Morphological Aspects of the Myocarditis and Myositis in *Calomys callosus* Experimentally Infected with *Trypanosoma cruzi*: Fibrogenesis and Spontaneous Regression of Fibrosis

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*Calomys callosus* is a wild rodent, is a natural host of Trypanosoma cruzi. Twelve *C. callosus* were infected with 10⁵ trypomastigotes of the F strain (a myotropic strain) of *T. cruzi*. Parasitemia decreased on the 21st day becoming negative around the 40th day of infection. All animals survived but had positive parasitological tests, until the end of the experiment. The infected animals developed severe inflammation in the myocardium and skeletal muscle. This process was pronounced from the 26th to the 30th day and gradually subsided from the 50th day becoming absent or residual on the 64th day after infection. Collagen was identified by the picro Sirius red method. Fibrogenesis developed early, but regression of fibrosis occurred between the 50th and 64th day. Ultrastructural study disclosed a predominance of macrophages and fibroblasts in the inflammatory infiltrates, with small numbers of lymphocytes. Macrophages had active phagocytosis and showed points of contact with altered muscle cells. Different degrees of matrix expansion were present, with granular and fibrillar deposits and collagen bundles. These alterations subsided by the 64th days. Macrophages seem to be the main immune effector cell in the *C. callosus* model of infection with *T. cruzi*. The mechanisms involved in the rapid fibrogenesis and its regression deserve further investigation.

Key words: Trypanosoma cruzi - Calomys callosus - myocarditis - myositis - fibrogenesis - fibroblasia - ultrastructure - collagen

*Calomys callosus* (Rodentia - Cricetidae), a natural reservoir of *Trypanosoma cruzi*, was first identified as harboring this parasite by Ribeiro (1973) and Mello and Teixeira (1977). The biology of *C. callosus* and its breeding in the laboratory have been described by Mello (1984). Since its identification as a natural reservoir, it has been used as a model for experimental *T. cruzi* infection (Mello et al. 1979, Mello & Borges 1981, Borges et al. 1982). Recently, a comparative study was made of the evolution of the infection induced by different strains of *T. cruzi* in this model and in mice, including the correlation between activation of peritoneal macrophages and the histopathological findings (Borges et al. 1992). The F strain of *T. cruzi* evoked early macrophage infiltration and fibroblastic proliferation in myocardium and skeletal muscle, with conspicuous fibrogenesis. This process subsided spontaneously, without the development of chronic lesions such as those seen in mice (Andrade & Grimaud 1986). Since myocarditis and myositis in *Calomys* showed peculiar features, with marked fibrogenesis followed by fibroblasia, the cellular changes and the responses of the extracellular matrix at different stages were investigated by light and electron microscopy in an attempt to clarify the pathogenesis of *T. cruzi* infection in this model.

MATERIALS AND METHODS

Twenty *C. callosus* raised in the Instituto de Medicina Tropical de São Paulo, Brazil, were used in the study. Twelve were infected with the F strain of *T. cruzi* and eight were maintained as uninfected controls. Inoculations were performed intraperitoneally with 10⁵ trypomastigotes (blood forms) obtained from infected mice.

The F strain was originally obtained from PA D'Alessandro (Deane & Kloetz 1974) and has been maintained at the Instituto de Medicina
Tropical de São Paulo, both in mice and in *C. callosus*. It is a myotropic strain, as previously shown by Borges et al. (1992). Parasitemia was evaluated daily by microscopic examination of peripheral blood under coverslips (22 x 22mm), counting the numbers of parasites in 50 high power (X400) fields. In animals in which direct blood examination was negative, blood subinoculation into newborn mice and xenodiagnosis were performed. For subinoculation, 0.1 ml of citrated blood was injected intraperitoneally. Xenodiagnosis was performed using five to 5 stage nymphs of *Rhodnius prolixus*. Infected and control animals were killed at 21, 26, 30, 50 and 64 days after infection.

For histopathological study, samples of myocardium and skeletal muscle were formalin-fixed, paraffin-embedded, and 5μm-thick sections were stained with hematoxylin and eosin. Sections were also stained for collagen with the picro Sirius red method of Junqueira et al. (1979), using Rot-Sirius 3 (Chroma Gessellschaft) in a saturated aqueous solution of picric acid and Fast green as a counterstain. Sections were examined with bright field and polarized light optics using a Zeiss photo microscope II.

For ultrastructural study, small blocks of right atrium and skeletal muscle from the thigh were fixed in 2% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4, for 2 hr and postfixed with 1% Osmium tetroxide in 0.15M cacodylate buffer. After dehydration with ethanol the tissues were embedded in Poly-bed 812 resin. 1μm-thick sections stained with Toluidine blue were examined by optical microscopy to select areas for electron microscopic study. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a JEOL 1200EX Electron Microscope at an accelerating voltage of 60 kV.

RESULTS

Infected *C. callosus* developed parasitemia, which peaked at the 21st day of infection. Then it declined, becoming negative from the 40th day until the end of the experiment (64 days). Xenodiagnosis and/or subinoculation were positive in all the animals.

