Anti-CD4 Abrogates Rejection and Reestablishes Long-Term Tolerance to Syngeneic Newborn Hearts Grafted in Mice Chronically Infected with Trypanosoma cruzi

By R. Ribeiro dos Santos,* Marcos A. Rossi,† J. L. Laus,‖ J. Santana Silva,§ Wilson Savino,¶ and José Mengel$ }

From the Evandro Chagas Hospital, Instituto Oswaldo Cruz, 20010 Rio de Janeiro; the Departments of †Pathology and §Immunology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, 14049 Ribeirão Preto; the ||Department of Surgery, Faculty of Veterinary Medicine, Unesp, 14870 Jaboticabal; and the ‡Department of Immunology, Instituto Oswaldo Cruz, 20010 Rio de Janeiro, Brazil

Summary

The contribution of autoimmunity in the genesis of chronic Chagas’ heart pathology is not clear. In the present study, we show that: (a) BALB/c mice chronically infected with Trypanosoma cruzi reject syngeneic newborn hearts; (b) in vivo treatment with anti-CD4 but not anti-CD8 monoclonal antibodies (mAbs) abrogates rejection; (c) CD4+ T cells from chronically infected mice proliferate in vitro to syngeneic myocardium antigens and induce heart graft destruction when injected in situ; (d) anti-CD4 treatment of chronically infected mice establishes long-term tolerance to syngeneic heart grafts; and (e) the state of tolerance is related to in vitro and in vivo unresponsiveness of the CD4+ T cells. These findings allow us to suggest that autoimmunity is the major mechanism implicated in the rejection of syngeneic heart tissues grafted into the pinna of the ear of mice chronically infected with T. cruzi. The similarity of the lesions to those found in humans suggests that autoimmunity is involved in the pathogenesis of chagasic cardiomyopathy in humans. Moreover, this could imply therapeutic strategies by reestablishing long-term tissue-specific tolerance with anti-CD4 mAb treatment, mediating anergy, or deleting the responder CD4+ T cells to heart tissue antigens.

Chagas’ disease (American trypanosomiasis) is caused by transmission of the protozoan Trypanosoma cruzi by a Triatoma bug. It is one of the leading causes of death in many countries of Latin America. The disease is characterized by three phases: acute, indeterminate, and chronic (1). The heart is the most severely and frequently affected organ. The degree of the cardiac involvement during the acute phase varies from mild to severe. The acute heart disease, which courses with parasitemia, is characterized histopathologically by foci of myocytolytic necrosis and an impressive mononuclear inflammatory infiltrate directly related to tissue parasitism. The indeterminate phase is usually of long duration (up to 10–30 yr) and is characterized by the absence of histopathological lesions. Progression from the acute to chronic form of Chagas’ disease coincides with clearance of parasites from blood stream and tissues. In the chronic phase variable degrees of cardiac hypertrophy and dilatation occur with or without thinning of the apical region (apical aneurism). Foci of myocardial necrosis and degeneration are present with an inflammatory infiltrate predominantly composed of mononuclear cells and interstitial fibrosis. Myofibers containing parasites are rarely observed.

The participation of autoimmune mechanisms in the genesis of the chronic myocarditis of Chagas’ disease has been suggested and is still a controversial matter (2). Organ-specific autoimmunity is often related to the outcome of many parasitic infections caused by virus, bacteria, and parasites (3–5). Thus, it would be of importance to find out how infection triggers autoimmunity and how the resultant autoimmunity contributes to the disease. This would improve therapeutical tools, thus contributing to the better understanding of the breaking of tissue-specific tolerance.

The present study was undertaken to investigate the autoreactivity against syngeneic heart tissue in vivo by grafting newborn hearts into the pinna of the ear of mice chronically infected with T. cruzi. The results demonstrate that syngeneic heart tissues once grafted in normal recipients are accepted, while they are invariably rejected in mice chronically infected...
of Immunology, University of São Paulo, Ribeirão Preto, Brazil.

**Materials and Methods**

**Mice.** BALB/c and C57BL/6 mice were bred and maintained under standard conditions in the animal colony at the Department of Immunology, University of São Paulo, Ribeirão Preto, Brazil.

**Parasites.** The Y and the Colombian strains of *T. cruzi* were used. Blood-derived trypomastigote forms were used in all experiments. Mice were infected subcutaneously with 10³ parasites. The PF strain is nonpathogenic, noninfective, and has been shown to protect mice challenged with virulent *T. cruzi* strains (6). The PF strain is essentially represented by epimastigote forms and is maintained in vitro in Warren's medium. In all experiments in which this strain was used, 2 × 10⁴ parasites/animal/wk were injected during four consecutive weeks.

