Activation-Induced T Cell Death Exacerbates Trypanosoma cruzi Replication in Macrophages Cocultured with CD4+ T Lymphocytes from Infected Hosts

Marise P. Nunes, Regis M. Andrade, Marcela F. Lopes and George A. DosReis

*J Immunol* 1998; 160:1313-1319; 
http://www.jimmunol.org/content/160/3/1313
Activation-Induced T Cell Death Exacerbates Trypanosoma cruzi Replication in Macrophages Cocultured with CD4⁺ T Lymphocytes from Infected Hosts

Marise P. Nunes, Regis M. Andrade, Marcela F. Lopes, and George A. DosReis

Activation-induced cell death (AICD) of CD4⁺ T lymphocytes was described in infection with Trypanosoma cruzi, but a role for AICD in modulating parasite spread in host cells has not been investigated. In this study, replication of T. cruzi in vitro in murine macrophage (Mφ) monolayers was investigated. Long term (5 to 13 day) replication of infective (trypomastigote) T. cruzi forms was blocked by supernatants from activated (anti-TCR) CD4⁺ T cells of infected mice or by rIFN-γ. However, when CD4⁺ T cells from infected mice were cocultured with Mφ and activated by anti-TCR, marked exacerbation of trypomastigote growth in Mφ ensued. The deleterious effect required contact between T cells and infected Mφ. Both anti-Fas and TCR activation killed a proportion of CD4⁺ T cells. Ly-6 activation did not induce AICD and did not exacerbate parasite growth. However, Fas-mediated killing of T cells before Ly-6 activation led to exacerbated parasite growth. Although a minor population, Fas-susceptible cells were the major source of IFN-γ production by activated T cells. Addition of a neutralizing anti-Fas ligand antibody blocked 50 to 60% of CD4⁺ T cell AICD and reduced trypomastigote growth in T/Mφ cocultures stimulated by anti-TCR. The results demonstrate that in CD4⁺ T cells from infected mice, the onset of AICD selectively ablates IFN-γ production and up-regulates parasite replication in Mφ in vitro. These findings suggest a deleterious role for AICD in T. cruzi infection. The Journal of Immunology, 1998, 160: 1313–1319.

Cell-mediated immune responses play a key role in host defense against the intracellular protozoan parasite Trypanosoma cruzi, the causative agent of Chagas’ disease (American trypanosomiasis) (reviewed in Ref. 1). Mice made genetically deficient in either CD4⁺ or CD8⁺ T cell subsets become highly susceptible to death after infection with T. cruzi (2–4). However, the mechanisms responsible for antiparasite resistance have not been precisely defined for CD4⁺ T cells. It has been suggested that protection could result from either T cell help for production of protective lytic Abs or secretion of cytokines that help infected macrophages (Mφ) to control intracellular parasite replication (4, 5). Supernatants from T cells activated by T. cruzi Ags or mitogens contain cytokines that restrict the intracellular growth of T. cruzi in infected Mφ cultures (6, 7). The cytokine IFN-γ plays a central role in Mφ microbicidal activity against intracellular forms of T. cruzi in vitro (8, 9) and also during the initial phase of infection in vivo (10, 11). Depending on parasite strain and host susceptibility in different models, CD4⁺ T cells can secrete large amounts of IFN-γ and TNF-α, but little or no IL-4 upon activation (12). Nonetheless, activation through the TCR results in suppressed T cell responses (12), a feature that could help the parasite to escape complete destruction and persist in the host. Different mechanisms of CD4⁺ T cell suppression in vitro have been described (12, 13). Infection with highly virulent blood form trypomastigotes suppresses CD4⁺ T cell responses through IFN-γ production and secretion of nitric oxide (NO) metabolites by cocultured activated Mφ (12). On the other hand, in infection with metacyclic trypomastigotes, a form of suppression was found that is independent of Mφ products (13) and results from activation-induced cell death (AICD) (14), a regulatory response of activated CD4⁺ T cells to TCR,CD3 engagement (15, 16). Both susceptibility of CD4⁺ T cells to AICD in vitro and CD4⁺ T cell apoptosis in vivo have been described in the course of experimental Chagas’ disease induced by metacyclic trypomastigotes (17). AICD is regarded as a mechanism for the elimination of activated T cells, reducing damage to host tissues (15, 16). However, in the case of T. cruzi infection, it has been suggested that AICD could play a deleterious role for the host by killing activated T cells and restricting the effectiveness of antiparasite effector responses (18). To investigate whether AICD plays a role in T cell modulation of parasite growth within infected Mφ, we developed an in vitro system of coculture of T. cruzi-infected Mφ monolayers and purified CD4⁺ T cells from infected donors. Activation of these T cells was elicited under various controlled conditions. We found that while addition of CD4⁺ T cell supernatants or IFN-γ reduced the load of infective T. cruzi forms, direct activation through the TCR of cocultured CD4⁺ T cells from infected donors exacerbated parasite accumulation in Mφ monolayers. In this investigation we demonstrate that the onset of CD4⁺ T lymphocyte AICD is responsible for exacerbation of parasite growth, with potentially deleterious consequences for the host.
Materials and Methods

