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Presence of anti-*Trypanosoma cruzi* antibodies in the sera of mice with experimental autoimmune myocarditis

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Summary. The existence of antigens shared in common by *T. cruzi* and heart muscle cells is suggested by the presence of antibodies binding to the parasite surface in the serum of mice with autoimmune myocarditis induced by immunization with syngeneic heart antigens.
Key words. Trypanosoma cruzi; Chagas' disease; myocarditis; autoimmunity.

South American trypanosomiasis (Chagas' disease) affects several million people in Latin America. About 10–20% of humans infected with *Trypanosoma cruzi* develop a severe chronic myocarditis 10 to 30 years after infection. On the basis of this long asymptomatic interval, and the fact that usually no parasites are seen at the site of the lesions, indirect mechanisms, mainly immunological, have been postulated for explaining the tissue damage [1]. Among them autoimmunity has been claimed to be a possible pathogenetic mechanism, but the conditions which may lead to the appearance of autoreactivity have not been clearly defined. Persistence of autoreactive clones after the polyclonal lymphocyte activation present during the acute phase of infection, or the existence of epitopes shared in common by the parasite and the mammalian host tissues, have been suggested as putative mechanisms of autoreactivity [2].

In the last few years most of the search for epitopes shared by the parasite and the host tissues has been carried out by developing monoclonal antibodies against nerve tissue, *T. cruzi* or closely related trypanosomatids, and determining their cross-reactivity with parasite and mammalian tissues. An alternative approach for investigating antigenic determinants common to the parasite and the host is to search for *T. cruzi* antibodies in patients with autoimmune disease, or in animals with experimentally-induced organ-specific autoimmune diseases. In the present report we investigate whether mice with experimental autoimmune myocarditis develop antibodies that recognize *T. cruzi* epitopes.

Materials and methods

Experimental autoimmune myocarditis (EAM) was induced in 3-month-old female BALB/c mice. Animals received, in multiple intradermal sites, 8 weekly injections (0.2 ml each) of a BALB/c heart homogenate incorporated in Freund's adjuvant (FA). For the first injection complete FA was employed; incomplete FA was used for the remainder. Fifteen days after the last injection a booster injection of 0.2 ml of the heart homogenate in phosphate buffered saline (PBS) was given intraperitoneally, and 7 days later the mice were bled to death by retroorbital puncture. Controls were mice receiving, according to a similar schedule, a BALB/c kidney homogenate prepared in the same way as the heart homogenate, or mice injected with FA alone. Eight animals were employed for each group.

For the preparation of the organ homogenates the organs were carefully dissected, sectioned into small pieces, washed repeatedly in Hanks' balanced salt solution, suspended 1/10 (w/v) in Hanks', and homogenized in a Potter-Elvehjem tissue homogenizer with a tightly-fitting Teflon pestle. The homogenate was centrifuged for 15 min at 100 × g to remove tissue clumps, divided into small aliquots and stored at −70 °C. Its protein concen-

tration was determined by Lowry's method and adjusted to 1 mg/ml.

To search for anti-*T. cruzi* antibodies we used as targets epimastigotes grown in a biphasic medium, dried on glass slides, fixed for 15 min in cold (4 °C) acetone, and washed repeatedly in phosphate buffered saline (PBS). Parasites were incubated with the mouse sera serially diluted in PBS for 30 min at room temperature, washed in PBS, post-incubated with a fluorescein-labeled goat antiserum against total mouse immunoglobulins (BioYeda, Rehovot, Israel), and mounted in buffered glycerine.

In order to test whether the sera recognized epitopes located on the parasite surface, living epimastigotes were incubated overnight at 4 °C with the mouse sera diluted 1/40 in Hanks' with 0.1% bovine albumin. After incubation the parasites were washed 3 times with cold (4 °C) Hanks' and fixed for 1 h in 4 °C buffered formaldehyde. After repeated washings in PBS the parasite suspension was incubated with a biotinylated goat IgG against mouse IgG (BioYeda) and post-incubated with streptavidin coupled with horseradish peroxidase (BioYeda). Enzyme activity was revealed with 3.4-diaminobenzidine in 0.01% hydrogen peroxide. The parasites were washed repeatedly in PBS, dried on glass slides and mounted in gelatine jelly.

