INFECTION AND IMMUNITY, Apr. 1994, p. 1484–1488
0019-9567/94/$04.00+0

Vol. 62, No. 4

Trypanosoma cruzi-Induced Immunosuppression: Blockade of Costimulatory T-Cell Responses in Infected Hosts due to Defective T-Cell Receptor–CD3 Functioning

MARCELA F. LOPES AND GEORGE A. DOS REIS*

Department of Immunology, Institute of Microbiology, Federal University of Rio de Janeiro, Rio de Janeiro, 21944-970, Brazil

Received 28 July 1993/Returned for modification 27 September 1993/Accepted 7 January 1994

A model of experimental *Trypanosoma cruzi* murine infection with chemically induced metacyclic forms (opossum clone Dm28c) showed a marked state of T-cell unresponsiveness during acute phase, but lacked evidence of suppressor cell activity. Spleen cells from infected mice were suppressed in vitro in responses to T-cell activators concanavalin A, anti-Thy1 monoclonal antibody (MAb), and anti-CD3 MAb compared with spleen cells from control littermates. Activation with accessory cell-independent stimulus provided by immobilized anti-CD3 was defective in splenic CD4-positive T cells from infected mice, but not in such cells from control mice. No evidence of splenic suppressor cell activity was found in cell-mixing experiments using nylon-passed T cells from control and infected donors. Kinetic experiments showed that there was a discrete stage in infection when T cells were already suppressed in response to anti-CD3 but still responded to anti-CD69 MAb. In these T cells, immobilized anti-CD3 failed to enhance simultaneous CD69 responses, although anti-CD3 enhanced CD69 responses in control T cells from uninfected donors. These results demonstrate an intrinsic defect in T-cell receptor-mediated T-cell activation, which could be a mechanism generating T-cell suppression during infection by *T. cruzi*.

Infection of humans by the intracellular protozoan *Trypanosoma cruzi* causes Chagas' disease, which by 1985 affected 16 million people in Latin America (22). Therefore, a great effort has been devoted to understanding the pathogenic mechanisms related to the dysfunction of the immune system induced by the parasite. Experimental murine models of Chagas' disease have identified a marked T-lymphocyte immunosuppression during acute infection (7, 14, 17) which could be involved in key pathogenic events in the host, such as continued parasite persistence (10), and immunoregulatory and repertoire disturbances leading to late autoimmune attack of target tissues such as cardiac and skeletal muscle or neuronal myoenteric plexuses (12). Distinct mechanisms for reduced T-cell responses have been described, including an accessory cell (AC) defect in interleukin 1 production (14), release of suppressive arachdonate metabolites (7, 17), and CD8 T-cell-derived suppressor activity (17). However, some of these and other studies also noted the concomitant presence of an endogenous T-cell defect (7, 18). We have developed an experimental model of murine infection with metacyclic forms of *T. cruzi* clone Dm28c, chemically transformed in vitro as described previously (4). In this system, we found a profound T-cell suppression in spite of undetectable suppressor cell activity. AC-independent T-cell responses to immobilized anti-CD3 antibody were markedly reduced in T cells from infected donors compared with those in cells from normal controls. However, there was a discrete stage in the course of infection when otherwise-suppressed T cells could still be activated via the early activation antigen CD69 (24). T cells from infected mice, but not from control littermates, failed to mount enhanced responses upon concomitant CD69 and T-cell receptor (TcR)-CD3 cross-linkage, indicating that the intrinsic T-cell defect can be antigen receptor mediated.

* Corresponding author.