Mice

Male BALB/c and C57.B16 (B6) mice, 6 to 8 wk of age, were obtained from the Oswaldo Cruz Institute (IOC-FIOCRUZ, Rio de Janeiro, Brazil) animal facility. Mice were infected s.c. with 10^5 chemically induced metacyclic trypomastigotes from the T. cruzi clone Dm28c in 0.1 ml, as previously described (17). Uninfected littersmates were used as controls and as a source of normal peritoneal Mφ. Chemically induced metacytic forms were obtained with triatamine artificial urino-proline medium as originally described (19). Parasitemia peaked between 18 and 26 days of infection. All T cell preparations used in this study were obtained between 18 and 26 days of infection.

Infection of Mφ monolayers with T. cruzi

Normal resident Mφ from uninfected BALB or B6 mice were obtained by peritoneal lavage with DMEM. Cells were washed and cultured at 37°C in 7% CO₂ in a humud atmosphere at 3 × 10^5 cells/well in 24-well vessels (Corning Glass Works, Corning, NY) in 1 ml of complete culture medium containing DMEM (Sigma Chemical Co., St. Louis, MO), supplemented with 10% heat-inactivated FCS, 2-ME (5 × 10⁻⁵ M), MEM nonessential amino acids, 10 mM HEPES, 2 mM l-glutamine, 1 mM sodium pyruvate, and 10 μg/ml gentamicin sulfate. After overnight incubation, nonadherent cells were discarded, and adherent cells were washed, leaving approximately 2 × 10^6 Mφ. These Mφ monolayers were infected by overnight incubation with Dm28c metacyclic trypomastigotes (19) at a 10:1 parasite: cell ratio in culture medium at 37°C in 7% CO₂. After 18 h, monolayers were extensively washed to remove extracellular parasites.

Coculture of Mφ monolayers with purified T cell subsets

Splenocytes obtained from either T. cruzi-infected or control mice syngeneic with the Mφ donors were depleted of erythrocytes by treatment with Tris-buffered ammonium chloride and filtered through nylon wool columns. Nonadherent cells were subjected to negative selection by magnetic cell sorting using a mixture of anti-CD8 (or anti-CD4, in the case of purified CD4 cells), anti-rat Ig-coated magnetic beads (Bio-Mag, Perseptive Diagnostics, Cambridge, MA). By flow cytometry, CD4^+ T cells had <1% contaminating CD8^- and MHC class II^- cells, in both control and infected groups. The Mφ monolayers provided an efficient source of accessory cells for anti-TCR activation of purified T cells. Since addition of phorbol ester does not interfere with CD4^+ T cell AICD in cells from infected mice (14), and since T cell activation by anti-Ly-6 strictly requires phorbol ester as a cofactor (20), most experiments were conducted in the presence of phorbol ester (0.5 ng/ml PMA; Sigma). Anti-TCR activation of T cells in the absence of phorbol ester yielded similar results (see Table II).