The search for antiheart antibodies was carried out as previously described[3], by incubating isolated syngeneic adult cardiocytes with the test sera. Test sera were adsorbed overnight at 4 °C with a heart or *T. cruzi* homogenate, and centrifuged for 1 h at 20000 × g at 4 °C. The heart homogenate was the same as that employed for the immunization, and the parasite homogenate was prepared as previously described[3]. For histological study the hearts were fixed in Bouin's fluid, sagitally sectioned in halves and embedded in paraffin. Serial sections were stained with hematoxylin – eosin.

Results and discussion

In the hearts of mice immunized with the heart homogenate a mononuclear inflammatory infiltrate was present in all the animals (fig. 1). The infiltrates were nodular and showed no preferential localization. In the mice immunized with kidney homogenate, or those which received FA alone, no inflammatory infiltrates were observed. The sera of mice immunized with heart antigens contained antibodies that bound to epitopes present in the cardiocyte surface, which showed a diffuse linear fluorescence (fig. 2). The same sera reacted with the acetone-fixed epimastigotes in dilutions up to 1/160 (fig. 3), and also with living epimastigotes; the latter showed a positive reaction on the parasite surface. The enzyme activity was not diffuse, and appeared to be localized in discrete zones with no preferential localization (fig. 4). When cardiocytes and fixed or living parasites were incubated with sera from the control mice immunized with kidney homogenate or injected with FA alone, no binding of antibodies was observed. After EAM sera had been adsorbed with the heart homogenate their reactivity with cardiocytes and parasites became negative, but adsorption of the same sera with the *T. cruzi* homogenate caused only their binding with epimastigotes to become negative.

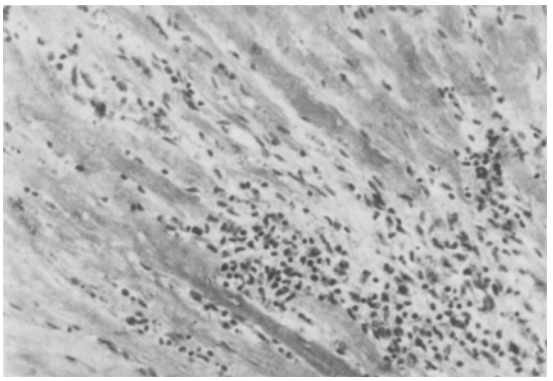

Figure 1. Mononuclear inflammatory infiltrate in the heart of a mouse immunized with heart homogenate. Hematoxylin-eosin. × 250.

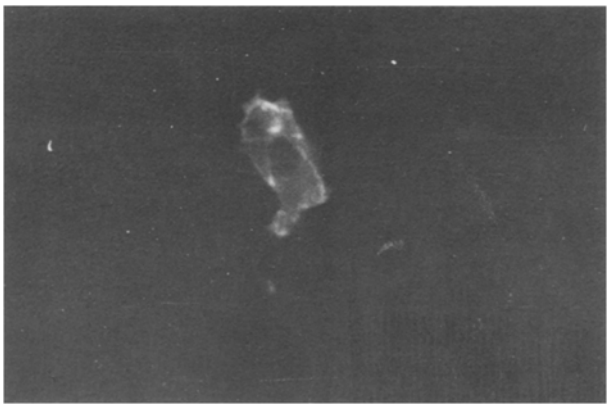

Figure 2. Diffuse linear fluorescence on the surface of an isolated cardiocyte incubated with serum from mice with chronic autoimmune myocarditis and post-treated with FITC-labeled anti-mouse IgG. × 400.

