ARTIGOS

KINETICS OF *TRYPANOSOMA CRUZI* DESTRUCTION IN THE MOUSE SPLEEN

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Massive destruction of parasitized splenic macrophages was histologically observed at the height of a virulent infection caused by Trypanosoma cruzi *(Y strain) in the mouse. This was coincident with a sudden drop in parasitemic curve. Most of the animals died at this point, probably due to the liberation of toxic products, such as TNF, following the massive destruction of parasitized cells. However, parasitized-cell destruction indicated the transition from susceptibility to resistance. Although it has been extensively studied* in vitro, *this study contributes with the morphological counterpart observed in vivo by optical and electron microscopy. When infected animals were specifically treated during early infection transition to chronic phase was immediately observed without splenic parasitism. Animals that apparently recovered from massive cell-destruction in the spleen showed evidences of a rapid restoration of splenic architecture.*

Key-words: Trypanosoma cruzi. *Macrophages. Spleen.*

During infection of mice with a virulent Y strain of *Trypanosoma cruzi*, macrophages appear as the predominant site of parasite multiplication. This macrophagotropism is a prominent feature of *Type 1* strains, which include the Y strain and the Peruvian strain as prototypes[1].

By sequentially and histologically studying the evolution of a virulent Y strain infection in young mice, two striking features were observed: a) the progressive increase of macrophage parasitism in the spleen followed by a massive destruction of both macrophages and parasites at the height of infection, which coincided with a sudden drop in parasitemia and which often preceded animal death; b) when mounting splenic parasitism was abrogated by chemotherapy, infected animals survived for prolonged periods of time, splenic parasitism completely disappearing, while chronic infection followed its course. Thus, the dramatic and massive parasite destruction represents a transition from susceptibility to immunity. Although several well-conducted sequential histological studies of the spleen in acute *T. cruzi* infection with the Y strain have been

made, most of the characteristics of this transitional phase here considered have been overlooked[2 5 9 10].

The present work attempts to investigate by light and electron microscopy the splenic changes leading or preceding the transition from susceptibility to resistance during a virulent *T. cruzi* infection *in vivo*.

MATERIAL AND METHODS

White swiss mice, 10/12g, of both sexes were intraperitoneally injected with 1,000 to 20,000 blood forms of *T. cruzi* in eight series of experiments, each involving about 50 animals. Parasitemia was evaluated daily for the first five groups and, for the other three groups, every other day, starting on day 5th. Mortality was very high. Even with small inoculum (5,000 trypomastigotes) mortality was total by the 10-11th day, eventually reaching the 15th/18th day in those receiving the smallest inoculum (1,000 trypomastigotes). Animals found dead were discarded. Following inoculation the animals were sacrificed on the 5th, 7th, 10th, 12th, 14th, 15th and 18th day.

To avoid high mortality, a group of 95 mice infected with 2,000 to 5,000 trypomastigotes was treated on the 7th day of infection with 50mg/kbw of benznidazole daily, during 5 days. They were sacrificed at different periods of time, up to 30 days after infection. Parasitemia was considerably reduced after treatment and

remained low thereafter (Figure 1) and the animals presented normal development with no signs of disease.