**Chronic Infection.** Mice infected with the Y strain of *T. cruzi* were obtained by selecting those animals that survived the acute phase. However, mice infected with the Colombian strain of *T. cruzi* had to be treated during the acute infection with n-benzyl-2-nitro-1-amidazolacetamide (Rochagan, Roche Lab., Brazil), orally administered, in a dose of 100 mg/kg/d during 10 d, beginning on the 10th day after infection. 5–6 mo after challenge, mice were considered in the chronic phase, with no parasites in the blood. Control mice were injected with saline instead of the PF, Y, and Colombian strains of *T. cruzi*.

**Heart Transplantation Technique.** We have modified the method originally described by Fulmer et al. (7). A full description appears elsewhere (8). Briefly, newborn mice were anesthetized by hypothermia, and the heart was immediately excised, rinsed in cold sterile saline (pH 7.2), and implanted into the dorsal base of the pinna of the ear. The implant was subcutaneous, and the surgical incision was gently closed with the aid of forceps. In the present study grafts were sex matched, usually using male recipients. In addition, in many experiments a given recipient had one graft in each ear, a procedure that allowed us to have in the same animal a control and an experimental heart transplant. The majority of grafts in normal syngeneic mice start to present contractile activity after 7–10 d posttransplantation. Rejection was defined macroscopically as the disappearance of the grafted tissue. This corresponded microscopically to perivascular, interstitial, and perimyocytic mixed inflammatory infiltrate (lymphomononuclear cells ± neutrophils) with myocyte necrosis.

**Monoclonal Antibodies.** Anti-L3T4 (GK1.5) (9), anti-Lyt-2 (53.6.75) (10), and anti-Thy-1.2 (HO13.4 from American Type Culture Collection, Rockville, MD) mAbs were obtained in the form of ascitic fluid. Gamma globulin fraction of the ascitic fluid was purified anti-mouse Fab antibody. T cells were obtained by 1 h depletion during the process of immersion fixation with glutaraldehyde (0.3% ruthenium red mixture in 0.1 M cacodylate buffer, pH 7.2), and implanted into the dorsal base of the pinna of the ear. The implant was subcutaneous, and the surgical incision was gently closed with the aid of forceps. In the present study grafts were sex matched, usually using male recipients. In addition, in many experiments a given recipient had one graft in each ear, a procedure that allowed us to have in the same animal a control and an experimental heart transplant. The majority of grafts in normal syngeneic mice start to present contractile activity after 7–10 d posttransplantation. Rejection was defined macroscopically as the disappearance of the grafted tissue. This corresponded microscopically to perivascular, interstitial, and perimyocytic mixed inflammatory infiltrate (lymphomononuclear cells ± neutrophils) with myocyte necrosis.

**Histological Examination.** The ears containing the heart grafts were fixed in Bouin’s solution. After fixation, all ears were sectioned transversally across the graft at 2-mm intervals, embedded in paraffin, sectioned, stained with hematoxylin and eosin or Masson’s trichrome, and examined under the light microscope. For electron microscopic study, small blocks of tissue were fixed in glutaraldehyde, postfixed in osmium tetroxide, dehydrated in acetone, and embedded in araldite. Semithin sections stained with toluidine blue were examined under the light microscope, and a suitable area was selected for preparation of ultrathin sections, which were double stained with uranyl acetate and lead citrate, and examined in an electron microscope at 80 kV (EM 109; Carl Zeiss, Oberkochen, Germany).

To visualize permeability alteration of the myocyte sarcolemma, small pieces of myocardium were immersed in a 1.2% glutaraldehyde (0.3% ruthenium red mixture in 0.1 M cacodylate buffer, postfixed in a 2% osmium tetroxide) 0.3% ruthenium red mixture in 0.1 M cacodylate buffer, dehydrated, and embedded in Epon (12–14). Unstained ultrathin sections selected on the basis of a light microscope were examined in the electron microscope. Since this technique is subjected to artifacts because ruthenium red is introduced during the process of immersion fixation with glutaraldehyde, random sampling and blind evaluation were performed as a control for these effects.
Results