Male BALB/c mice (6 to 8 weeks) were subcutaneously infected with 10^5 metacyclic Dm28c forms, obtained in vitro as described previously (4). After 20 to 60 days of infection, spleens were removed from infected and normal littermates. Whole-splenocyte suspensions and nylon wool-enriched T cells were prepared as described previously (8). These cells still contained residual AC sufficient to mount proliferative responses to concanavalin A (ConA) and to anti-CD69 plus phorbol myristate acetate (PMA), although the responses were of limited amplitude. In addition, the experiment shown in Fig. 1 was done with highly purified normal splenic T cells, obtained by two cycles of nylon wool purification followed by lysis with anti-Ia monoclonal antibody (MAb) and complement. These cells are depleted of AC, since they fail to respond to ConA and to anti-CD69 plus PMA (Fig. 1 and data not shown). CD8-depleted T cells were nylon-passed splenic T cells obtained by lysis with anti-CD8 MAb and complement followed by a second cycle of cytotoxicity with anti-rat immunoglobulin MAb and complement. By flow cytometry, the T cells in this population (80 to 85% of total cells) contained more than 92% CD4-positive cells and less than 3% CD8-positive cells in both control and infected donors. Cultures were done in 0.2 ml of Dulbecco modified Eagle medium (Sigma Chemical Co., St. Louis, Mo.) supplemented with bicarbonate, 2-mercaptoethanol (5×10^{-5} M), L-glutamine (2 mM), gentamycin (10 μg/ml), and 10% fetal calf serum (FCS) (Cultilab, São Paulo, Brazil) in 96-well flat-bottom microtiter plates (Linbro, Hamden, Conn.) for 3 days in a humid incubator with 5% CO_2 at 37°C. Eighteen hours before harvesting, 0.5 μCi of tritiated thymidine ([^{3}H]TdR; 6.7 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was added to each well. Cultures were harvested with a Mini-Mash II harvesting device (M.A. Bioproducts, Walkersville, Md.) onto fiberglass filters, and the amount of [^{3}H]TdR incorporated into DNA was measured by liquid scintillation spectroscopy. Cultures were set up in triplicate. Reagents added to cultures included

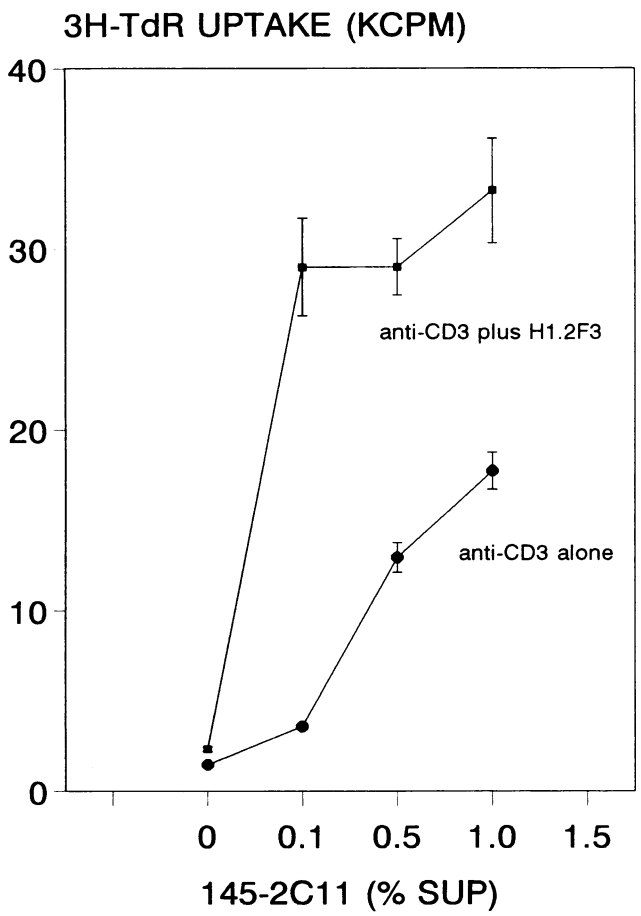

FIG. 1. Potentiation of T-cell activation by costimulation with anti-CD3 and anti-CD69 antibody H1.2F3. Splenocytes from normal BALB/c mice were purified over two nylon wool columns, depleted of Ia-positive cells, and stimulated (2×10^5) in the presence of 5 ng of PMA per ml in wells coated overnight with the indicated doses of anti-CD3 (145-2C11 MAb) supernatant fluid, which were or were not co-coated with anti-CD69 (10 μg of H1.2F3 salt-cut ascites per ml). Proliferation was assessed by [^{3}H]TdR uptake after 3 days in culture. Bars indicate the standard errors of the mean of triplicate cultures. Bars are hidden by circles where the error is too small.