Sacrificed animals were submitted to complete autopsies. The spleen was weighted. Fragments of the spleen, liver, heart and
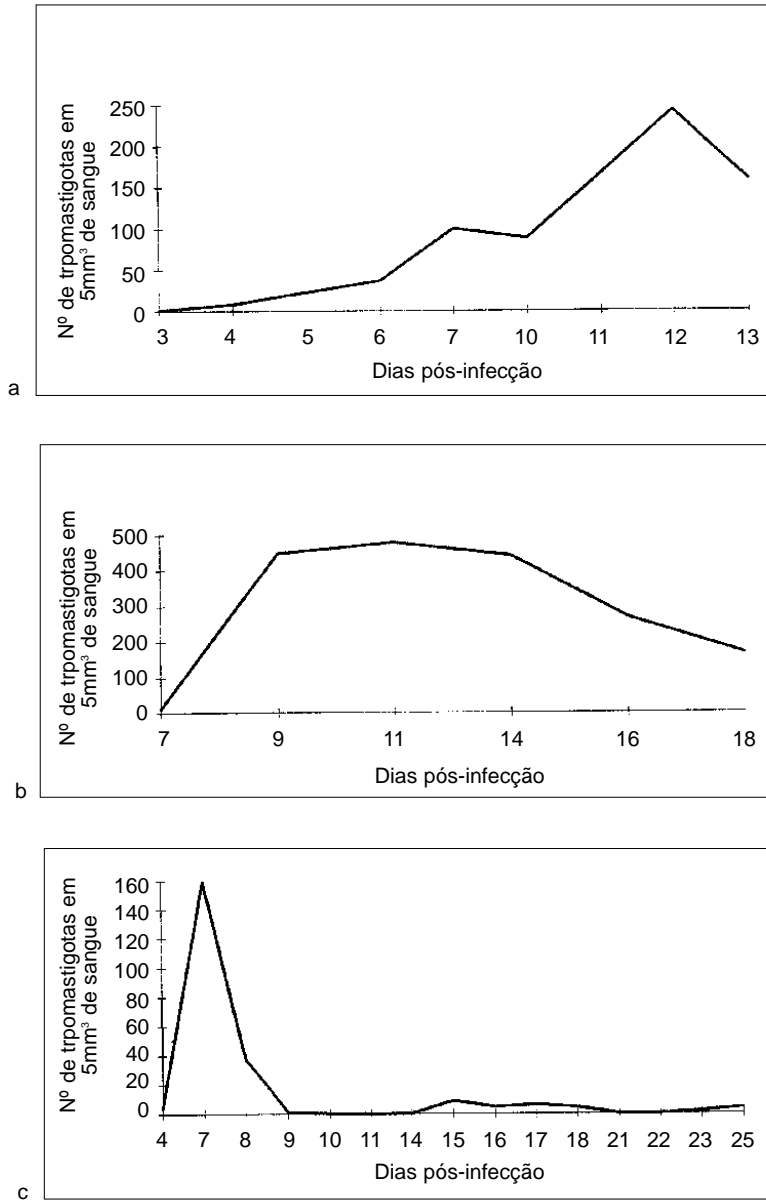

Figure 1 - Parasitemic curves: a) animals inoculated with 20,000 trypomastigotes; b) shows a somewhat less severe course seen in animals inoculated with 4,000 trypomastigotes; c) it demonstrates the effect of treatment, which was administered from the 7th up to the 12th day of infection.

intestines were fixed in neutral formalin (Millonig fixative). and later embedded in paraffin. The sections obtained were stained with hematoxilyn and eosin. Small pieces of splenic tissues were immediately fixed in 4% gluteraldehyde in 0.2M sodium cacodylate buffer, pH 7.4, during one hour, post-fixed for one hour in 1% pH 7.4 osmium tetroxide in 0.3M cacodylate buffer. These fragments were embedded in 812 polybed resin. The ultra-thin sections were contrasted with lead citrate and 7% uranyl acetate, and examined under an M-9 Zeiss electron microscope at 50Kv.

Parasitemia was performed in a drop of blood obtained from the mouse tail, covered with a cover slip and immediately examined with 40x objective x 10x ocular. One-hundred fields were counted and results were expressed as averages (Figure 1).

RESULTS

Parasitemic curves are shown in Figure 1.

At light microscopy parasitism of splenic macrophages was detected on day 5th in isolated cells present specially within the marginal zone of the lymph follicles. Occasionally, macrophages in the red pulp also appeared parasitized at this time. Parasitism became more evident from the 7th day on. Amastigotes in cells of the white pulp appeared later (15th day). Progressive accumulation of parasitized cells in the spleen was followed at first by relatively mild changes in splenic histology. Only after the 7th day of infection, three main changes became progressively more constant: hyperplasia of non-parasitized macrophages, depletion of lymphoid cells in the red pulp and activation of germinal centers of the white pulp. Depletion of lymphocytes was probably due to apoptosis, since many of them exhibited cytoplasmic condensation, nuclear clumping, picnosis and cariorrhexis. Non-parasitized macrophages, presented clear and enlarged cytoplasm, tending toward an epithelioid appearance. The center of the lymphoid follicles and the marginal perifollicular zones were expanded and the latter gradually fused with the red pulp. Dark dots, probably nuclear fragments, were frequently found within phagocytes in the center of the white pulp. After the 7th day of infection a few, isolated, parasitized macrophages disintegrated and the liberated amastigotes were seen within the shrunken and vacuolated macrophage cytoplasm or free in the interstitial tissue. This was regularly accompanied by an influx of polymorphonuclear neutrophils, which sometimes were seen with phagocytized amastigotes (Figure 2).
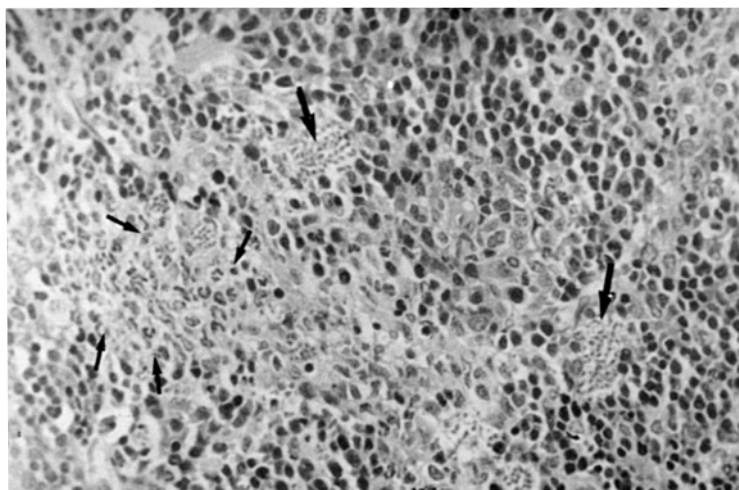

