

A *Trypanosoma cruzi* Membrane Protein Shares an Epitope with a Lymphocyte Activation Antigen and Induces Crossreactive Antibodies

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Summary

Chagas' disease results from the infection of the protozoan parasite *Trypanosoma cruzi* and affects several million people in South America. Several alterations of the immune response have been described in this disease, such as severe immunosuppression of both cellular and humoral responses and massive polyclonal stimulation with the generation of autoantibodies crossreacting with host cells and tissues. We have obtained monoclonal antibodies (mAbs) from *T. cruzi*-infected mice that recognized a 50/55-kD antigen (GP50/55) on the *T. cruzi* membrane, but not in other parasites of the family *Trypanosomatidae*. One of these GP50/55-specific mAbs (C10) crossreacts with a 28-kD antigen (p28) expressed on the membrane of >85% of activated mouse T and B lymphocytes, after in vitro activation with concanavalin A, *Salmonella typhosa* lipopolysaccharide, phorbol dibutyrate ester, or antigen, and on several murine T and B lymphocyte cell lines. Human T and B lymphocytes also express upon activation with phytohemagglutinin or *Staphylococcus aureus* Cowan I (SAC) a similar antigen recognized by mAb C10, although in a lower proportion of cells (30–40%). Furthermore, this mAb was able to suppress mouse and human T and B cell proliferation to any of those stimuli. In addition, sera from chagasic patients and *T. cruzi*-infected mice, but not from control patients or littermates, contain antibodies that recognize a similar p28 antigen on B lymphocytes. Furthermore, the immunoglobulin fractions of some chagasic sera also suppress the proliferation of human T lymphocytes. These results suggest a possible pathological role of autoantibodies as an alternative mechanism for *T. cruzi*-associated immunosuppression.

T*rypanosoma cruzi* is the causal agent of the Chagas' disease, which affects several million people in Central and South America (1). This protozoan parasite has a complex life cycle and exists in at least three morphologically distinct forms: infective (metacyclic or blood trypomastigotes), insect borne (epimastigotes), and intracellular replicative (amastigotes), which grows and replicates inside a variety of mammalian cells (1).

From a clinical point of view, the *T. cruzi* infections proceed in two phases. In the acute phase, circulating blood trypomastigotes are observed and there is a local inflammation at the sites of infection (1). During the chronic phase, circulating parasites cannot be observed by inspection of blood but progressive tissue damage occurs involving the esophagus, colon, and heart (1).

An autoimmune mechanism in the pathogenesis of human and experimental models of chronic Chagas' disease has been proposed (2, 3), but the exact role that this mechanism plays in disease production is not clear. A massive polyclonal acti-

vation of T and B lymphocytes (4) with an increased production of autoantibodies (5), together with a severe immunosuppression of the humoral and cellular responses (6) have been described in the acute phase of Chagas' disease, although the importance of those phenomena on the pathology of the disease is still poorly understood (6, 7).

On the other hand, there are many examples of antibodies from sera of *T. cruzi*-infected patients or animals that have been shown to react with host nervous and cardiac tissue antigens (8–15). In addition, T cells that are crossreactive with cardiac or nerve cells have been also found in mice with experimental Chagas' disease (16, 17), which can transfer the tissue pathology to naive animals (16). This has led to the hypothesis that *T. cruzi* has crossreactive antigens that mimic host molecules, which are responsible for the tissue damage associated with the chronic phase of Chagas' disease (2, 3), when virtual absence of parasites exists. The origin of the autoimmunity in Chagas' disease is not yet understood but it is likely that the polyclonal stimulation caused by the para-

site in the acute phase of *T. cruzi* infection (4) is responsible for the origin of this apparent breakdown of self-tolerance (18). Thus, it has been proposed that the combined effects of the polyclonal stimulation and the chronic presence of cross-reactive antigens give rise to the autoimmune pathology of Chagas' disease (18). Therefore, it is important to characterize these crossreactive antigens in order to get a clearer understanding of their function in the etiology of this disease in the chronic phase.

In a previous work (19), we described four mAbs that recognize a 50/55-kD antigen (GP50/55) on *T. cruzi* membrane. We describe in this manuscript that one of these GP50/55-specific mAbs crossreacts with an activation antigen present on T and B lymphocyte membranes and is able to suppress polyclonal T and B proliferation. Furthermore, antibodies with similar specificity are present in the sera from chagasic patients and from infected mice. This is the first description of a *T. cruzi*-specific mAb that recognizes an activation antigen of T and B lymphocytes, and our results suggest a possible pathological role of autoantibodies as an alternative mechanism for *T. cruzi*-associated immunosuppression.

Materials and Methods

Parasites and Cell Lines. The strain of *T. cruzi* used was originally obtained from a patient with Chagas' disease in the Instituto Nacional de la Salud (Madrid, Spain). It was cloned and named strain G. Promastigote forms of *Leishmania mexicana* were obtained from Dr. Luis Rivas (Instituto de Investigaciones Biológicas, Madrid, Spain). *T. cruzi* epimastigotes and *L. mexicana* promastigotes parasites were grown in liver infusion tryptose medium (20) with 10% of FCS (Flow Laboratories, Inc., McLean, VA) at 27°C.

The murine T hybridoma DO-11.10 (21) and the murine B plasmacytoma P3X63Ag8 and Sp2/0*Ag14 (Sp2/0⁺) (22) cell lines were cultured in complete RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 10% FCS. The human B lymphoblastomas Daudi and JY, the human B hybridomas Sp0/0 and HMA.A (a gift from Dr. F. Gambón, Hospital Puerta de Hierro, Madrid), the murine lymphoma Yac-1, the murine helper T cell clone Cl.Ly1⁺2⁻/9 (D9V) (23), and the human T lymphoblastoma CEM cell lines were routinely grown in the same media. The human T lymphoblastomas Jurkat and MOLT-4, the human monocyte cell line U-937, the murine macrophage-like cell line J774-G8 derived from murine reticulum cell sarcoma, the murine plasmacytoma P815, the murine neuroblastomas N2A and N115, the murine fibroblast L-929, and the human carcinoma HeLa were cultured in DMEM (Gibco Laboratories) containing 10% FCS. The murine IL-2-dependent T cell line, CTLL-2, was maintained in continuous culture in RPMI 1640 supplemented with 5% FCS, 5 × 10⁻⁵ 2-ME, and 2% conditioned medium from Con A-treated murine spleen T lymphocytes. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

Antibodies. The mAb C10 hybridoma was obtained in our laboratory from *T. cruzi*-infected BALB/c mice (19). The mice were infected (10⁶ parasites/mouse) with a mixture of morphologies of live *T. cruzi* (50% epimastigotes, 30% trypomastigotes, and 20% amastigotes) with the immunization protocol described previously (19). The mAb M1/9.3.4.HL.2 anti-mouse CD45 and the mAb 34.1.2 anti-H-2^d were obtained from Dr. J. M. Rojo (Centro de Investigaciones Biológicas, Madrid, Spain), anti-Ia^d antibodies were purchased from Becton Dickinson & Co. (Erembodegem-Aalst, Bel-

gium). The irrelevant mouse IgG1 (P3) was obtained from the hybridoma P3X63Ag8. The rabbit F(ab')₂ anti-mouse Ig-FITC and the goat anti-rat Ig-FITC antibodies were purchased from Southern Biotechnology (Birmingham, AL).