PMA (Sigma), ConA (Pharmacia, Uppsala, Sweden), MTT reagent (Sigma), rat anti-mouse Thy1 MAb G7 (6), hamster anti-mouse CD3ε MAb 145-2C11 (11), and hamster anti-mouse CD69 MAb H1.2F3 (23) (provided by Ethan Shevach, Laboratory of Immunology, National Institutes of Health, Bethesda, Md.). To stimulate cells with plastic-immobilized antibodies, microtiter plates were treated for either 2 h or overnight at 37°C with MAb 145-2C11, diluted in 50 μl of serum-free medium at the desired dosage or with a mixture of 145-2C11 and 10-μg/ml H1.2F3. Before use, wells were washed three times with Hanks solution-10% fetal calf serum and reincubated until the cells were added. In one series of experiments, nylon-passed splenic T cells were cultured in vitro with different stimuli and, after 3 days, activation and/or proliferation was measured by an MTT reduction colorimetric assay as described previously (13). Color resulting from formazan crystal formation was measured at 590 nm in an enzyme-linked immunosorbent assay (ELISA) plate reader.

Murine CD69, like its human counterpart (2, 3, 19), is absent in resting T cells but is rapidly induced following in vitro activation with phorbol ester and in the presence of AC, cross-linkage with anti-CD69 MAb H1.2F3 induces murine polyclonal T-cell but not B-cell activation (23). In addition, human CD69 also enhances T-cell activation induced with anti-CD3 MAb (3). In highly purified mouse splenic T cells treated with phorbol ester (Fig. 1), immobilized anti-CD69 MAb H1.2F3 enhances T-cell activation induced by coimmobilized anti-CD3. Soluble anti-CD69 failed to enhance immobilized anti-CD3 responses in these highly purified T cells (data not shown). However, when responding cells still contained residual AC, as do splenic T cells passed over one nylon column, a cooperative response was seen with soluble anti-CD69 and limiting doses of immobile anti-CD3 (data not shown). Therefore, CD3 and CD69 pathways cooperate to costimulate normal murine T-cell activation, as occurs in human T cells. In a second experiment, BALB/c nylon-passed T cells gave responses of 3,828 cpm to PMA alone, 8,737 cpm to PMA and immobile H1.2F3, and 29,079 cpm to PMA and immobile anti-CD3. However, in microtiter wells coated with both anti-CD3 and H1.2F3, the T-cell response was 89,835 cpm. Addition of an equal number of irradiated AC (10^5) had no effect on the response to immobile anti-CD3 plus PMA (30,423 cpm), probably because this stimulus is AC independent. Addition of AC and soluble H1.2F3 in the presence of PMA resulted in a response of 413,090 cpm by these T cells in the absence of anti-CD3, indicating that anti-CD69 is a potent mitogen when added in soluble form in the presence of excess AC and PMA. We have developed a model of experimental Chagas' disease by infecting BALB/c mice with chemically induced metacyclic forms of the opossum *T. cruzi* clone Dm28c. Very similar alterations were induced in mice infected with either chemically induced or triatomine-derived metacyclic forms of clone Dm28c (11a). During acute phase, a profound state of immunosuppression develops among spleen T cells (Fig. 2A); this is characterized by markedly reduced responses to polyclonal T-cell stimuli like ConA, mitogenic anti-Thy1 MAb G7, and mitogenic anti-CD3ε chain MAb 145-2C11. However, stimulation of otherwise-suppressed splenocytes with anti-CD69 MAb H1.2F3 in the presence of PMA resulted in a vigorous proliferative response, almost comparable to that of control littermates (Fig. 2A). Since splenic T cells were collected at various time points during infection, this differential response of suppressed cells to anti-CD69 was not observed in all experiments. Some animals presented a generalized T-cell defect, including suppression to anti-CD69 stimulation. To bypass AC function in T-cell responses, we used immobilized anti-CD3 to stimulate nylon-passed spleen T cells in the presence or absence of PMA. This form of T-cell activation is considered to be AC-independent (5, 21). As mentioned above, nylon-passed, CD8-negative spleen cells from either control or infected donors had similar numbers of T cells expressing CD3 and CD4 markers and were essentially devoid of CD8-bearing cells, as assessed by flow cytometry. Nylon-passed CD8-negative T cells from control littermates responded both to high doses of immobilized anti-CD3 in the absence of PMA and to lower doses of immobilized anti-CD3 in the presence of PMA, as shown in Fig. 2B. On the other hand, as shown in Fig. 2B, nylon-passed, CD8-negative T cells from infected mice failed to respond to immobilized anti-CD3, even in the presence of a potent costimulator like PMA. Thus, an intrinsic defect in responsivity through CD3 is likely to occur in T cells from infected mice. Besides CD8 depletion, additional experiments also indicated a lack of suppressor cell activity in immunosuppressed mice. As shown in Table 1, experiment 1, splenic nylon-passed T cells