Figure 2 - Infiltration of polymorphonuclear neutrophil leukocytes around disintegrating parasitized macrophages (small arrows) as observed at the 7th day of infection. Heavily infected but well preserved macrophages are also present (larger arrows). Hematoxylin & Eosin, 250X.

Macrophage parasitism, lymphoid-cell depletion and macrophage hyperplasia became accentuated in the following days. However, the most striking change was observed around the 12th-15th days when intracellular parasitism was at its highest point. Then, all parasitized cells appeared swollen, fragmented, containing disintegrating parasites (Figure 3). No polymorphonuclear leukocytes were seen at this time, when a single parasitized macrophage could ever be detected, even after a thorough search of the histological sections. As indicated before these changes were coincident with a sharp decline in parasitemic curve and this event was regularly followed by the death of the animal.

Macrophages in the liver (Kupffer cells) also contained amastigotes and revealed the same changes observed in splenic macrophages (Figure 4). However, sections of the myocardium showed well preserved parasites within myocytes at the same time parasitized cells in the spleen and liver were undergoing destruction (Figure 4).

Some animals were sacrificed next day after parasitemic drop and so the degradative changes could be analyzed by electron microscopy. Then, every intra and extra-cellular parasites showed evidences of degradation (Figure 5). Amastigotes were found within the cytoplasm of disintegrating macrophages or free in the interstitial tissue. In both cases they exhibited
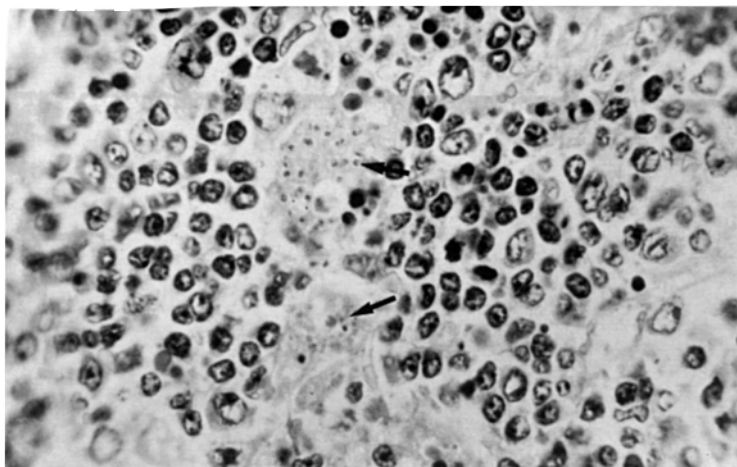

Figure 3 - Aspect of the red splenic pulp during the phase of massive cellular destruction. Parasitized macrophages appear swollen, pale and vacuolated. Internalized parasites exhibit irregularities in nuclear size and staining (arrows) Hematoxylin & Eosin, 400X.

nuclear and cytoplasmic degenerative alterations. They usually presented an irregular outline and shrunken, dark, vacuolated cytoplasm and nuclear picnosis or cariorrhexis (Figure 6). The kinetoplast/mitochondrial complexes were markedly swollen and/or vacuolated. All parasitized macrophages were partially or totally destroyed, but the non-parasitized ones appeared normal. Frequently aggregated and partially degranulated platelets were observed within splenic capillaries (Figure 7), which showed excessive signs of pinocytosis and irregular vacuolization. Sometimes cells with

the characteristics of myofibroblasts (fusiform cells with dark submembranar contractile apparatus) exhibited focal proliferation, forming clusters in the middle or in close proximity to disintegrated macrophages. (Figure 8).