The mAbs C10 and P3 were purified by affinity chromatography with protein A-Sepharose CL4B (Sigma Chemical Co., St. Louis, MO), and they were biotinylated with *N*-hydroxysuccinimido biotin (Sigma Chemical Co.).

Human chagasic sera were obtained from Argentina and Colombia and were a gift from Drs. C. Alonso, J. M. Requena (Centro de Biología Molecular, Madrid, Spain), and M. López (Instituto López-Neyra, Granada, Spain). The Ig fraction was purified by affinity chromatography with anti-human Ig (Nordic, Tilburg, UK) covalently coupled to protein A-Sepharose CL4B by using dimethyl-dipimelimidate as crosslinker agent.

Cytofluorimetry Analysis. Cells (1–2 × 10⁵) or parasites (1–2 × 10⁶) were centrifuged three times in PBS with 2% BSA and 0.1% sodium azide. They were subsequently incubated with 50 μg/ml of biotin-labeled mAb C10, or biotin-labeled control mAb P3, or nonlabeled antibodies diluted in the same buffer, for 30 min at 4°C. Cells or parasites were washed in the above buffer and incubated again with 50 μl of streptavidin-PE or 50 μl rabbit anti-mouse F(ab')₂ Ig-FITC or goat anti-rat Ig-FITC (Southern Biotechnology) diluted in the same buffer, for 30 min at 4°C in the dark. After three rinses, the parasites or cells were resuspended in the same buffer containing 1% paraformaldehyde, and the fluorescence was analyzed in an EPICS cytofluorimeter (Coulter Scientific, Harpenden, UK). Detailed combination of reagents for the double-color staining is described in the legend of Fig. 3.

Immunoprecipitations. Parasites (2 × 10⁸ epimastigotes) or cells (2 × 10⁷) were labeled with ¹²⁵I by the lactoperoxidase method (19). The immunoprecipitations of *T. cruzi* antigens were carried out using preformed protein A-Sepharose CL4B (Sigma Chemical Co.) antibodies complexes as described (19). For immunoprecipitation from labeled murine cells, these were divided in two aliquots of 2 ml in PBS, and 50–100 μg of purified mAb C10 or irrelevant mouse IgG1 mAb were added. The samples were incubated for 2 h at 4°C. Cells were washed three times with PBS and were disrupted in 1 ml of lysis buffer containing 1% NP-40 (19). The immunoprecipitation of cell antigen was carried out as described (19).

Proliferation Assays. BALB/c mice were immunized intraperitoneally with a *T. cruzi* extract obtained from 10⁷ epimastigotes per mouse in PBS mixed with CFA at day 0. At day 28, mice were immunized in the same way but substituting CFA for IFA. At the day 42, mice were finally injected intraperitoneally with the *T. cruzi* extract without adjuvants. Mice were killed by CO₂ inhalation 15 d after the last immunization. Spleens were removed, the RBC were eliminated by hypotonic shock, and the spleen cells were activated in vitro with Con A, phorbol dibutyrate ester (PDBu),¹ LPS, or parasite lysates at the indicated concentrations in the presence of different concentrations of antibodies.

Human PBL were obtained from heparinized venous blood of healthy volunteers by Ficoll-Hypaque centrifugation as described (24). Human T cells were activated with different concentrations of PHA (Sigma Chemical Co.) in the presence of the indicated concentrations of the antibodies. Human B cells were obtained from human tonsils of children 4–10 yr old, and the cells were activated with *Staphylococcus aureus* Cowan I (SAC) (Calbiochem-Behring Corp., San Diego, CA) as described (25).

¹ Abbreviation used in this paper: PDBu, phorbol dibutyrate ester.

The proliferation assays were carried out in 96-well U-shaped microtiter plates (Flow Laboratories, Inc.) containing 100 μ l/well with $1-5 \times 10^5$ cells in complete RPMI 1640 with 10% FCS and 5×10^{-5} M 2-ME. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂ for 24–96 h. Cell proliferation was estimated by the amount of [³H]thymidine (New England Nuclear, Boston, MA) incorporated during the last 4–16 h of culture. Cells were harvested, and the radioactivity incorporated into DNA was measured in a liquid scintillation counter.

Results

The T. cruzi-specific mAb C10 Is Crossreactive with an Activation Antigen Present on T and B Lymphocytes. We have obtained mAbs from *T. cruzi*-infected mice with the aim of searching for those that were crossreactive with host cells. For this purpose, BALB/c mice were infected with the *T. cruzi* G strain and hybridomas were first selected by their ability to secrete antibodies that bind specifically to intact parasites (19). To search for autoreactivity, the binding of these mAbs against a panel of transformed mouse cell lines from distinct tissue origins was tested by cytofluorimetry analysis. One of these mAbs, termed mAb C10, strongly reacted with 99% of the intact *T. cruzi* epimastigotes (Fig. 1). mAb C10 reacted with all the morphological forms of the parasite: trypomastigotes, amastigotes, and epimastigotes (19). This mAb was *T. cruzi* specific since it did not react with other closely related protozoa belonging to the *Trypanosomatidae* family, such as *L. mexicana* (Fig. 1), *T. brucei*, and *L. infantum* (data not shown). Among the cell lines tested, the cell membrane of some T cells, such as DO-11.10 (21), and of B cell plasmacytomas, such as SP2/0⁺ (22), was strongly stained with mAb C10 (Fig. 1). Cell lines from origins other than T and B lymphocyte lineage were negative. T or B cell lines of human

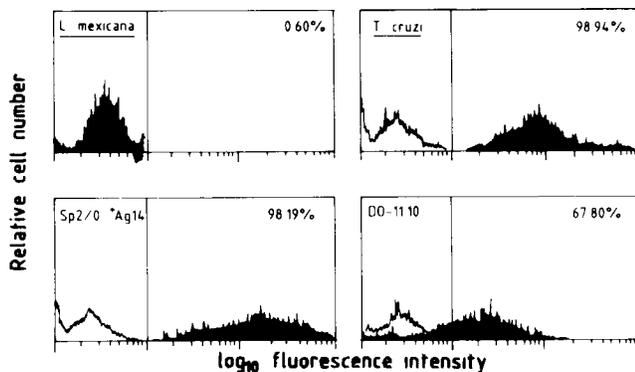


Figure 1. Reactivity of mAb C10 with *T. cruzi* and mouse lymphocyte cell lines. For the cytofluorimetry analysis, cells ($1-2 \times 10^5$) or parasites ($1-2 \times 10^6$) were washed as described in Materials and Methods. Then, they were stained with biotin-labeled mAb C10 or biotin-labeled control mouse IgG1 for 30 min at 4°C. Cells or parasites were incubated with streptavidin-PE diluted 1/300 for 30 min at 4°C in the dark. The fluorescence was analyzed in an EPICS cytofluorimeter. The histograms represent the number of cells versus the logarithm of the fluorescence intensity. The white and black profiles represent the fluorescence staining obtained with the control P3 mAb or mAb C10, respectively. The percent of positive cells and the cells for each case are shown inside the corresponding panel.