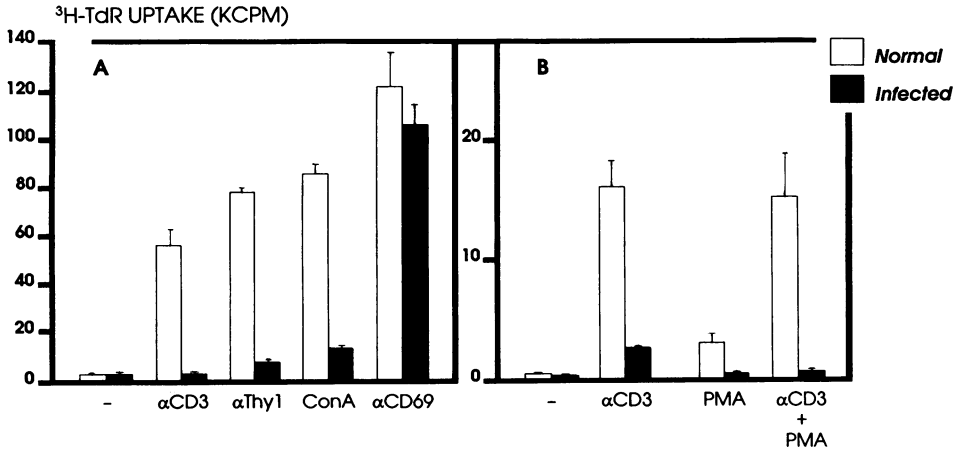

FIG. 2. Characterization of T-cell immune defects in *T. cruzi*-infected mice. (A). Sparing of CD69 activation pathway in suppressed T cells. Splenocytes (3×10^5) from control littermates or infected (51 days) BALB/c mice were stimulated with medium, 2 μg of ConA per ml, 0.002% anti-CD3 ascites, 10 μg anti-Thy1 (G7) salt-cut ascites per ml, or 10 μg of anti-CD69 (H1.2F3) salt-cut ascites per ml in the presence of PMA (5 ng/ml). (B). Immobilized anti-CD3 fails to activate CD4-positive T cells from infected donors. Control littermates or infected (41 days) BALB/c splenocytes were purified over nylon wool columns and depleted of CD8 T cells. CD4 T cells were cultured (2×10^5) either in the absence of PMA in anti-CD3-coated (0.1% ascites) wells or in the presence of PMA in anti-CD3-coated (0.005% ascites) wells. Proliferation was assessed by [^{3}H]TdR uptake after 3 days in culture. Standard errors of the mean are shown.

from infected mice were completely unresponsive to ConA, compared with the control donor. However, upon being mixed at a 1:1 ratio, nylon-passed T cells from the infected donor did not suppress the T-cell response of control donor to ConA. In fact, an enhancement of the ConA response was observed. Presumably, the enhancement was caused by an increase in the number of endogenous AC available for costimulation of the ConA response after the mixing of equal amounts of nylon-passed cells from control and infected donors. Moreover, as shown in Table 1, experiment 2, mixing nylon-passed T cells from an infected donor, which were unresponsive, with a responsive T-cell population from a control littermate did not result in any suppression of the control T-cell response to immobile anti-CD3 plus PMA. In this experiment, addition of an equal number of irradiated AC from a control donor did not enhance the response to immobile anti-CD3 in T cells from both control and infected donors, presumably because the stimulus was AC independent (data not shown). These results strongly suggest that the T-cell defect was intrinsic and not due to suppressor cell activity contained in residual AC or T-cell splenic populations. It was possible that the variable behavior of T cells upon anti-CD3 and anti-CD69 stimulation of individual infected mice resulted from differential kinetics of suppression in response to these stimuli during infection. To investigate this possibility, one group of infected mice and their control littermates was monitored at distinct stages postinfection. Activation and/or proliferation of nylon-passed splenic T cells (2×10^5) were measured by an MTT colorimetric assay, as described previously (13), after 3 days in culture supplemented with PMA and with or without stimulus. After 27 days of infection, an infected animal's T-cell response to immobile anti-CD3 was 144% above that of its control (0.793 change in optical density [ΔOD] versus 0.549 for control T cells). In a previous kinetic study, we had already noted this hyperresponsiveness in infected T cells just before the onset of immunosuppression. One week later (day 34), although control T-cell response to immobile anti-CD3 was maintained (0.670 ΔOD), T cells from the infected animal were already markedly unresponsive to immobile anti-CD3 (0.191 ΔOD). On the same day (day 34), we measured anti-CD69 (soluble) re-