**Histologic study** - Until the 21st day post infection parasitism was scarce in cardiac and skeletal muscle and was not detected in macrophages. From the 26th to the 30th day post infection, myocardial inflammatory lesions varied from mild to very severe, with few intracellular parasites. In one animal, diffuse necrosis of cardiac myocytes and marked inflammatory infiltration (mainly of macrophages and fibroblasts and some lymphocytes), were present together with abundant deposit of eosinophilic material in the interstitial connective tissue matrix (Figs 1a, b). From the 50th through the 64th day inflammatory lesions were reduced to focal mononuclear infiltrates and scarce interstitial matrix deposits. Inflammatory and degenerative lesions in skeletal muscle were severe from the 26th to the 30th day, with numerous intracellular parasites (amastigotes) forming large clusters. Marked infiltration of mononuclear cells was seen in the perivascular spaces, in the interstitium and around necrotic cells, sometimes in association with neutrophils, disintegrated parasites and cell debris (Fig. 2a). Macrophages and fibroblasts predominated in the infiltrate, with some small lymphocytes and plasmocytes and loose matrix deposits. Interstitial lesions formed by epithelioid cells were also seen (Fig. 2b). From the 50th to the 64th day, focal areas of muscle cells necrosis and mononuclear cell infiltration were seen. From the 50th to the 64th day, the interstitial spaces in skeletal muscle were free of inflammation and abnormal matrix deposits.

**Staining for collagen** - Examination of sections stained with picro Sirius red by direct and polarized light microscopy was used to identify fibrillar collagen. In the heart, small deposits of collagen were seen in the interstitium and in perivascular spaces, corresponding to focal inflammatory infiltrates. In the areas of severe inflammatory lesions, Sirius red-positive interstitial matrix deposits were abundant (Figs 4a, b). Occasionally, dense bundles of collagen extended from the subepicardium into the subjacent myocardium, in association with perivascular inflammatory infiltrates. In skeletal muscle, deposits with positive staining and birefringence were seen in the perivascular spaces and extended into other interstitial areas, forming long strands of collagen that delimited groups of muscle cells (Fig. 5c). On the 50th day, deposits of collagen were limited to the perivascular spaces and focal areas of the interstitium. By the 64th day, only small focal perivascular collagen deposits were present.

Sections of the heart and skeletal muscles from uninfected control animals showed essentially normal structure. Fine strands of collagen were seen as isolated bundles in the epicardium and in the perivascular and interstitial spaces of skeletal muscle (Figs 5a, b).

**Ultrastructural study - Heart**: from the 24th to the 30th day of infection sections of right atrium showed a predominance of macrophages, with abundant lysosomes, in the inflammatory infiltrates. These lysosomes contained electron-dense materials that sometimes were identified as parasite debris by the presence of submembranous microtubules (Fig. 6). Points of contact between cytoplasmic processes of macrophages and the basement membranes of myocytes were seen (Fig. 6). Fibroblasts showing prominent cisterns of endoplasmic reticulum
were present in small numbers by the 26th through the 30th day of infection. Lymphocytes were present only in small numbers and were mostly of the small, agranular type. Myocardial capillaries appeared normal. Abundant matrix deposits formed by granular and fibrillar material and dense collagen bundles were seen (Fig. 7). The collagen deposits in subepicardial areas were more prominent and were admixed with elastic fibers.

Myocytes showed focal alterations such as disruption of Z bands, mitochondrial vacuoliza-
tion, formation of intramitochondrial amorphous densities and myofibrillar disorganization (Fig. 8). These changes were associated with the presence of focal inflammatory infiltrates. By the 50th day, macrophages and fibroblasts were less numerous. Interstitial spaces contained amorphous or finely fibrillar material associated with isolated collagen fibrils which showed indistinct periodicity and a marked increase in electron density (Fig. 9). By the 64th day of infection, small focal inflammatory infiltrates were seen in association with altered myocytes.

Skeletal muscle - From the 26th to the 30th day after infection, ultrastructural alterations
were more severe in skeletal muscle than in cardiac muscle. Inflammatory infiltrate consisted mainly of macrophages with abundant lysosomes and phagosomes, fibroblasts and a few lymphocytes. Macrophages with epithelioid differentiation were found in the interstitial spaces (Fig. 10). In some animals, the predominance of fibroblasts in the interstitium was remarkable, and these were almost the only type of cell present in such areas. Extracellular matrix deposits were abundant and consisted of fibrillar and granular material and collagen bundles (Fig. 11). Disintegrated parasites were rarely seen within the cytoplasm of altered muscle cells. By
the 50th and the 64th day, a decrease in the numbers of macrophages and fibroblasts was detected. Interstitial matrix was scarce, with few collagen bundles. Focal cellular infiltrates were seen and these correlated with the presence of necrosis of muscle cells in contact with macrophage processes (Fig. 12).