Two kinds of experiments required modifications of the above procedure. First, in cultures performed with membrane-separated culture inserts (MilliCell inserts for 24-well vessels; 0.4 μm; Sigma), endogenous splenic accessory cells were not depleted to ensure proper T cell activation in the compartment separated from Mφ. Therefore, CD4^+ T cells were obtained by treatment of nylon wool-nonadherent cells with anti-CD4 mAb and, followed by a second cycle of indirect cytotoxicity as previously described (17). Second, in all experiments involving anti-Ly-6 mAb, purified T cells were supplemented with 10% irradiated (3000 rad) normal syngenic splenocytes regardless of the stimulus used. This treatment restored anti-Ly-6-mediated T cell activation, which was deficient with adherent Mφ.

Quantitation of T cell viability

Purified CD4^+ T cells were cultured overnight with anti-Fas or isotype control as described above and were stimulated, or not, with anti-TCR the next day. Supernatants were collected 48 h after TCR activation and stored at −80°C until cytokine measurement. 

In experiments comparing the effect of anti-Fas treatment (using the Fas agonist mAb Jo2) (24) on Ly-6 activation, a two-step culture procedure was used to avoid coincidence of both CD4^+ T cell AICD and the onset of lymphokine secretion (25). Purified CD4^+ T cells (2 × 10^6, plus 10% irradiated splenocytes) were cultured alone in 1 ml of complete culture medium containing PMA plus 2 μg/ml hamster anti-mouse Fas mAb Jo2 or 2 μg/ml of control hamster IgG mAb. After overnight incubation, the entire content of individual wells was aspirated and transferred to wells containing decanted T. cruzi-infected Mφ monolayers. Cocultures were then initiated in the absence or the presence of newly added activating anti-TCR or 0.5% (v/v) ascites of anti-Ly-6 A/E mAb D7 (20). Extracellular trypomastigote accumulation was measured as described above. Controls included infected Mφ cultured with PMA in the absence of T cells and in the presence or the absence of anti-Fas or anti-Ly-6. No changes in Mφ viability or number of motile trypomastigotes were noticed with any of these treatments. Suspensions of CD4^+ T cells from infected mice contained a limited (<10^5) number of motile trypomastigotes. Following activation in the absence of adherent Mφ, no detectable parasites were recovered after 5 days or more, even when partially purified T cells were strongly activated.

Assay for IFN-γ production

Purified CD4^+ T cells were cultured overnight with anti-Fas or isotype control as described above and were stimulated, or not, with anti-TCR the next day. Supernatants were collected 48 h after TCR activation and stored at −80°C until cytokine measurement. Purified CD4^+ T cells were cultured overnight with anti-Fas or isotype control as described above and were stimulated, or not, with anti-TCR the next day. Supernatants were collected 48 h after TCR activation and stored at −80°C until cytokine measurement. 

Abs and cytokines

Anti-CD8 mAb 53-6.7, anti-B220 mAb 6B2, anti-MHC class II mAb AMS 32-1, anti-MAc-1, and anti-CD16/CD32 mAbs as previously described (17) plus anti-TCR g8o to yield highly purified CD4^- or CD8^- T cells after three rounds of separation with anti-rat Ig-coated magnetic beads (Bio-Mag, Perseptive Diagnostics, Cambridge, MA). By flow cytometry, CD4^- T cells had <1% contaminating CD8^- and MHC class II^- cells, in both control and infected groups. The Mφ monolayers provided an efficient source of accessory cells for anti-TCR activation of purified T cells. Since addition of phorbol ester does not interfere with CD4^- T cell AICD in cells from infected mice (14), and since T cell activation by anti-Ly-6 strictly requires phorbol ester as a cofactor (20), most experiments were conducted in the presence of phorbol ester (0.5 ng/ml PMA; Sigma). Anti-TCR activation of T cells in the absence of phorbol ester yielded similar results (see Table II).