TABLE 1. Lack of suppressor cell activity in splenic AC and T cells from infected donors

Expt no.	Nylon-passed T-cell origin	Mean (SEM) [^{3}H]TdR incorporation (cpm) in response to[a]:				
		Medium	ConA	Immobile anti-CD3	Δcpm ConA	Δcpm anti-CD3
1						
	Control	539 (166)	36,401 (2,251)	—[b]	35,862	—
	Infected	239 (94)	448 (132)	—	209	—
	Control + infected	748 (94)	66,014 (2,789)	—	65,266	—
2						
	Control	2,411 (263)	—	103,923 (3,562)	—	101,512
	Infected	363 (103)	—	6,116 (522)	—	5,753
	Control + infected	12,455 (576)	—	140,458 (3,220)	—	128,003

[a] Nylon-passed splenic T cells (1.5×10^5 in experiment 1, 1×10^5 in experiment 2) were cultured either alone or with an equal number (1.5×10^5 or 1×10^5, respectively) of T cells from control or infected animals. Proliferation was measured after 3 days in culture by [^{3}H]TdR uptake. Numbers are means of triplicate cultures. All cultures in experiment 2 received 5 ng of PMA per ml, and immobile anti-CD3 was used at an concentration of 0.1% 145-2C11 ascites.

[b] —, not done.

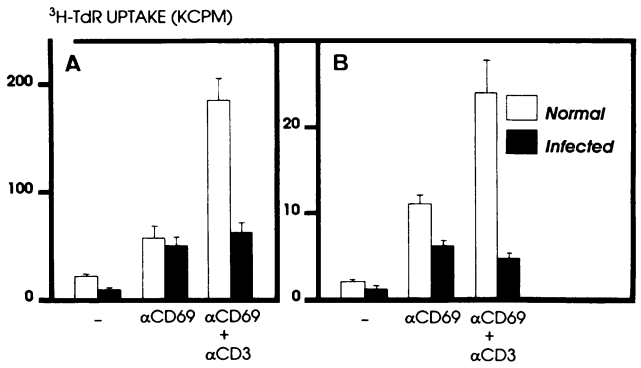

FIG. 3. CD3 engagement fails to enhance CD69 T-cell responses in *T. cruzi*-infected mice. Splenocytes (2×10^5) from control littermates or infected (29 days for panel A and 32 days for panel B) BALB/c mice were passed over nylon wool columns and stimulated in the presence of PMA in anti-CD69-coated (10 μg/ml) wells. Part of the cultures (A and B) also contained immobilized anti-CD3 MAb (0.005% ascites for coating). Coating was done by a 2-h incubation at 37°C. Proliferation was assessed by [^{3}H]TdR uptake after 3 days in culture. Panels A and B represent independent experiments done with distinct lots of infected animals. Standard errors of the mean are shown. Statistical analysis was done by comparing T-cell responses to anti-CD69 without or with immobile anti-CD3 (paired *t* test, using log transformation of [^{3}H]TdR uptake data). The differences caused by including anti-CD3 were significant ($P < 0.05$) for control T cells in both panels A and B. However, the differences caused in T cells from infected donors were not significant in either panel A or B.

sponses and found that they were almost comparable in control (0.138ΔOD) and infected (0.112ΔOD) animals. At a later stage in infection (day 55), the response to anti-CD3 in the control was 0.553ΔOD but was only 0.061ΔOD in the infected donor. However, by this time the response to soluble anti-CD69 was also suppressed in the infected animal's T cells (0.034) compared within control T cells (0.435ΔOD). As an additional control, although T-cell responses to ionomycin plus PMA were comparable in control and infected animals up until 45 days after infection, T cells from the infected group lost responsiveness to this mitogenic stimulus at day 55 (0.172 compared to 0.661ΔOD in control T cells). Therefore, T-cell unresponsiveness seems to progress during infection, and loss of T-cell responses occurs in the following order: CD3 (and ConA [not shown]), CD69, ionomycin. These results confirm that there is a discrete stage during infection when T cells still respond to soluble anti-CD69, although they are already defective to stimulation via immobile anti-CD3. Therefore, our previous variable results might have been due to testing animals either before or after this critical stage.