Table 1. mAb C10 Expression on Several Cell Lines

| Cell lineage | Origin | Percent positive cells |
|--------------------------|--------|------------------------|
| B cells: | | |
| Sp2/0 ⁺ Ag14 | Mouse | 99.3 |
| P3X63 Ag8 | Mouse | 69.7 |
| Daudi | Human | 5.0 |
| Sp0/0 | Human | 2.3 |
| JY | Human | 3.5 |
| HMA.A | Human | 6.8 |
| T cells: | | |
| DO-11.10 | Mouse | 98.0 |
| D9V | Mouse | 0.0 |
| CTLL-2 | Mouse | 0.0 |
| Yac-1 | Mouse | 1.4 |
| Jurkat | Human | 1.3 |
| MOLT-4 | Human | 2.6 |
| CEM | Human | 3.7 |
| Monocytic cells: | | |
| U937 | Human | 3.9 |
| Neuronal cells: | | |
| N2A | Mouse | 2.0 |
| N115 | Mouse | 4.0 |
| Epithelial cells: | | |
| Hela S3 | Human | 0.0 |
| L929 | Mouse | 0.0 |

Binding of mAb C10 to cell lines was assayed by flow cytometry. Cells ($1-2 \times 10^5$) were washed as described in Materials and Methods. Then, they were stained with biotin-labeled mAb C10 or biotin-labeled control mouse IgG1, for 30 min at 4°C. Cells were incubated with streptavidin-PE diluted 1/300 for 30 min at 4°C in the dark. The fluorescence was analyzed in an EPICS cytofluorimeter. The percent of positive cells shown was calculated by subtracting the binding of the irrelevant control.

origin were negative (Table 1). This result prompted us to test the reactivity of the mAb C10 with normal T and B lymphocytes from BALB/c mouse spleen. As shown in the Fig. 2, a very small percent (6–13%) of cells in a normal spleen were positive for this antibody. By contrast, this percentage rose to 85% or more after 96 h of in vitro activation with PDBu, with the T cell mitogen Con A, or with the B cell mitogen *Salmonella typhosa* LPS (Fig. 2). The binding of mAb C10 to the membrane of activated mouse lymphocytes was not due to the recognition of the Con A or LPS used in the stimulation, because the crossreactive mAb C10 antigen was also induced by phorbol ester stimulation, and Con A or LPS were unable to affect the mAb C10 binding to either DO-10.11 or Sp2/0⁺ cells (data not shown). The binding of mAb C10 to T and B cells was not due to the Fc receptor since binding of isotype-matched irrelevant antibodies was

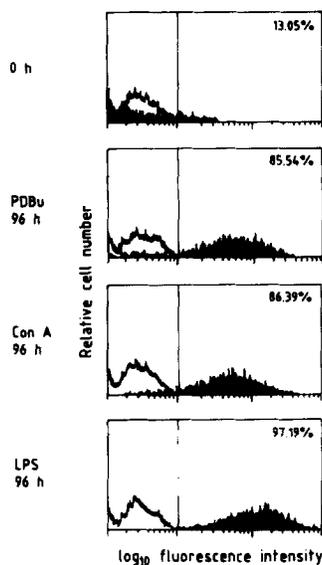


Figure 2. Reactivity of mAb C10 with mouse spleen cells. Spleen cells from normal BALB/c mice, obtained as described in Materials and Methods, were activated in vitro by incubating with PDBu (20 ng/ml), Con A (1.5 μ g/ml), or *Salmonella typhosa* LPS (50 μ g/ml) in complete RPMI 1640 containing 10% FCS. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂, for 96 h. The top panel shows the stain pattern of resting spleen cells with the mAb C10, and the other histograms show the stain pattern of activated spleen cells with the different stimuli used. The cells were stained and analyzed as described in Fig. 1. The histograms represent the number of cells versus the logarithm of the fluorescence intensity. The white and black profiles represent the fluorescence staining obtained with the control P3 mAb or mAb C10, respectively. The percent of positive cells is shown inside the corresponding panel.

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negative. Therefore, the lymphocyte antigen recognized by mAb C10 is present in both activated mouse B and T cells.

By double-color cytofluorimetry analysis, the mAb C10 antigen was first clearly detected on the membrane of Ig⁺ B mouse cells after 48 h of LPS activation. This expression rose strongly after 72 h and it was maximal 96–120 h after the addition of the stimulus (Fig. 3). These results also indicated that all Ig⁺ cells expressed mAb C10 antigen. Similar results in the kinetic of mAb C10 expression were obtained after PDBu or Con A stimulation (data not shown).

Despite the lack of reactivity of human T and B cell lines with mAb C10, we tested the effect on normal human PBL stimulated with T and B cell mitogens. As seen in Fig. 4, resting human lymphocytes were weakly stained with the antibody. However, after 48 h of in vitro activation with PHA or SAC, 30–40% of both T and B cells were positive, and this percent, in contrast to the mouse system, did not increase with longer stimulation times. These activated cells were strongly positive for other activation antigen markers, such as 4F2 or CD25, 65–85%, (data not shown), therefore discarding inefficient activation as the cause of the lower percent observed with human lymphocytes. Interestingly, mAb C10 antigen expression on the membrane of activated human T cells was stronger than activated human B cells.

mAb C10 recognized a GP50/55 *T. cruzi* glycoprotein that is linked to the membrane by a glycosyl-phosphatidylinositol anchor (19). This antigen can be immunoprecipitated by mAb C10 from ¹²⁵I-labeled *T. cruzi* lysates (Fig. 5). By contrast, a faint but detectable band ~28 kD was specifically immunoprecipitated by mAb C10 from intact Sp2/0⁺ (Fig. 5) or DO-11.10 (data not shown) ¹²⁵I-labeled cells. Similar results were obtained under reducing or nonreducing conditions (data not shown).