Two kinds of experiments required modifications of the above procedure. First, in cultures performed with membrane-separated culture inserts (MilliCell inserts for 24-well vessels; 0.4 μm; Sigma), endogenous splenic accessory cells were not depleted to ensure proper T cell activation in the compartment separated from Mφ. Therefore, CD4^- T cells were obtained by treatment of nylon wool-nonadherent cells with anti-CD4 mAb and, followed by a second cycle of indirect cytotoxicity as previously described (17). Second, in all experiments involving anti-Ly-6 mAb, purified T cells were supplemented with 10% irradiated (3000 rad) normal syngenic splenocytes regardless of the stimulus used. This treatment restored anti-Ly-6-mediated T cell activation, which was deficient with adherent Mφ. Purified CD4^- T cells (2 × 10^5) in 1 ml of complete culture medium, were added to prewashed, T. cruzi-infected syngenic Mφ monolayers (2 × 10^6) in the presence of PMA and in the presence or the absence of 10% (v/v) culture supernatant of anti-TCRαβ MAb H57.597 (21). Additional Abs added along with anti-TCR included mouse (gld strain) anti-mouse FasL (B6 strain) mAb K10 (10 μg/ml), a neutralizing IgG2b/anti-FasL Ab that reacts with an allotopic determinent on mouse FasL that is present in B6- but not in BALB-activated T cells (22); and mouse isotype control mAb 156-8 (10 μg/ml), an IgG2b/Ab specific for hepatitis virus B (AYW3 strain, produced at FIGOCRUZ, Rio de Janeiro, Brazil). Cultures were set up in duplicate and maintained for up to 13 days. On the indicated days, individual wells were gently pipetted up and down, and 20-μl aliquots of the supernatant were removed for assessment of viable extracellular T. cruzi trypomastigote numbers. Motile trypomastigotes were easily identified in the counting chamber, based on established morphologic criteria (23). Results are presented as the mean and SE of trypomastigote number in the duplicate cultures.

Downloaded from http://www.jimmunol.org/ by guest on April 19, 2017
FIGURE 1. Blockade of trypomastigote growth in Mφ by IFN-γ and supernatants from activated CD4+ T cells. BALB peritoneal Mφ, infected with metacyclic T. cruzi trypomastigotes (Dm28c), were treated with an excess of the indicated recombinant murine cytokines or with 10% (v/v) anti-TCR-activated T cell supernatants in a single addition on day 0. Cultures were maintained for 8 days (Exp 1) or 5 days (Exp 2), and viable trypomastigote number was determined. Results are the mean and SE of duplicate cultures. Control indicates infected Mφ alone. Significant differences were obtained with IFN-γ, GM-CSF, and supernatant from infected CD4+ T cells only.