In order to assess the role of TcR-CD3 pathway in establishing the low-responsiveness state, we investigated CD3-CD69 cooperation in animals which still responded to anti-CD69. Figure 3A and B shows two independent experiments comparing T cells from infected donors and matched control T cells, both of which were stimulated with immobilized anti-CD69 with or without coimmobilized anti-CD3 in the presence of the CD69-inducing agent PMA. The experiments were done on different occasions, using two distinct lots of infected animals. As shown in the figure, T-cell responses to anti-CD69 were still detectable in infected mice. Costimulation with immobile anti-CD3 enhanced the CD69 response in control T cells but failed to enhance T-cell responses in the infected group. The results shown in Fig. 3 strongly suggest that TcR-CD3 function is defective or abnormal in T cells from infected mice, being unable to costimulate activation with CD69. The possibility of a TcR signalling defect, such as the one described in peripheral T-cell tolerance to a self Mls 1^a ligand (1), is currently being investigated.

In murine T cells, the responses to both ConA and anti-Thy1 are TcR-CD3 dependent (16). CD69-induced activation in human T cells is also dependent on concomitant TcR-CD3 expression (15). However, T cells from infected mice could respond to anti-CD69 at a stage when they were unresponsive to TcR-CD3. Perhaps differences in the extent of TcR-CD3 engagement by CD69 or additional unique properties of CD69 signalling could explain this differential behavior. In this study, we used immobile anti-CD3 to observe that an intrinsic functional defect in the TcR-CD3 pathway occurs in T cells from *T. cruzi*-infected hosts. Therefore, T-cell anergy is a likely mechanism of immunosuppression in the host. During polyclonal activation and parasite replication, most of the murine T cells in vivo (12) and about 15% of human T cells in vitro (20) are activated, which seems paradoxical in light of the finding of an unresponsive state. However, vigorous expansion of T cells in vivo in the periphery can lead to T-cell anergy and/or programmed cell death, as has been demonstrated for T-cell subsets stimulated in vivo with self (1) and bacterial (9) superantigens. We are currently investigating, in this model of Chagas' disease, the relationship between polyclonal T-cell activation and the onset of TcR-CD3-mediated defects in T-cell activation.

We are indebted to Samuel Goldenberg (FIOCRUZ, Rio de Janeiro, Brazil) for the gift of clone Dm28c and for transfer of the metacyclogenesis procedure to us. We also thank Ethan Shevach (NIH, Bethesda, Md.) for the gift of H1.2F3 MAb.

This work was supported by Fundação José Bonifácio/UFRJ and Financing Agency of Studies and Projects (FINEP/MCT). M.F.L. is a CAPES/ME graduate student, and G.A.D.R. is a fellow of CNPq/MCT.

REFERENCES

1. **Blackman, M. A., T. H. Finkel, J. Kappler, J. Cambier, and P. Marrack.** 1991. Altered antigen receptor signaling in anergic T cells from self-tolerant T cell receptor beta-chain transgenic mice. Proc. Natl. Acad. Sci. USA **88:**6682–6686.
2. **Cebrián, M., J. M. Redondo, A. López-Rivas, G. Rodriguez-Tarduchy, M. O. De Landázuri, and F. Sánchez-Madrid.** 1989. Expression and function of AIM, an activation inducer molecule of human lymphocytes, is dependent on the activation of protein kinase C. Eur. J. Immunol. **19:**809–815.
3. **Cebrián, M., E. Yague, M. Rincón, M. O. López-Botet, M. O. De Landázuri, and F. Sánchez-Madrid.** 1988. Triggering of T cell proliferation through AIM, an activation inducer molecule expressed on activated human lymphocytes. J. Exp. Med. **168:**1621–1637.
4. **Contreras, V. T., J. M. Salles, N. Thomas, C. M. Morel, and S. Goldenberg.** 1985. *In vitro* differentiation of *Trypanosoma cruzi* under chemically defined conditions. Mol. Biochem. Parasitol. **16:**315–327.
5. **Geppert, T. D., and P. E. Lipsky.** 1987. Accessory cell-independent proliferation of human T4 cells stimulated by immobilized monoclonal antibodies to CD3. J. Immunol. **138:**1660–1666.
6. **Gunter, K. C., T. R. Malek, and E. M. Shevach.** 1984. T cell activating properties of an anti-Thy1 monoclonal antibody: possible analogy to OKT3/Leu-4. J. Exp. Med. **159:**716–730.
7. **Harel-Bellan, A., M. Joskowicz, D. Fradelizi, and H. Eisen.** 1985. T lymphocyte function during experimental Chagas disease: production of and response to interleukin 2. Eur. J. Immunol. **15:**438–442.
8. **Julius, M. H., E. Simpson, and L. H. Herzenberg.** 1973. A rapid method for the isolation of functional thymus derived murine lymphocytes. Eur. J. Immunol. **3:**645–649.