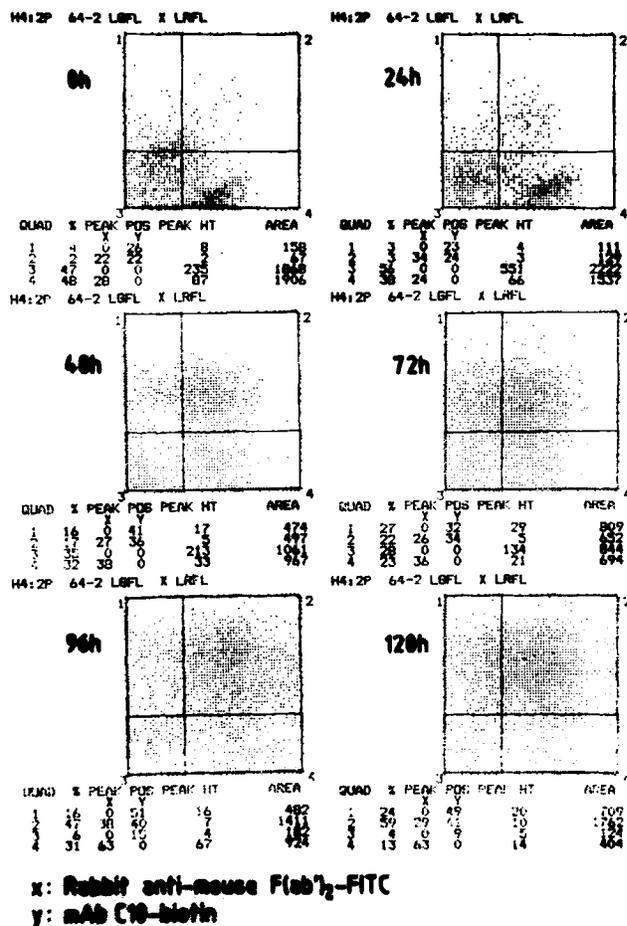


Figure 3. mAb C10 antigen and Ig expression on activated mouse B lymphocytes. B lymphocytes were obtained by lysis of T cells by incubating with anti-mouse T cell serum and C (Cedarlane, Hornby, Ontario, Canada). The cells were activated by incubation with LPS (50 μ g/ml) for several hours (0, 24, 48, 72, 96, or 120 h). Cells were first incubated with rabbit F(ab')₂ anti-mouse Ig-FITC (x-axis). Residual binding sites and Fc receptors were saturated by incubating with 100 μ g/ml mouse IgG. After washing, cells were further stained with biotin-conjugated mAb C10. PE-conjugated streptavidin was added at the end (y-axis). The percent of cells with positive or negative fluorescence for each group is shown below each panel. Similar results have been observed in two other independent experiments.

mAb C10 Is Able to Inhibit T and B Cell Proliferation. Since the antigen detected by mAb C10 was present in almost 90% of activated mouse spleen cells, we tested the functional effects of mAb C10 on T and B cell activation. Concentrations of purified antibody as low as 0.5 μ g/ml were able to induce maximum inhibition of the mouse spleen cell proliferation to Con A as measured by incorporation of [³H]thymidine into DNA 72 h later (Fig. 6). Spleen cells from BALB/c mice previously immunized with *T. cruzi*, as described in Materials and Methods, were activated in vitro with either Con A, LPS, PDBu, *T. cruzi*, or *L. mexicana* extracts. As seen in Fig. 7, these immune spleen cells proliferated in response to Con A, LPS, PDBu, or the appropriate antigen (*T. cruzi* extracts), but not to *L. mexicana* extracts. When purified mAb C10

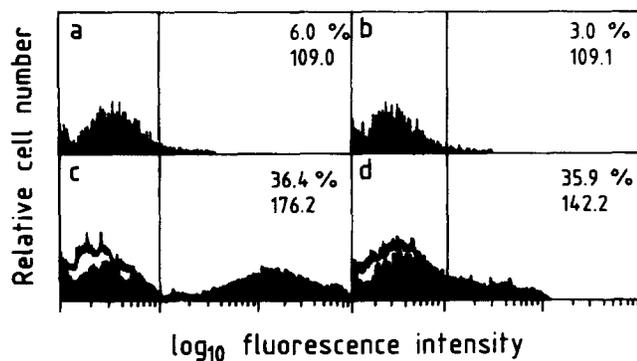


Figure 4. mAb C10 antigen expression on activated human T and B lymphocytes. Human T and B cells were obtained as described in Materials and Methods and were activated in vitro by incubating with PHA (2 μ g/ml) or *Staphylococcus aureus* Cowan I (1 μ g/ml) in complete RPMI 1640 containing 10% FCS. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂, for 48 h. The cells were stained and analyzed as described in Fig. 1. The staining pattern of resting PBL or tonsil lymphocytes is shown in *a* and *b*, respectively; *c* and *d* show the staining of PHA-activated PBL and SACI-activated tonsil lymphocytes. The white and black profiles represent the fluorescence staining obtained with the control P3 mAb or mAb C10, respectively. The percent of mAb C10-positive cells and the mean fluorescence intensity for each cell population are shown inside the corresponding panel.

(1 μ g/ml) was added to the cultures, a similar inhibition of the proliferation to all stimuli was observed.

The kinetic of this proliferative inhibition by the mAb C10 correlated with the kinetic of appearance on the cell membrane of the mAb C10 crossreactive antigen in those immune spleen cells (Fig. 8). The inhibition of T cell proliferation to Con A by mAb C10 was not observed during the first 24 h of culture. It was first detected after 48 h and was maximal after 72 or 96 h (Fig. 8). Similar results were obtained with the stimulation of B cells with LPS (data not shown).

Antibodies with Similar Specificity to mAb C10 Exist in Human Chagasic Sera and in *T. cruzi*-infected Mice Sera. To test the presence of antibodies with similar antigenic specificity to mAb C10 in the sera of chagasic patients and infected mice, inhibition experiments of the binding of biotinylated mAb C10 to intact parasites and cells were carried out. As seen in Fig. 9 and Table 2, sera of *T. cruzi*-infected mice and humans were able to block specifically mAb C10 binding both to lymphocytes and to *T. cruzi* epimastigotes, detected by cytofluorimetry analysis. Furthermore, the binding of other antibodies to different surface molecules such as mAb M1/9.3.4.HL.2 (anti-murine CD45) (Fig. 9 and Table 2), anti-H-2^d or anti-Ia^d (data not shown) on lymphocyte membranes was unaffected. All sera from the chagasic patients tested (5/5) were able to, specifically and significantly, block the binding of mAb C10 to *T. cruzi* and Sp2/0⁺ independently of their titer of antibodies against *T. cruzi*. Sera from human controls of the same area were negative (2/2). Furthermore, sera from *T. cruzi*-immune mice were less effective than sera from infected mice in blocking mAb C10 binding, despite the fact that immune sera had 300 times higher anti-*T. cruzi* antibody titer (Table 2).