Syngeneic Heart Graft Rejection in Chronically Infected Mice. Newborn hearts grafted in syngeneic recipients showed grossly visible contractile activity, usually within 2 wk after surgical procedure. The heterotopic hearts persisted up to 100 d. On the contrary, grafting of syngeneic newborn hearts in mice already developing chronic Chagas' disease was consistently rejected and completely absorbed at most by the 20th day posttransplantation. Actually, 70% of the grafts were acutely rejected and disappeared in 2 wk. Similar results were obtained regardless of which T. cruzi strain (Y or Colombian) was used to generate chronic infection. Rejection of allogeneic hearts by normal recipients was delayed in relation to infected groups (Fig. 1). Furthermore, it should be pointed out that heart contractile activity was never detected in the organs grafted into chronically infected animals. Syngeneic grafts were not rejected in either normal or PF strain–hyperimmunized mice (Fig. 1). In fact, these grafts persist for long periods (up to 6 mo) without significant changes.

Morphological Findings. Newborn mouse syngeneic hearts transplanted into the ear of normal recipients became vascularized from the auricular artery at the base of the ear. The central cavities of the grafts were filled with adipose tissue (fatty infiltration). Elongated myocardial cells with abundant myofilaments were seen. In most of the areas, myocytes were arranged in parallel bundles. The nuclei were elongated. No mitotic figures were seen. The interstitial space was diffusely widened, particularly around vessels, due to collagen fibrils, fibroblasts, and areas of mononuclear cells (Fig. 2). These hearts showed a mature ultrastructural appearance with par-

Figure 1. Engraftment or rejection of syngeneic newborn hearts in different groups of mice. Normal BALB/c mice ( ), PF strain–hyperimmunized BALB/c mice ( ), or BALB/c mice chronically infected with the Colombian strain of T. cruzi ( ) were grafted with syngeneic newborn hearts in both ears. A group of normal BALB/c mice was grafted with C57BL/6 newborn hearts as an allogeneic control ( ). Groups were of 10 animals each.

Figure 2. Newborn myocardium 2 mo after transplantation into the ear of syngeneic control mice. In most of the areas, the myocytes are arranged in parallel bundles. The nuclei are elongated. No mitotic figures are seen. The interstitial space is diffusely widened due to mild interstitial fibrosis. Fatty infiltration (arrows) is noted at the ventricular cavity and the periphery of the graft. s, skin; c, ear cartilage. Hematoxylin and eosin; A, x 170; B, x 340.
allel bundles of myofibrils alternating with rows of mitochondria and differentiated intercalated discs. The findings in the myocardium stained with ruthenium red were similar to those previously described (12-14). None of the cardiac cells had been penetrated by the tracer, and the density was confined to the extracellular space, particularly the pericellular material and collagen bundles.

The syngeneic heart grafted into the ear of chronically infected mice showed a marked inflammatory infiltrate composed of lymphomononuclear cells associated with multiple and diffuse foci of myocytolytic necrosis (Fig. 3), quite similar to the pattern of allogeneic heart grafts (Fig. 4). When the relationship between mononuclear cells and cardiocytes was examined by electron microscope, a close proximity between the two cells could be seen. The sarcolemma of the myocardial cells had a wavy outline. The basal lamina was loosely adherent, with a relatively low electron density and finely granular appearance. The mononuclear cells commonly showed convolution of their surface in the vicinity of the myofibrils. Numerous cytoplasmic projections come into close apposi-

![Figure 3. Syngeneic newborn myocardium 13 d after implantation in BALB/c mouse chronically infected with T. cruzi. Focal myocytolytic necrosis and extensive interstitial lymphomononuclear infiltrate and interstitial fibrosis. Hematoxylin and eosin; x410.](image1)

![Figure 4. C57BL/6 newborn myocardium 13 d after implantation in normal BALB/c mouse. Diffuse lymphomononuclear infiltrate associated with severe interstitial replacement fibrosis. Hematoxylin and eosin; x410.](image2)
tion with the myocytes (Fig. 5). The ruthenium red staining in syngeneic myocardium transplanted into chronically infected mice demonstrated, similarly to the allogeneic transplanted hearts, that the cytoplasmic components of myocytes were labeled at the site of macrophage contact, denoting alteration of the plasma membrane permeability (Fig. 6).