Two animals survived up to the 18th day of infection, 4 days following parasitemic drop. One was agonizing, but the other seemed in fair condition. No parasites were seen in their spleens, but numerous viable macrophages containing nuclear and other debris within the cytoplasm were observed. Lymphoid-cell
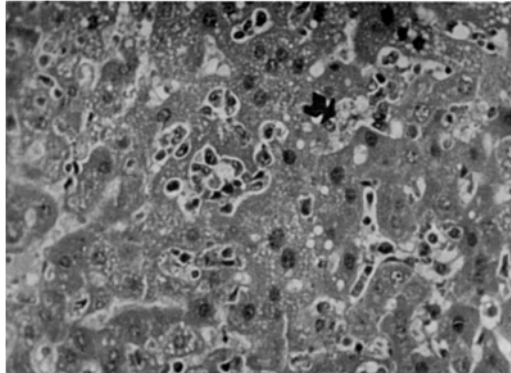
Figure 4 - Parasitized macrophages inside the hepatic sinusoids show disintegration in a similar way and at the same time as the splenic macrophages during the phase of massive cell destruction (arrow). Hematoxylin & Eosin, 160X.
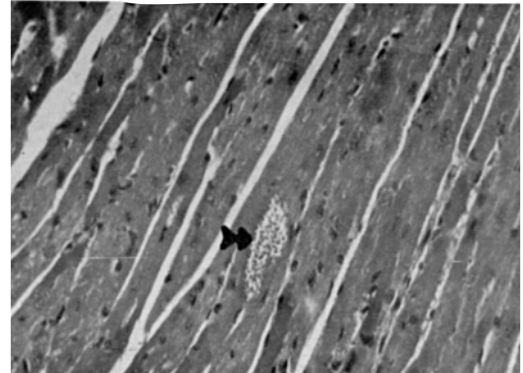

Figure 5 - Section of the myocardium showing a large well preserved amastigote collection inside a myocyte. There is no reaction within the myocardial area seen in this picture. Hematoxylin & Eosin, 160X.
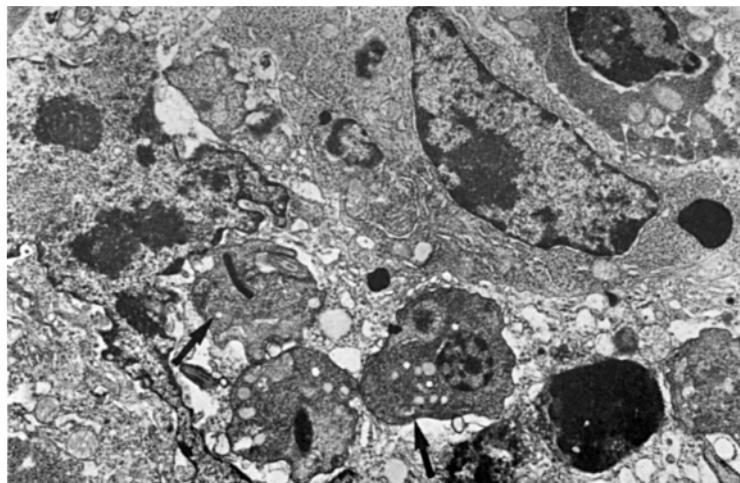

Figure 6 - Electron micrograph showing disintegrating amastigotes within a vacuolated splenic cell cytoplasm. The parasites exhibit an irregular outline, condensation and vacuolation of the cytoplasm, tumefaction of the mitochondrial-kinetoplasm apparatus and picnosis (arrows). 7,000X.

depletion was not a striking feature as in the previous cases. Accumulation of plasmocytoid and other basophilic lymphoid cells appeared at several scattered foci within the red pulp. Focal accumulation of macrophages, sometimes with a micro-granulomatous structure, was also observed. The marginal zone and the lymph follicles became more apparent, suggesting a returning to the normal splenic structure. However, a few clear, empty areas were present within the red pulp, suggesting the dropping out of splenic cells.

The spleens taken from treated mice were essentially within normal limits and no parasites were seen within macrophages or elsewhere in the spleen after 5 days of

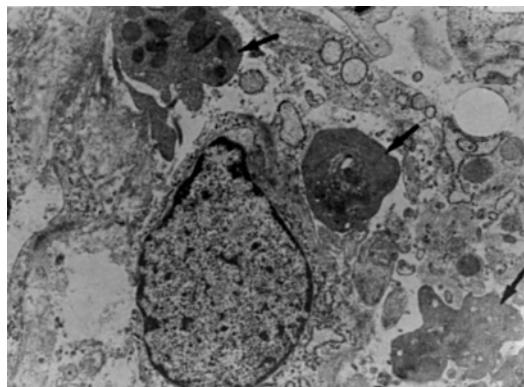benznidazole treatment. Parasites were detected in other organs within muscle cells, but not in macrophages.