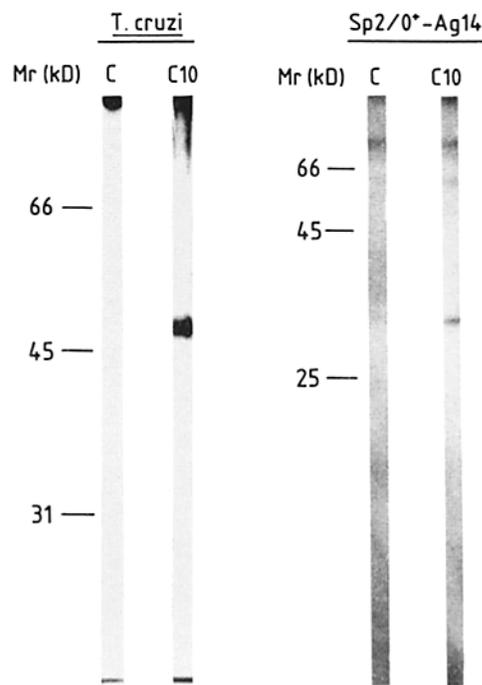


Figure 5. Immunoprecipitation with mAb C10 from *T. cruzi* and Sp2/0⁺. Immunoprecipitation from *T. cruzi* or from Sp2/0⁺ cells extracts: (C) with an irrelevant P3 mouse IgG1 mAb and (C10) with the mAb C10. Parasites (2 \times 10⁸ epimastigotes) or cells (2 \times 10⁷) were labeled with ¹²⁵I by the lactoperoxidase method and then lysed in 1% NP-40. The immunoprecipitations were carried out as described in Materials and Methods. The immunoprecipitated antigens were subjected to electrophoresis on a 10–15% acrylamide/SDS gels. The molecular mass markers are indicated.

To further confirm the specificity of the antibodies present in the chagasic sera, we purified the Ig fraction of the sera, and that fraction was tested in the proliferation of human PBL. mAb C10 was able to inhibit (40–50%) PHA-dependent proliferation of human T cells, in a percent similar to the percent of mAb C10-positive lymphocytes (40%) among these activated cells (Fig. 10). Similarly, purified Igs from the sera of two chagasic patients, but Igs not from normal sera, also inhibited, in a dose-response manner, PHA proliferation (Fig. 10). Of purified Igs from 14 chagasic sera tested, 50% of them had similar inhibition to the two shown in Fig. 10, whereas four were without effect and three were weakly inhibitory (data not shown).

Discussion

It is likely that the combined effects of the polyclonal stimulation and the chronic presence of crossreactive antigens could account for the autoimmune pathology of Chagas' disease (18). To get a clearer understanding of the function of self-reactive antibodies in the etiology of this disease, we have obtained mAbs from *T. cruzi*-infected mice with the aim of characterizing the relevant parasite antigens.

Although several *T. cruzi*-specific antibodies that crossreact

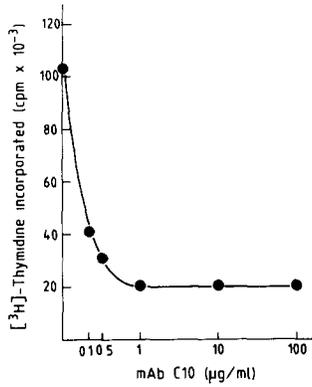


Figure 6 Dose-response inhibition of mAb C10 in the mouse spleen cell proliferation to Con A. Normal spleen cells were activated in vitro with Con A (1.5 µg/ml) and incubated in presence of mAb C10 at the indicated concentrations. The proliferation assays were carried out as described in Materials and Methods. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂ for 72 h. Cell proliferation was estimated by the amount of [³H]thymidine incorporated during the last 4–16 h.

with molecules expressed in host cells have been described (8–15), and sera from chagasic patients have been shown that recognize neurotransmitter receptors on lymphocytes (26), the present work is to our knowledge the first description of a mAb that crossreacts with an activation antigen present in human and mouse T and B lymphocytes.

Our data also indicate that antibodies with similar reactivity to mAb C10 may exist in the sera of infected individuals and animals. This hypothesis is supported by the fact that (a) antibodies in chagasic sera but not in control sera were able to block specifically mAb C10 binding, both to lymphocytes and to *T. cruzi* epimastigotes (Table 2). It is very unlikely that the competition for the binding of mAb C10 to two different molecules, GP50/55 in *T. cruzi* and p28 on lymphocytes, by chagasic sera was due to steric reasons since the binding of other surface molecules, such as MHC antigens or CD45, highly abundant on the lymphocyte surface, were unaffected. Therefore, it is likely that the Igs of the chagasic sera contain antibodies of similar specificity to mAb C10, although it is very difficult to completely ascertain if the antibodies in chagasic sera recognize or not the same epitope that the mAb C10 recognizes. (b) Antibodies in some chagasic sera but not in control sera were able to suppress human T cell proliferative responses as mAb C10 did. Obviously, this inhibition could be due to many other Igs with different specificities in those sera, since it is very difficult to prove beyond a reasonable doubt that the antibodies are binding

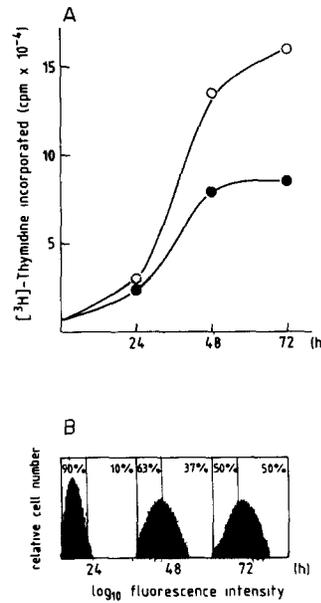


Figure 8 Correlation between the kinetics of inhibition of lymphocyte activation and of the expression of the mAb C10 antigen. Normal mouse spleen cells were activated with Con A (1.5 µg/ml) in 96-well microtiter plates as described in the legend to Fig. 5, in the presence of mAb C10 (●) or P3 (○). After 24, 48, or 72 h of culture, the cells were (A) pulsed with [³H]thymidine during the last 4 h to measure cell proliferation or (B) stained with mAb C10 as described in the legend to Fig. 1. The percent of positive and negative cells for each case is shown inside the corresponding panel.

to the same epitope that mAb C10 does. The reason that only 50% of the sera were inhibitory could be related to the different titers of Igs with mAb C10-like reactivity in the different sera. In general, the sera that inhibited T cell proliferation were the ones that had stronger inhibitions of mAb C10 binding to lymphocytes. Alternatively, since it is known that there are at least two clinical manifestations of the disease, it is possible that this is potentially related to our results. Furthermore, although this inhibition was low, it correlated with the percent of mAb C10-positive cells in those activated populations, which also supported the notion that the Igs of those sera were blocking proliferation through mAb C10 binding. Taking all together, our data support the notion that antibodies against p28 exist in chagasic patients and mice and could contribute at least in part to the observed immunosuppression.