9. **Kawabe, Y., and A. Ochi.** 1991. Programmed cell death and extrathymic reduction of V-beta 8+ CD4+ T cells in mice tolerant to *Staphylococcus aureus* enterotoxin B. Nature (London) **349:** 245–248.
10. **Kierszenbaum, F.** 1981. On evasion of *Trypanosoma cruzi* from the host immune response. Lymphoproliferative responses to trypanosomal antigens during acute and chronic experimental Chagas' disease. Immunology **44:**641–648.
11. **Leo, O., M. Foo, D. H. Sachs, L. E. Samelson, and J. A. Bluestone.** 1987. Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc. Natl. Acad. Sci. USA **84:**1374–1377.
11a. **Lopes, M. F., et al.** Unpublished data.
12. **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 TcR I T cells. Immunol. Rev. **112:**184–206.
13. **Mosmann, T.** 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **65:**55–63.
14. **Reed, S. G., D. L. Pihl, and K. H. Grabstein.** 1989. Immune deficiency in chronic *Trypanosoma cruzi* infection: recombinant IL1 restores Th function for antibody production. J. Immunol. **142:**2067–2071.
15. **Rincón, M., M. Cebrián, F. Sánchez-Madrid, and M. López-Botet.** 1989. Induction of T cell function via the gp 33/27 activation inducer molecule (AIM) requires co-expression of the CD3/TcR complex. Eur. J. Immunol. **19:**959–965.
16. **Schmitt-Verhulst, A. M., A. Guimezanes, C. Boyer, M. Poenie, R. Tsien, M. Buferne, C. Hua, and L. Leserman.** 1987. Pleiotropic loss of activation pathways in a T cell receptor alpha chain deletion variant of a cytolytic T cell clone. Nature (London) **325:**628–631.
17. **Tarleton, R. L.** 1988. *Trypanosoma cruzi*-induced suppression of IL2 production. II. Evidence for a role for suppressor cells. J. Immunol. **140:**2769–2773.
18. **Tarleton, R. L., B. H. Kohler, A. Latour, and M. Postan.** 1992. Susceptibility of beta 2-microglobulin-deficient mice to *Trypanosoma cruzi* infection. Nature (London) **356:**338–341.
19. **Testi, R., J. H. Phillips, and L. L. Lanier.** 1989. Leu 23 induction as an early marker of functional CD3/T cell antigen receptor triggering. J. Immunol. **142:**1854–1860.
20. **Van Voorhis, W. C.** 1992. Coculture of human peripheral blood mononuclear cells with *Trypanosoma cruzi* leads to proliferation of lymphocytes and cytokine production. J. Immunol. **148:**239–248.
21. **Williams, I. R., and E. R. Unanue.** 1990. Costimulatory requirements of murine TH1 clones; the role of accessory cell-derived signals in responses to anti-CD3 antibody. J. Immunol. **145:**85–93.
22. **World Health Organization.** 1991. Control of Chagas disease, p. 27–30. WHO Technical Report 811. World Health Organization, Geneva.
23. **Yokoyama, W. M., F. Koning, P. J. Kehn, G. M. Pereira, G. Stingl, J. E. Coligan, and E. M. Shevach.** 1988. Characterization of a cell surface-expressed disulfide-linked dimer involved in murine T cell activation. J. Immunol. **141:**369–376.
24. **Yokoyama, W. M., S. R. Maxfield, and E. M. Shevach.** 1989. Very early (VEA) and very late (VLA) activation antigens have distinct functions in T lymphocyte activation. Immunol. Rev. **109:**153–176.