S.C. & Calabrese, K.S. (1992) Schizotrypanids: the occurrence of dermatitis in immunodeficient animals infected with *Trypanosoma cruzi*. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro* **87, (Suppl. I),** 81–85.

Gonçalves da Costa, S.C., Calabrese, K.S., Alencar, A.A. & Lagrange, P.H. (1986a) *Trypanosoma cruzi* invasion of structure related to development and central nervous system. *Rev. Bras. Neurol.* **22,** 183–190.

Gonçalves da Costa, S.C., Calabrese, K.S., Bauer, P.G., Savino, W. & Lagrange, P.H. (1991) Studies of the thymus in Chagas' disease. III Colonization of the thymus and other lymphoid organs of adult and newborn mice by *Trypanosoma cruzi*. *Path. Biol.* **39,** 91–97.

Gonçalves da Costa, S.C., Lagrange, P.H., Hurtrel, B., Kerr, I. & Alencar, A. (1984) Role of T lymphocyte in the resistance and immunology of experimental Chagas' disease. I Histopathological studies. *Ann. Immunol. (Inst. Pasteur)* **135C,** 317–332.

Gonçalves da Costa, S.C., Palhares, P.E.S., Calabrese, K.S., Alencar, A.A. & Lagrange, P.H. (1986b) *Trypanosoma cruzi* histopathology and time course infection in indomethacin-treated mice. *Rev. Bras. Neurol.* **22,** 161–164.

Henry, K. (1981) The human thymus in disease with particular emphasis on thymitis and thymoma. In *The Thymus Gland.* Ed. M.D. Kendall, Academic Press, London, pp. 85–111.

Jarpa, A., Agosin, M., Christen, R. & Atias, A. (1951) Ensaios de quimioterapia de la enfermidad de Chagas experimental. VII-Cortisona y fosfato de pentaquina. *Bol. Inf. Parasit. Chile* **6,** 25–27.

Lamano Carvalho T.L., Lopez R.A., Carraro A.A. & Ribeiro R.D. (1990) The male reproductive organs in the chronic phase of Chagas' disease. *Mem. Inst. Oswaldo Cruz* **85 (Suppl. 1),** 9.

Lomonaco, D.A., Oliveira, A.L., Kieffer, J. & Pieroni, R.R. (1966) Abnormal regulation of thyroid function in patients with chronic Chagas' disease. *Acta Endocr.* **63,** 162–176.

Low, T.L.K & Goldstein, A.L. (1979) Thymosin and other thymic hormones and their synthetic analogues. *Springer Semin. Immunopathol.* **2,** 169-186.

Melo, R.C. & Brener, Z. (1978) Tissue tropism of different *Trypanosoma cruzi* strains. *J. Parasitol.* **64,** 475–482.

Mineiro, V. (1958) Contribuição à etiologia do megaesôfago. *Rev. Goiana Med.* **4,** 29–37.

Nery-Guimarães, F. & Lage, H. (1970) Ação da cortisona e da betametasona na doença de Chagas experimental. I Camundongos. *O Hospital* **77,** 119–132.

Nery-Guimarães, F., Silva, N.N., Clausell, D.T., Melo, A.L., Rapone T., Snell, T. & Rodrigues, N. (1968) Um surto epidêmico de doença de Chagas de provável transmissão digestiva, ocorrido em Teutonia (Estrela, Rio Grande do Sul). *O Hospital* **73,** 1767–1804.

Penna de Azevedo A. (1936) Histopathologia da glândula thyreoide na forma chronica da doença de Chagas (Trypanosomose Americana). *Folha Médica* 1–14.

Pereira da Silva, L.H. & Nussensweig, V. (!953) Sobre uma cepa de *Trypanosoma cruzi* altamente virulenta para camundongos brancos. *Folia Clin. Biol. (São Paulo)* **20,** 191–207.

Pizzi, T. (1957) *Immunologia de la enfermidad de Chagas.* Universidad de Chile, p. 183.

Reis, L.C.F. & Vichi, F.L. (1965) Estudos sobre o metabolismo dos hidratos de carbono na molestia de Chagas. II A prova de glucagon. *Rev. Assoc. Med. Bras.* **11,** 61–67.

Ribeiro dos Santos, R. & Hudson, L. (1980a) Trypanosoma cruzi immunological consequences of parasite modification of host cells. *Clin. Exp. Immunol.* **40,** 36–41.

Ribeiro dos Santos & Hudson, L., (1980b) *Trypanosoma cruzi*: binding of parasite antigens to mammalian cell membrane. *Parasite Immunol.* **2,** 1–10.

Robinson, H.J. (1956) Adrenal cortical hormones and infection. *Pediatrics* **17,** 770–780.

Rubio, M. (1952). Estudio de los factores que intervienen en la virulencia de una cepa de *Trypanosoma cruzi.* – Accion de la cortisona en la capacidad de invasion y multiplicacion del parasito. *Biologica, Santiago* **20,** 89–125.

Savino W. (1990) The thymic microenvironment in infectious diseases. *Mem. Inst. Oswaldo Cruz* **85,** 255–260.

Shoemaker, J.P., Hoffman, Jr., R.V. & Hoffman, D.G. (1970) *Trypanosoma cruzi*: preference for brown adipose tissue in mice by the Tulahuen strain. *Exp. Parasitol.* **27,** 403–407.

Stolf, N.A.G., Higushi, L., Bocchi, E., Bellotti, G., Auler, J.O.C., Uip, D., Amato Neto, V., Pileggi, F. & Jatene, A.D. (1987) Heart transplantation in patients with Chagas' disease cardomyopathy. *J. Heart Transplant.* **6,** 307–312.

Taliaferro, H. & Pizzi, T. (1955) Connective tissue reaction in normal and immunized mice to a reticulotropic strain of *Trypanosoma cruzi. J. Infect. Dis.* **96,** 199–226.

Vernon-Roberts B. (1969) The effects of steroid hormones on macrophage activity. *Int. Rev. Cytology* **5,** 131–159.

Vianna, G. (1911) Contribuição para o estudo da anatomia patológica da moléstia de Chagas. *Mem. Inst. Oswaldo Cruz* **3,** 276–294.

Vieira, C.B., Camara-Lopes, L.H. & Menegucci, W. (1962) Variações de volume das glândulas salivares e de amilasemia em pacientes com megaesôfago chagásico operado. *Rev. Goiana Med.* **8,** 11–19.

Vieira, C.B. & Meneghelli, U.G. (1970) Pecularidades da hipoglicemia insulinica na forma crônica da doença de Chagas. I Estudo Clínico. *Rev. Inst. Med. Trop. S. Paulo* **12,** 175–178.

Watkins, R. (1966) Comparison of infections produced by two strains of *Trypanosoma cruzi* in mice. *J. Parasitol.* **52,** 958–961.

Wolf, A., Kabat, E.A., Bezer, A.E. & Fonseca, J.R.C. (1951) Activation of trypanosomiasis in Rhesus monkeys by cortisone. *Fed. Proc.* **10,** 375.

Yaeger, R.G. & Miller, N. (1960) Effect of malnutrition on susceptibility of rats to *Trypanosoma cruzi.* I Thiamin deficiency. *Exp. Parasitol.* **9,** 215–222.