It is worth noting that the sera from *T. cruzi*-immune mice were less effective than serum from infected mice in blocking mAb C10 binding, despite the fact that immune serum had 300 times higher anti-*T. cruzi* antibody titer. This is in agree-

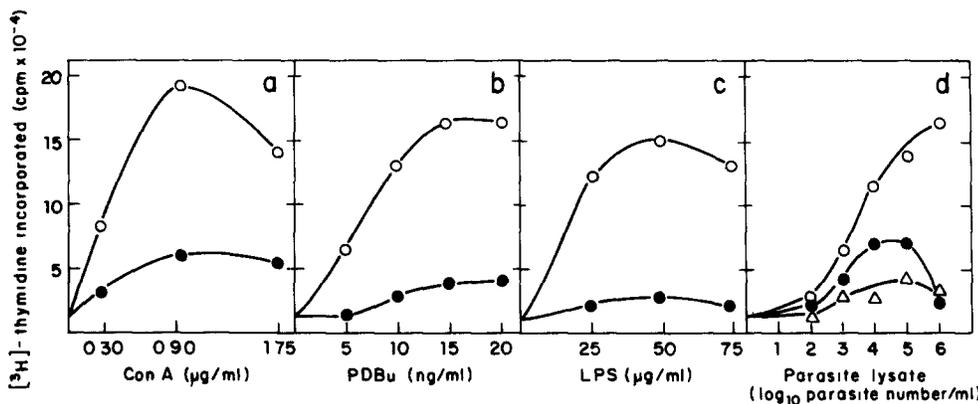
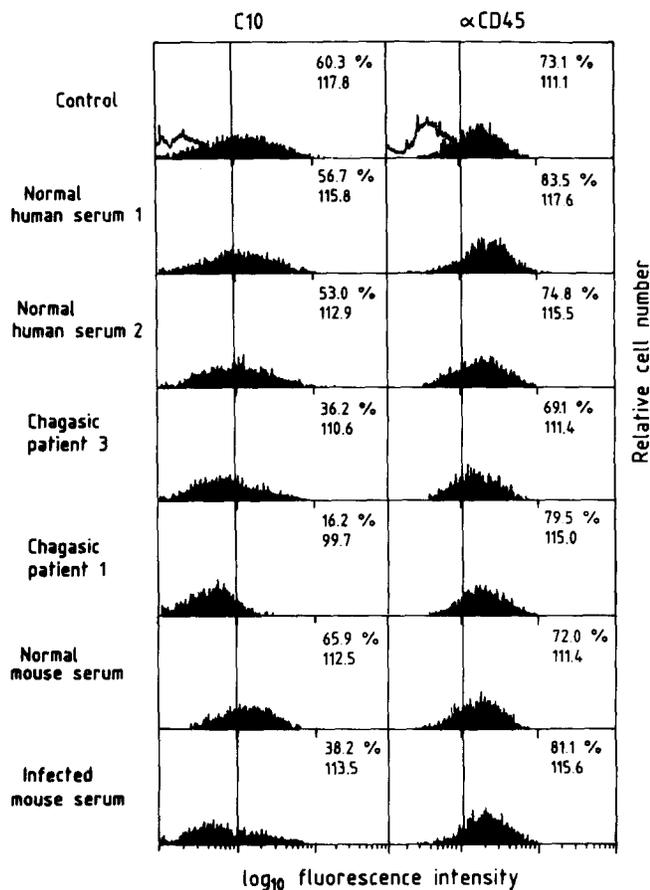


Figure 7. Inhibition of mouse T and B cell proliferation by mAb C10. *T. cruzi*-immune mouse spleen cells were activated in vitro with Con A, PDBu, *Salmonella typhosa* LPS, or *T. cruzi* lysates at the indicated concentrations, in the presence of 1 µg/ml purified mAb C10 IgG1 (●) or a control mouse IgG1 mAb (○). *L. mexicana* lysates were used as control of proliferation to *T. cruzi* lysates (Δ). The mice were immunized against *T. cruzi* as described in Materials and Methods. The proliferation assays were carried out as in Fig. 5.



ment with the fact that only a small percent (1/4) of mAbs against GP 50/55 (19) are crossreactive with p28 (Hernández-Munáin et al., unpublished results). Besides, these results confirm the importance of studying antibody repertoire from infected animals instead of from immunized animals. It has been shown that a *T. cruzi* infection does not have the same effects in the immune system as an exposition to dead microorganisms (27). Therefore, our results confirm that an active infection induces antibodies with a different repertoire of antigenic specificities to those of classical immunizations.

Our data suggest that *T. cruzi* presents antigens on its membrane that can induce crossreactive antibodies to the surface antigens of *T. cruzi*-activated lymphocytes during the infection and inhibit their proliferation. This phenomenon is of particular interest in as much as the specific recognition of lymphocyte membrane receptors by autoantibodies may result in modulation of immune response in Chagas' disease. It has been suggested that Chagas' disease may have an immunological basis (2, 3, 6, 7). Severe immunosuppression of the immune responses in the acute phase of the infection

Figure 9. Inhibition of mAb C10 or mAb M1/9.3.4.HL.2 (anti-murine CD45) binding to *T. cruzi* or Sp2/0⁺ membrane by cytofluorimetry analysis. The assay was performed as described in the legend to Table 2. The histograms represent the number of cells versus the logarithm of the fluorescence intensity. The white profiles represent the staining with the irrelevant mAb. The percent of positive cells and the mean fluorescence intensity for each case are shown inside the corresponding panel.

Table 2. Specific Inhibition of mAb C10 Binding by Chagasic Sera

| Source of blocking antibodies | Titer | Percent inhibition of binding | | |
|---------------------------------------|--------|-------------------------------|--------------------|--------------------|
| | | mAb C10 | | mAb M1/9 |
| | | <i>T. cruzi</i> | Sp2/0 ⁺ | Sp2/0 ⁺ |
| Normal mouse serum | <10 | 0 | 0 | 0 |
| <i>T. cruzi</i> -immune mouse serum | 10,500 | 30 | 18 | 0 |
| <i>T. cruzi</i> -infected mouse serum | 325 | 60 | 42 | 0 |
| Normal human serum 1 | <10 | 0 | 0 | 0 |
| Normal human serum 2 | <10 | 0 | 0 | 0 |
| Chagasic patient 1 | 220 | 40 | 77 | 0 |
| Chagasic patient 2 | 180 | 45 | 73 | 0 |
| Chagasic patient 3 | 110 | 52 | 44 | 0 |
| Chagasic patient 4 | 240 | 51 | 83 | 0 |
| Chagasic patient 5 | 80 | 62 | 53 | 0 |
| mAb C10 supernatant | 1,800 | 80 | 75 | 0 |

Binding of mAbs C10 or M1/9.3.4.HL.2 (anti-murine CD45) to *T. cruzi* or SP2/0⁺ membrane was studied by flow cytometry analysis. 1–2 × 10⁶ *T. cruzi* epimastigotes or 1–2 × 10⁵ Sp2/0⁺ cells were resuspended and incubated with 100 μl of each sera diluted 1/10 in PBS with 2% BSA and 0.1% sodium azide for 1 h at 4°C. After three washes with the same buffer, the parasites or cells were incubated with 10 μg/ml biotin-labeled mAb C10 or mAb M1/9.3.4.HL.2 for 30 min at 4°C. The parasites or cells were washed and incubated for 30 min at 4°C in the dark with streptavidin-PE or goat anti-rat IgFITC, respectively. The fluorescence was analyzed in an EPICS cytofluorimeter. The titer shown refers to the antibody titer against *T. cruzi* determined by ELISA as described (19).