Ultrathin sections of myocardium and skeletal muscle of uninfected control animals showed normal structure.
DISCUSSION

A peculiar evolution of *T. cruzi* (F strain) infection in *C. callosus* as compared with that in the mouse, has been previously described (Borges et al. 1992). *C. callosus* was more resistant and survived the infection, while a high mortality was observed in the infected mice in the acute phase. The present investigation shows that, although being "resistant" in the sense of having a low mortality, *C. callosus* developed severe lesions in
Fig. 6: myocardium - widened interstitial space in the myocardium (26th day). Macrophage contains degenerate parasites with conspicuous microtubules within phagosomes (short arrows). Macrophage processes make contact with the basement membrane of a cardiac myocyte (long arrow). 10,000X
skeletal muscle and myocardium. This infection was associated with a severe fibrogenic process, mainly in skeletal muscle, since the early phase (26 to 30 days). From the 50th day of infection regression of the lesions was observed, and inflammatory and fibrotic processes were focal and residual by the 64th day. Although direct parasitemia was negative, this did not indicate eradication of the infection, since parasitological tests still yielded positive results. Survival of all the animals throughout the experiment indicated that C. callosus controls the infection and develops a host-parasite equilibrium, an ideal condition for becoming a reservoir of parasites. The strain of T. cruzi is important in determining the severity of the histopathological lesions in C. callosus. As previously shown (Borges et al. 1992), the F strain induced severe lesions up to a late phase after infection; however, the lesions induced by the Y strain subsided entirely 20 days
after infection. The present ultrastructural study disclosed details of the progressive and spontaneously regressive processes that occurred during the infection of *C. callosus* with the F strain of *T. cruzi*. Macrophages were the most prominent cells in the inflammatory infiltrate and appear to be responsible for the clearance of parasites from infected tissues. These cells showed morphological signs of activation, as indicated by long cytoplasmic processes and abundant lysosomes, which often contained phagocytosed, degenerating parasites. These findings suggest that parasite clearance and control of the infection are dependent upon activation of the macrophages. Contacts between cytoplasmic processes of macrophages and the basement membranes of cardiac myocytes constituted evidence of direct interactions between these two types of cells. A damaging effect of macrophages on the membranes of cardiac myocytes has been previously described in the mouse model (Rossi 1990). Macrophages appear
Fig 9: myocardium - enlarged interstitial space of the myocardium (50th day) with fibroblast and scanty matrix deposit and few collagen bundles with increased electron density (arrows). 6000X.

to be the main immune effector cells in the Calomys model of infection with T. cruzi. Activation and proliferation of fibroblasts probably were induced by the release of fibrogenic cytokines by the macrophages involved in the inflammatory process. Delayed type hypersensitivity has been shown by several authors to be involved in chronic infection with T. cruzi (Santos-Buch & Teixeira 1974, Ribeiro dos Santos et al. 1991, Andrade 1991). It is possible that a significant immunosuppression of delayed type hypersensitivity, as described in mice (Scott 1981) and in dogs (Andrade et al. 1987), is involved in the rapid regression of the inflammatory lesions in the C. callosus model. The reversibility of collagen deposition in chronically infected mice, under curative chemotherapy, has been previously shown (Andrade et al, 1991). Although morphological evidence of fibroclasia was present in C. callosus, subsiding fibrosis seemed to be more dependent on decreased in fibrogenesis than
Fig. 10: skeletal muscle - epithelioid cell is present in a granulomatous reaction in the interstitial space of skeletal muscle and show abundant cytoplasm in which numerous ribosomes, and few mitochondria are found (30th day). Lysosomes are not present. One nucleus contains a thin rim of peripherally located chromatin and two nucleoli. 8,000X.

was the case in mice. A down regulation of fibrogenesis, mediated by suppression of delayed type hypersensitivity, may be involved in the reduction in collagen. A similar mechanism has been described in schistosomiasis (Wyley et al. 1992). Fibrogenesis and spontaneous regression of fibrosis in this model deserve further investigations. Fibrosis is one of the most important features of chronic Chagas’ cardiomyopathy and shows a progressive evolution in the mouse model (Andrade et al. 1989). According to Weber et al. (1993), fibrillar collagen deposits in the myocardial interstitium are of paramount importance in the development of cardiac failure, and particularly of diastolic dysfunction, in different cardiac diseases. These observations on the highly unusual features of T. cruzi infection in C. callosus, suggest that this species may become an useful animal model for the study of myocardial inflammation and fibrosis.
Fig. 11: skeletal muscle - enlarged interstitial space of skeletal muscle showing several fibroblasts with prominent endoplasmic reticulum, cell processes and granular and fibrillar matrix deposits (30th day). The fibroblasts are the only type of cell present in this area. 8,000X.
REFERENCES


Fig 12: skeletal muscle - necrotic skeletal muscle cell in an area of focal residual damage shows focal cytoplasmic disintegration, myofibrillar loss, lysosomes and mitochondrial condensation (50th day). Cytoplasmic processes of a macrophage are in contact with the myocyte. 10,000X.