In recent years, several reports have described the presence of anti-heart autoantibodies in the sera of humans with Chagas' disease[4] or experimentally infected animals[5]. The origin of those antibodies, and the role that they may play in the pathogenesis of tissue lesions, have been the object of much debate, and it has not been established whether they appear as a consequence of the tissue destruction provoked by the infection[6], are due to the persistence of autoreactive clones after the polyclonal expansion that occurs during the acute phase of infection[7] or arise as a response to epitopes cross-reacting between the parasite and the heart cells. This last possibility has been explored previously by means of raising monoclonal antibodies against nervous tissue, *T. cruzi* and closely related trypanosomatids, and testing their cross-reactivity with parasites and different mammalian tissues. Monoclonal IgM antibodies that recognize both

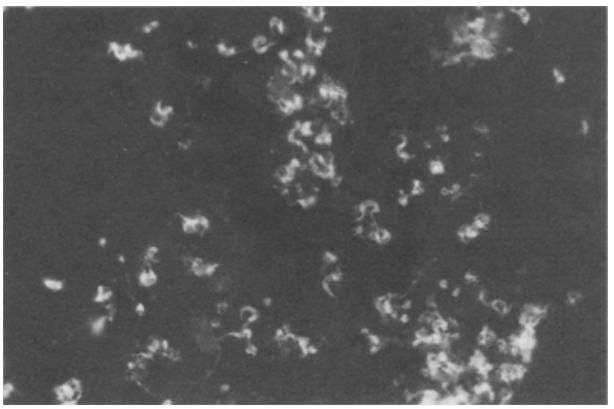

Figure 3. Intense fluorescence stain of *T. cruzi* epimastigotes incubated with the serum from a mouse with autoimmune myocarditis. × 250.

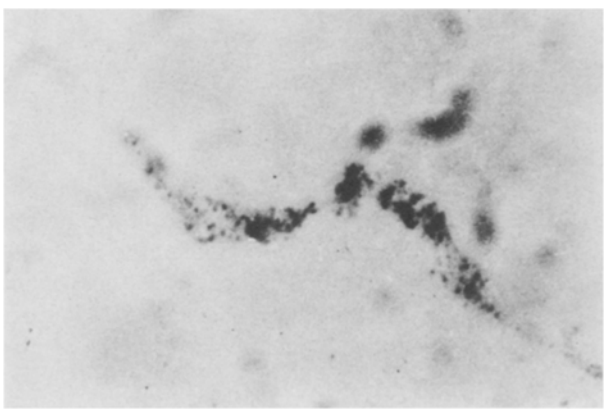

Figure 4. Microphotograph of epimastigotes incubated with serum from EAM and post-treated with biotinylated anti-mouse IgG and horseradish peroxidase-labeled streptavidin. The enzyme activity indicating the sites of bound IgG appears as discrete zones on the parasite membrane. × 1250.

parasite, neuronal and striated muscle epitopes [8] and IgG antibodies that bind to nerves, neurones and endothelial cells of capillaries have been reported [9–12]. In those studies no cross-reactivity between the parasite and the cardiocyte surface was described. Since the targets of the tissue damage present in the course of chagasic cardiopathy are the heart muscle cells, it seems relevant to establish whether the cross-reactivity antibodies bind to epitopes present on the cardiocyte membrane or to cytoplasmic components, or to other tissue structures present in the heart.

Previous work from our laboratory showed that mice chronically infected with *T. cruzi* develop antibodies that bind to the surface of living, isolated cardiocytes and that those antibodies are able to elicit antibody-dependent cytotoxicity [3]. The results presented here show that during the course of EAM, autoantibodies reactive with the cardiocyte surface are also synthesized. The facts that the same sera also recognize structures present on the parasite surface, and that this reactivity can be abolished by adsorption either with heart or with *T. cruzi* homogenates, suggest the existence of epitopes shared in common by the heart muscle cells and *T. cruzi*. Whether they are responsible for the heart autoreactivity present in Chagas' disease remains to be determined. The fact that myocarditis can be induced by immunization of rabbits [13] or mice [14] with subcellular fractions of *T. cruzi* or other protozoan flagellates [15] gives support to the possibility that immunization with cross-reacting epitopes may lead to myocarditis. In addition, since our studies show that anti-*T. cruzi* antibodies appear during the course of an autoimmune myocarditis in animals neither immunized nor infected with the parasite, and since the diagnosis of *T. cruzi* infection in humans is based on serological reactions, the possibility must be borne in mind that Chagasic cardiopathy may be diagnosed in humans who are not in fact infected, but have heart disease. Studies are in progress to test this possibility.

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