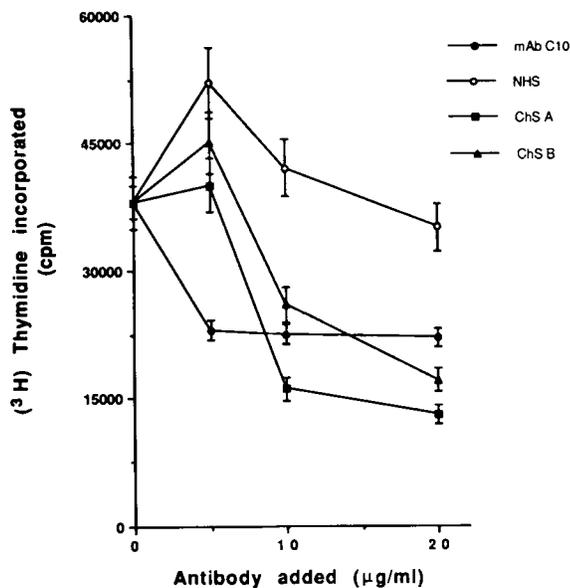


Figure 10. Inhibition of human T cell proliferation by mAb C10 and Igs from chagasic sera. Human T cells were activated with 1 µg/ml PHA in the presence of the indicated concentrations of purified Ig from normal human serum (○), two chagasic sera (▲ and ■), and mAb C10 (●) for 72 h. Cell proliferation was estimated by the amount of [³H]thymidine incorporated during the last 4–16 h of culture.

has been reported (28–36). This immunosuppression is thought to diminish the resistance of the host to the establishment and dissemination of the disease. However, the molecular basis for this immunosuppression is not well understood. These effects have been attributed to impaired IL-2 production due to the presence of suppressor cells: either macrophages (28–30) or T cells (31–35), or to *T. cruzi* immunosuppressive factors (36). Another mechanism could be the polyclonal activation of lymphocytes that may alter the reactivity against foreign antigens causing the immune system to respond poorly to further antigenic challenge due to a state of refractoriness (4). The results shown in this manuscript add a new potential mechanism to explain this immunosuppression. The presence in chagasic sera of *T. cruzi*-specific

antibodies with similar properties as mAb C10 could theoretically be able to suppress T and B cell activation like mAb C10 does in vitro and may account for the suppression of the immune response. Alternatively, they may indirectly contribute to the immunosuppression by eliminating activated lymphocytes through complement-mediated killing.

Recently, a mAb against another pathogen with similar functional properties to mAb C10 has been described (37). This antibody, specific for a measles virus protein, crossreacts with a T lymphocyte subset also causing immunosuppression. This crossreactivity has been also suggested to play a role in the etiology of measles-induced immunosuppression in vivo (37).

The 28-kD antigen recognized by mAb C10 on T and B lymphocytes seems to represent a yet undescribed activation antigen at least in mice. This antigen appears late after activation with several mitogenic activators and seems to be involved in the regulation of the late stages of lymphocyte proliferation. Among those, mAb C10 blocks IL-2-dependent proliferation of T cells (Hernández-Munáin et al., manuscript in preparation). A somewhat similar activation antigen, CDw70, has been described in human T and B lymphocytes (38); this antigen, like mAb C10 antigen, is present in T- and B-activated lymphocytes but absent in thymocytes (38) (Fig. 2 and data not shown). The described molecular mass of CDw70, detected by immunoblot techniques, is ~30 kD, similar to that of mAb C10 antigen. Furthermore, anti-CDw70 antibodies have inhibitory activity on lymphocyte proliferation like mAb C10. Recently, a 26-kD TAPA-1 antigen has been described in human T and B cells (39) that also shares some similarities with mAb C10 lymphocyte antigen. Experiments are in progress to further characterize the p28 antigen.

In summary, our results show the existence of an antibody specific for *T. cruzi* that is crossreactive with an activation antigen present on T and B lymphocytes. This may have implications in the understanding of the complex pathology of this disease. Our data suggest that *T. cruzi* presents antigens on its membrane that can induce crossreactive antibodies to the surface antigens of *T. cruzi*-activated lymphocytes during the infection altering their functionality.