Figure 7 - Partially degranulated platelets (arrows) appeared frequently within the splenic capillaries, as shown in this electron micrograph. 7,000X,
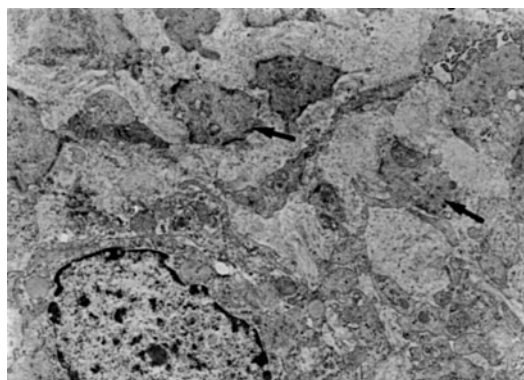

Figure 8 - Focal proliferation of fusiform connective cells, identified as myofibroblasts, seen in the spleen of a mouse during the phase of massive parasitized macrophage disintegration. Electron Micrograph, 4.400X.

DISCUSSION

Sequential observation of splenic changes during a virulent *T. cruzi* infection in the mouse revealed that parasitism of splenic macrophages progressed up to a point of massive involvement and then, suddenly, there was a diffuse destruction of parasitized cells and parasites. Significance of this finding involves immunologic and pathogenetic aspects of great importance. Immune destruction of infected cells probably means the expression of foreign antigen(s) in the macrophage external membranes. Such antigens are then a target to sensitized cytotoxic lymphocytes, specific antibodies, and/or antibody-mediated cell citotoxicity. Why the destruction occurred suddenly is a matter probably related to the reaching of an equivalence or critic point amongst immunologic factors[15].

This would mean that the host became strongly immune, but paradoxically this transition is often fatal, probably due to the liberation of toxic products by the disintegrating cells. One of such product could well be TNF (tumor necrosis factors), since it is synthesized in great amount by macrophages parasitized by *T. cruzi*[13] and has been hold responsible for generalized toxic symptoms and even shock[10]. Eventual disintegration of parasitized macrophages occurring before the episode of massive cell destruction may depend on a different pathogenesis, since the host reaction was then limited to a focal infiltration by polymorphonuclear leukocytes. Similar findings observed in the connective tissue of mice have been correlated with local antigen-antibody reaction and hypocomplementemia[12].

Macrophages are probably the first cells to be infected by *T. cruzi*. Trypomastigotes entering or being phagocytized by naive macrophages readly escape from the phagocytic vacuole and get into the cell cytosol, where they successively multiply as amastigotes. However, if macrophages are stimulated by sensitized T-cell lymphocyte soluble products, they become able to rapidly destroy internalized trypomastigotes[6 7]. These early events are crucial and may determined the outcome of the infection[14]. Suppressive chemotherapy accelerates this transitional phase and thus allows for a direct passage to the chronic stage, as has been demonstrated here and by others[3]. Probably, the elimination of parasites from the spleen would expose adequate antigens to the immune system and/or would interferes with the generation of suppressive splenic cells or other suppressive factors[4].

Findings observed in two animals that apparently survived the critical period of massive parasitized-cell destruction are suggestive that reconstitution of splenic structure may be quite rapid and complete. That capacity for regeneration shown by the spleen is in

keeping with observations made in other lymphoid tissues[8].

RESUMO

Um estudo histologico sequenciado mostrou que o parasitismo dos macrófagos esplênicos por uma cepa virulenta (cepa Y) do Trypanosoma cruzi *tem um curso progressivo, mas chega até um ponto em que todos as células parasitadas são subitamente destruídas. Tal achado coincidiu com uma quéda brusca da curva parasitêmica e com a morte da maioria dos animais, provavelmente devido à liberação de produtos tóxicos (como o TNF) pelas células desintegradas. O achado foi interpretado como o auge da transição entre uma fase de susceptibilidade e outra de resistência. Embora esta transição tenha sido bem estudada* in vitro*, este estudo contribui com os dados do substrato morfológico observados* in vivo*, através da microscopia ótica e eletrônica. O tratamento específico e supressivo feito na fase inicial da infecção acarreta uma transição imediata para a fase crônica e aí o parasitismo esplênico desaparece completamente. Os animais que aparentemente se recuperaram expontaneamente após a fase de destruição maciça dos macrófagos parasitados exibiram evidências de que a reconstituição da estrutura esplênica pode se fazer rapidamente.*

Palavras-chaves: Trypanosoma cruzi. *Macrófagos. Baço.*

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