Differential Effects of In Vivo T Cell Subset Monoclonal Antibody Depletion on the Rejection of Syngeneic Heart Grafts. We have analyzed the cell population that was necessary for the rejection of syngeneic heart grafts in chronically infected mice by depletion of CD4⁺ or CD8⁺ single-positive T lymphocytes with mAbs. Fig. 7 shows that rejection of newborn
Reestablishment of Heart Tissue Tolerance before transplantation. In contrast, all mice receiving anti-CD4 mAb accepted 90% of syngeneic grafts (18/20) over a period of 100 d of observation, but are still able to reject full allogeneic heart transplants at most 23 d postengraftment. Histopathology of these mice have confirmed that no inflammatory reaction was present in the tolerant groups.

In Vivo Pretreatment with Anti-CD4 mAb

Heart tissue grafted into chronically infected mice could be prevented by treating the animals with anti-CD4 mAb 2 wk before transplantation. In contrast, all mice receiving anti-CD8 treatment rejected the heart grafts within 20 d after transplantation.

Reestablishment of Heart Tissue-specific Tolerance in Chronically Infected T. cruzi Mice by In Vivo Treatment with Anti-CD4 mAb

Chronically infected mice that had been previously treated with anti-CD4 mAb and naturally reconstituted thereafter, in order to check if such a reactivity would persist. We found that no graft destruction could be induced by these cells and grafts persisted for long periods (Table 2). Moreover, histopathology revealed a dramatic inflammatory reaction surrounding and within the grafts, when CD4 or total T cells from chronically infected mice were injected (Fig. 8).

Table 1. In Vivo Pretreatment with Anti-CD4 mAb Reestablishes Heart Tissue Tolerance

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Anti-CD4</th>
<th>Syngeneic</th>
<th>Allogeneic*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>19/20 (100)</td>
<td>0/20 (22)</td>
</tr>
<tr>
<td>Normal</td>
<td>+</td>
<td>20/20 (100)</td>
<td>0/20 (23)</td>
</tr>
<tr>
<td>Chronically infected</td>
<td>-</td>
<td>0/20 (20)</td>
<td>0/20 (19)</td>
</tr>
<tr>
<td>Chronically infected</td>
<td>+</td>
<td>18/20 (100)</td>
<td>0/20 (23)</td>
</tr>
</tbody>
</table>

* Hearts from newborn C57BL/6 mice were grafted in BALB/c mice from the different experimental groups. Numbers shown in parentheses represent the total time of graft observation in days.

Discussion

The pathogenesis of chronic Chagas' heart disease remains incompletely understood. Different mechanisms have been
Table 2. Injection of Different Cell Populations into Heart Grafts

<table>
<thead>
<tr>
<th>Donor groups</th>
<th>Cell population*</th>
<th>Incidence of graft survival</th>
<th>Period of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Total T cells</td>
<td>18/20</td>
<td>100 d</td>
</tr>
<tr>
<td></td>
<td>CD4</td>
<td>20/20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>CD8</td>
<td>19/20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Non-T cells</td>
<td>20/20</td>
<td>100</td>
</tr>
<tr>
<td>Hyperimmunized</td>
<td>Total T cells</td>
<td>19/20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>CD4</td>
<td>20/20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>CD8</td>
<td>18/20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Non-T cells</td>
<td>17/20</td>
<td>100</td>
</tr>
<tr>
<td>Chronically infected</td>
<td>Total T cells</td>
<td>0/20</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>CD4</td>
<td>0/20</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>CD8</td>
<td>20/20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Non-T cells</td>
<td>18/20</td>
<td>100</td>
</tr>
<tr>
<td>Anti-CD4-tolerized mice</td>
<td>Total T cells</td>
<td>20/20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>CD4</td>
<td>18/20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>CD8</td>
<td>19/20</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Non-T cells</td>
<td>19/20</td>
<td>100</td>
</tr>
</tbody>
</table>

* Purified cell populations from the different experimental groups were injected within the hearts grafted in normal recipients at a cell number of $5 \times 10^4$ in a total volume of $10 \mu l$. Hyperimmunized mice were inoculated four times in intervals of 7 d with $2 \times 10^7$ epimastigote forms of the PF strain of *T. cruzi* and challenged 30 d after the last inoculation with $10^3$ trypomastigote forms of the Y or Colombian strains of *T. cruzi*.