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References

1. Brener, Z.A. 1973. The biology of *Trypanosoma cruzi*. *Annu. Rev. Microbiol.* 27:347.
2. Hudson, L. 1985. Autoimmune phenomena in chronic Chagasic cardiopathy. *Parasitol. Today.* 1:6.
3. Kierszenbaum, F. 1985. Is there autoimmunity in Chagas' disease? *Parasitol. Today.* 1:4.
4. Minoprio, P., S. Itohara, C. Heusser, S. Tonegawa, and A. Coutinho. 1989. Immunobiology of murine *T. cruzi* infection: the predominance of parasite-nonspecific responses and the activation of TCRI T cells. *Immunol. Rev.* 112:183.
5. Ternynck, T., C. Bleux, J. Gregoire, S. Avrameas, and C. Kanellopoulos-Langevin. 1990. Comparison between autoantibodies arising during *Trypanosoma cruzi* infection in mice and natural autoantibodies. *J. Immunol.* 144:1504.
6. Kierszenbaum, F., and M.B. Szein. 1990. Mechanisms underlying immunosuppression induced by *Trypanosoma cruzi*. *Parasitol. Today.* 6:261.
7. Brener, Z. 1980. Immunity to *Trypanosoma cruzi*. *Adv. Parasitol.* 18:247.
8. Cossio, P.M., R.P. Leguens, C. Díez, A. Szarfman, A. Segal, and R.M. Arana. 1974. Chagasic cardiopathy: antibodies reacting with plasma membrane of striated muscle and endothelial cells. *Circulation.* 50:1252.
9. Szarfman, A., V.P. Terranova, S.I. Renard, J.M. Foidant, M. de Fátima Lima, J.I. Scheinman, and G.R. Martin. 1982. Antibodies to laminin in Chagas' disease. *J. Exp. Med.* 155:1161.
10. Towbin, H., G. Rosenfelder, J. Weislander, J.L. Avila, M. Rojas, A. Szarfman, K. Esser, H. Nowack, and R. Timpl. 1987. Circulating antibodies to mouse laminin in Chagas' disease, American cutaneous leishmaniasis and normal individuals recognize terminal galactosyl-(α 1-3)-galactose epitopes. *J. Exp. Med.* 166:419.
11. Wood, J.N., L. Hudson, T.M. Jessell, and M. Yamamoto. 1982. A monoclonal antibody defining antigenic determinants on subpopulations of mammalian neurones and *Trypanosoma cruzi* parasites. *Nature (Lond.)* 296:34.
12. Snary, D., J.E. Flint, J.N. Wood, M.T. Scott, M.D. Chapman, J. Dodd, T.M. Jessell, and M.A. Miles. 1983. A monoclonal antibody with specificity for *Trypanosoma cruzi*, central and peripheral neurones and glia. *Clin. Exp. Immunol.* 54:617.
13. Petry, K., E. Nudelman, H. Eisen, and S. Hakomori. 1988. Sulfated lipids represent common antigens on the surface of *Trypanosoma cruzi* and mammalian tissues. *Mol. Biochem. Parasitol.* 30:113.
14. Alcina, A., and M. Fresno. 1987. A tubulin-related 55 kD surface antigen recognized by different *Trypanosoma cruzi* stage-specific monoclonal antibody from infected mice. *Mol. Biochem. Parasitol.* 29:181.
15. Van Voohris, W.C., and H. Eisen. 1989. A surface antigen of *Trypanosoma cruzi* that mimics mammalian nervous tissue. *J. Exp. Med.* 169:641.
16. Hontebeyrie-Joskowicz, M., G. Said, G. Millon, G. Marschall, and H. Eisen. 1987. L3T4⁺ T cells able to mediate a parasite-specific delayed type of hypersensitivity play a role in the pathology of experimental Chagas' disease. *Eur. J. Immunol.* 17:1027.
17. Gattass, C.R., M.T. Lima, A.F. Nobrega, M.A. Barcinski, and G.A. Dos Reis. 1988. Do self-heart-reactive T cells expand in *Trypanosoma cruzi*-immune host? *Infect. Immun.* 56:1402.
18. Eisen, H., and S. Kahn. 1991. Mimicry in *Trypanosoma cruzi*: fantasy and reality. *Curr. Opin. Immunol.* 3:507.
19. Hernández-Munáin, C., M.A. Fernández, A. Alcina, and M. Fresno. 1991. Characterization of a glycosyl-phosphatidylinositol anchored protein from *Trypanosoma cruzi*. *Infect. Immun.* 59:1409.
20. Gutteridge, W.E., J. Knowles, and J.D. Combes. 1969. Growth of *Trypanosoma cruzi* in human heart tissue cells and effects of aminonucleosides, puromycin, trypanocidin and aminopterin. *J. Protozool.* 21:512.
21. Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler, and P. Marrack. 1983. The major histocompatibility complex-restricted antigen receptor on T cells. I. Isolation with a monoclonal antibody. *J. Exp. Med.* 157:1149.
22. Márquez, C., A. De la Hera, E. Leonardo, L. Pezzi, A. Strasser, and C. Martínez-A. 1990. Identity of PB76 differentiation antigen and lymphocyte alkaline phosphatase. *Eur. J. Immunol.* 20:947.
23. Fresno, M., H. Der Simonian, G. Nabel, and H. Cantor. 1982. Proteins synthesized by inducer T cells: evidence for a mitogenic peptide shared by inducer molecules that stimulate different cell types. *Cell.* 30:707.
24. Muñoz-Fernández, M.A., F.X. Pimentel-Muñoz, M.A. Alonso, M. Campanero, F. Sánchez-Madrid, A. Silva, J.L. Alonso, and M. Fresno. 1990. Synergy of tumor necrosis factor with protein kinase C activators on T cell activation. *Eur. J. Immunol.* 20:605.
25. Postigo, A., R. Pulido, M. Campanero, A. Acevedo, A. Garcia-Pardo, A.L. Corbí, F. Sánchez-Madrid, and M.O. de Landázuri. Differential expression of VLA-4 integrin by resident and peripheral blood lymphocytes. Acquisition of functionally active α 4 β 1-fibronectin receptors upon B-cell activation. *Eur. J. Immunol.* 21:2347.
26. Sterin-Borda, L., G. Gorelik, A. Genaro, J.C. Goin, and E.S. Borda. 1990. Human chagasic IgG interacting with lymphocyte neurotransmitter receptors triggers intracellular signal transduction. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 4:1661.
27. D'Imperio Lima, M.R., M. Joskowicz, A. Coutinho, T.L. Kipnis, and H. Eisen. 1985. Very large and isotypically atypical polyclonal plaque-forming cell responses in mice infected with (*Trypanosoma cruzi*). *Eur. J. Immunol.* 15:201.
28. Kierszenbaum, F. 1982. Immunological deficiency during experimental Chagas' disease (*Trypanosoma cruzi* infection): role of adherent nonspecific esterase-positive splenic cells. *J. Immunol.* 129:2202.
29. Cunningham, D.S., and R.E. Kuhn. 1980. *Trypanosoma cruzi*-induced suppression of the primary immune response in murine cell cultures to T-cell-dependent and independent antigens. *J. Parasitol.* 66:16.
30. Ritter, D.M., and R.E. Kuhn. 1990. Antigen-specific T-helper cells abrogate suppression in *Trypanosoma cruzi*-infected mice. *Infect. Immun.* 58:3248.
31. Ramos, C., I. Schädler-Siwon, and L. Ortiz-Ortiz. 1979. Suppressor cells present in the spleens of *Trypanosoma cruzi* infected mice. *J. Immunol.* 122:1243.
32. Reed, S.G., S.B. Roters, and E.A. Goild. 1983. Spleen cell-mediated suppression of IgG production to non-parasite antigen during chronic *Trypanosoma cruzi* infection in mice. *J. Immunol.* 131:1878.
33. Reed, S.G., J.A. Inverso, and S.B. Roters. 1984. Suppressed antibody responses to sheep erythrocytes in mice with chronic *Trypanosoma cruzi* infections are restored with interleukin 2. *J. Immunol.* 133:3333.

34. Serrano, L.E., and J.A. O'Daly. 1987. Protein fraction from *Trypanosoma cruzi* infected spleen cell supernatants with immunosuppressive activity in vitro. *Int. J. Parasitol.* 17:851.
35. Gao, X.M., J.A. Schmidt, and F.Y. Liew. 1988. Suppressive substance produced by T cells from mice chronically infected with *Trypanosoma cruzi*. *J. Immunol.* 141:989.
36. Kierszenbaum, F., M.B. Sztein, and L.A. Beltz. 1989. Decreased human IL-2 receptor expression due to a protozoan pathogen. *Immunol. Today.* 10:129.
37. Oldstone, M.B.A. 1989. Molecular mimicry as a mechanism for the cause and as a probe uncovering etiologic agent(s) of autoimmune disease. *Curr. Top. Microbiol. Immunol.* 145:127.
38. Stein, H., R. Schwarting, G. Niedobitek, and F. Dallenbach. 1989. Cluster report: CDw70. In *Leucocyte Typing IV. White Cell Differentiation Antigens*. W. Knapp, B. Dorken, W.R. Gilks, E.P. Rieber, R.E. Schmidt, H. Stein, and A.E.G.Kr.v.d. Borne, editors. Oxford University Press, Oxford. 446-449.
39. Oren, R., S. Takahashi, C. Doss, R. Levy, and S. Levy. 1990. TAPA-1, the target of anti-proliferative antibody, defines a new family of transmembrane proteins. *Mol. Cell. Biol.* 10:4007.