proposed. The lesions could be due to continuing parasitic invasion of the heart, local release of toxins, or both. The low levels of parasitemia and the very rare findings of tissue parasitic pseudocysts are arguments against the hypothesis that myocardial lesions are directly correlated with the presence of parasites. The chronic cardiomyopathy of Chagas' disease could be a neurogenic form of heart disease promoted by destruction of the parasympathetic ganglion cells in the...
In vivo studies with CD4⁺ T cells from chronically infected mouse with anti-CD4, show unresponsiveness to myocardial antigens. Purified CD4⁺, CD8⁺, total T cells, or non-T cells from naive mice, mice hyperimmunized with PF strain of *T. cruzi*, mice chronically infected with the Colombian strain of *T. cruzi*, or mice that had been treated with anti-CD4 were cultured (2 x 10⁶/well) together with irradiated filler cells (2 x 10⁶/well) in the presence of heart antigens from syngeneic animals (a previous study showed that 20 μg/ml was the optimal antigen concentration) for 4 d with [3H]thymidine added for the final 8 h. Data are expressed as the mean of triplicate wells in cpm. Data represent one of five similar experiments.

A set of experiments were done to determine the phenotype of the cells involved in the phenomenon of syngeneic heart rejection. In vivo depletion of CD4⁺, but not CD8⁺, T cells abrogates rejection. Heart grafts were retained by chronically infected mice during the course of anti-CD4 mAb treatment. After 2 mo of stopping antibody injections, the CD4⁺ population returned to normal levels, as judged by indirect immunofluorescence (not shown). However, grafts were not rejected. Instead they persisted for >6 mo, indicating that the recipients became tolerant. Thereafter, to differentiate between authentic tolerance and graft adaptation due to loss of the acute inflammatory reaction just after transplantation, a group of chronically infected mice was treated with the same mAb for 2 wk, and 2 mo after withdrawal of the antibody and reconstitution of the CD4⁺ compartment, heart grafts were implanted. These grafts were not rejected, surviving for >6 mo, clearly showing that a state of tolerance had been reestablished. In contrast, allogeneic grafts were not accepted and have been rejected in the maximum of 22 d posttransplantation. Anti-CD4 mAb was successfully used in similar experiments dealing with induction of tolerance to islet allografts in the mouse, or allogeneic heart grafts in the rat (20, 21).

The role of CD4 molecules in T cell activation has been analyzed. However, there has been much controversy in this regard over the part played by the CD4 glycoprotein. Recently, it has been reported that when preceded by ligation of CD4, signaling through TCR-α/β results in mature T cell unresponsiveness in vitro, due to induction of activation-dependent cell death by apoptosis (22). In contrast, it has been observed that in vivo treatment with anti-CD4 mediates proliferative clonal anergy of Vβ11 T cells during allotransplantation tolerance induction to pancreatic islets of Langerhans (23). The self reactivity of CD4⁺ T cells from chronically infected mice could also be shown by in vitro studies, where these lymphocytes proliferate in the presence of heart antigens plus irradiated filler cells. Non-T cell populations or CD8⁺ T lymphocytes failed to show any activity in this assay. Moreover, CD4⁺ T cells obtained from mice previously treated with anti-CD4 could not reject syngeneic heart grafts when injected in situ, thus indicating that tolerance after anti-CD4 treatment correlates with the disappearance of myocardial reactivities in vitro and in vivo. Our results do not allow further distinction between deletion or proliferative anergy as the basis for tolerance in this model, since in vitro unresponsiveness to myocardium antigens detected after in vivo anti-CD4 treatment could not be reverted by addition of IL-2-rich supernatants (not shown), and no molecular marker can be used, so far.

In vivo studies with CD4⁺ T cells from chronically infected mice have shown that these cells are able to mediate syngeneic heart graft destruction when injected in situ, whereas CD8⁺ or non-T cells were not effective. The bulk of results establishes that autoreactivity is restricted to the CD4⁺ T cell compartment, which is clearly different from allogeneic skin graft rejection, that has been attributed to both subsets of T lymphocytes (24, 25). This is in agreement with other experimental models of organ-specific autoimmune

**Figure 9.** CD4⁺ T cells from chronically infected BALB/c mice, tolerized with anti-CD4, show unresponsiveness to myocardial antigens. Purified CD4⁺, CD8⁺, total T cells, or non-T cells from naive mice, mice hyperimmunized with PF strain of *T. cruzi*, mice chronically infected with the Colombian strain of *T. cruzi*, or mice that had been treated with anti-CD4 were cultured (2 x 10⁶/well) together with irradiated filler cells (2 x 10⁶/well) in the presence of heart antigens from syngeneic animals (a previous study showed that 20 μg/ml was the optimal antigen concentration) for 4 d with [3H]thymidine added for the final 8 h. Data are expressed as the mean of triplicate wells in cpm. Data represent one of five similar experiments.
diseases in which CD4+ T cells play a major role in the induction of tissular lesions (26–28). Importantly, this is also in keeping with the data showing that the majority of T cells infiltrating the heart in both acute and chronic phases of experimental T. cruzi infection actually correspond to CD4+ T cells (29). Besides, these cells have been reported to modulate antibody production (30), macrophage activation (31, 32), and peripheral nerve cell injury (33). One might still argue that the results obtained by injecting cells in situ was a consequence of previously in vivo activated CD4+ T lymphocytes, independently of their putative antiheart antigens specificity. Actually, this does not seem to be the case since CD4+ T cells taken from 15-d infected mice, which have been described to be already activated in vivo (34), did not lead to any lesion when injected within the heart grafts. Furthermore, using the in situ injections, we could demonstrate the presence of CD4+ T lymphocytes against heart tissues as soon as 25–30 d after initial infection, but not earlier (not shown). In addition, in vivo treatment with anti-IFN-γ mAb, one of the important lymphokines released by activated inflammatory CD4+ T cells (35), was not able to block the graft damage induced by these cells (not shown).

It is known that expression of CD4 correlates with the ability of the cell to recognize antigens bound to class II molecules (36, 37) and that CD4 binds to class II molecules coaggregate with the TCR/CD3 complex and aids the activation of T cells. However, since there is no formal demonstration that myocardial cells can express class II molecules on the membrane, it would be difficult to accept that the CD4+ T cell could account for the final effector cytotoxic cell activity. The ruthenium red staining suggests that the mononuclear cell with an activated macrophage appearance could be the final effector cell that destroys the cardiomyocytes. In this case, CD4+ T cells would recognize myocardial antigens together with class II expressed on resident tissue macrophages or dendritic cells, thus activating these CD4+ T cells with consequent production of interleukins such as the early T lymphocyte activation 1 (Eta-1) protein (38). This could initiate an inflammatory process leading to the destruction of heart tissue.

One interesting question raised from these data refers to the triggering of those autoreactive CD4+ T lymphocytes. Molecular mimicry between heart and T. cruzi antigens could eventually be responsible for further stimulation of such a reactivity (39). However, T. cruzi–hyperimmunized mice were not able to reject syngeneic heart grafts, and CD4+ T cells from these mice could not destroy the grafts when injected in situ or proliferate in vitro in the presence of heart antigens. The acute infection with massive lesions of the target organ would be necessary to provoke the break of tissue-specific tolerance. For instance, during the acute phase of the disease, T. cruzi antigens would decorate the myocardium cells and augment tissue damage induced by the immune response against the parasite itself (40). Furthermore, polyclonal activation of T and B cells is an important component during the acute phase of T. cruzi infection. It has been suggested that such an activation could play an important role in the development of autoimmune disease a posteriori (34). So far, we favor the hypothesis that extensive tissue damage and immune dysfunction during the acute infection, rather than cross-reactivity between T. cruzi and self antigens, are absolutely necessary for the appearance of organ-specific autoimmunity during the chronic phase.

In conclusion, the findings in the present study allow us to suggest that autoimmunity is the major mechanism implicated in the rejection of syngeneic heart tissues grafted into the pinna of the ear of mice chronically infected with T. cruzi. The similarity of the lesions to those found in humans suggests that autoimmunity is involved in the pathogenesis of chagasic cardiomyopathy in humans. Moreover, this could imply therapeutic strategies by reestablishing long-term tissue-specific tolerance with anti-CD4 mAb treatment, mediating anergy, or deleting the responder CD4+ T cells.

We thank Drs. Montchillo Russo and Fabiola Cardillo for critical reading of the manuscript. We are pleased to acknowledge Maria M. O. Rossi, Renata A. Soares, Monica A. Abreu and Ligia V. Baroza for their excellent technical assistance.

R. Ribeiro dos Santos and Marcos A. Rossi are Senior Investigators of the Conselho Nacional de Desenvolvimento Científico Técnológico. This work was supported by grants from FAPESP, CAPES, and CNPq.

Address correspondence to Marcos A. Rossi, Professor of Pathology, Department of Pathology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, 14049 Ribeirão Preto, São Paulo, Brazil.

Received for publication 23 July 1991 and in revised form 9 September 1991.

References
2. Kierszenbaum, F. 1985. Is there autoimmunity in Chagas' dis-

Ribeiro dos Santos et al.


37. Swain, S.L. 1983. T cell subsets and the recognition of MHC