Results
Decrease in T. cruzi replication in macrophage monolayers induced by rIFN-γ and by supernatants from activated CD4+ T cells
Peritoneal resident Mφ monolayers from BALB/c mice were infected by 18-h exposure to T. cruzi metacyclic trypomastigotes, washed, and recultured for up to 13 days. Extracellular motile trypomastigote (infective) forms were first observed after 3 days in culture, and their number continuously increased thereafter. At the Mφ density used, trypomastigotes comprised the predominant parasite form in culture. There was variability in the number of trypomastigotes produced between different experiments, probably related to the period of time that epimastigotes are maintained in the laboratory before induction of metacyclogenesis. However, trypomastigote growth in replicate cultures within the same experiment was quite similar. Several recombinant murine cytokines were tested to determine the kinetics of extracellular trypomastigote accumulation in Mφ cultures (Fig. 1). Among all cytokines tested (rIL-1β, rIL-2, rIL-4, rIL-10, rTNF-α, rGM-CSF, and rIFN-γ), only rIFN-γ had a clear protective and long term effect against replication of T. cruzi in Mφ monolayers, as detected after 5 to 8 days in culture (Fig. 1). On the other hand, rGM-CSF consistently increased the number of accumulated T. cruzi trypomastigotes (Fig. 1). It should be noted that at earlier time points, such as 3 days in culture, IFN-γ completely blocked the emergence of motile parasites in the medium (not shown). We also tested the effect of supernatants from anti-TCR-activated CD4+ T cells from either control or T. cruzi-infected mice (Fig. 1). Only CD4+ T cells from infected hosts produced supernatants active against T. cruzi replication. In agreement with previous studies (12), we found that CD4+ T cells from acutely infected mice produced 18 times more IFN-γ than control CD4+ T cells following activation with anti-TCR mAb (not shown).

Table I. Exacerbation of T. cruzi replication in Mφ induced by activated CD4+ T cells

<table>
<thead>
<tr>
<th>Addition to Mφ</th>
<th>Viable Parasite Number (×10⁴)/Well ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>CD4 T</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>CD4 T + anti-TCR</td>
<td>4.8 ± 0.2</td>
</tr>
</tbody>
</table>

* Motile trypomastigote forms were counted after the indicated periods in culture. Results indicate mean ± SE of duplicate 1-ml cultures. Significant differences (compared with Mφ alone) are italicized.

Direct CD4+ T cell activation exacerbates T. cruzi replication in cocultured Mφ monolayers
Given the above results, we expected to find significant containment of parasite replication in Mφ following activation of cocultured CD4+ T cells with anti-TCR mAb. However, coculture of purified CD4+ T cells from infected mice by itself increased trypomastigote replication 2-fold in the absence of exogenous stimulation (Table I). Moreover, anti-TCR activation of these T cells resulted in a marked increase (12-fold in this experiment) in the number of T. cruzi trypomastigote forms recovered (Table I). Enhancement of parasite replication was observed at all time points investigated throughout a 13-day period. Growing parasites were derived from infected Mφ, since activation of CD4+ T cells alone did not result in detectable parasite growth (data not shown). The ability to potentiate parasite replication was a characteristic of CD4+ T cells from T. cruzi-infected donors, since activation of CD4+ T cells from control mice did not result in enhanced parasite replication or resulted in much less replication compared with that in T cells from infected mice (data not shown). Exacerbation of parasite replication required CD4+ T cell activation by anti-TCR, but addition of PMA was not necessary even though the number of trypomastigotes increased (Table II). However, since we studied accessory activation pathways that required phorbol ester, addition of PMA was maintained in the remaining experiments.

We compared CD4+ and CD8+ T cell subsets from infected mice for their ability to potentiate T. cruzi replication in Mφ monolayers (Fig. 2A). Activation of CD4+ T cells markedly potentiated trypomastigote growth, while activation of CD8+ T cells resulted in much less trypomastigote replication in Mφ (Fig. 2A). However, while CD4+ T cells invariably exacerbated trypomastigote growth, use of activated CD8+ T cells gave variable results. In some experiments, CD8+ T cells had little effect on trypomastigotes and increased the number of noninfective epimastigotes, while in other experiments, CD8+ T cells actually suppressed trypomastigote growth. The reason for this variable behavior of CD8+ T cells is being investigated. In any case, CD8+ T cells

Table II. Exacerbation of T. cruzi replication by CD4+ T cell activation does not require phorbol ester

<table>
<thead>
<tr>
<th>Addition to Mφ</th>
<th>Viable Parasite Number (×10⁴)/Well ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 T</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>CD4 T + anti-TCR</td>
<td>9.0 ± 1.0</td>
</tr>
<tr>
<td>CD4 T + PMA</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>CD4 T + anti-TCR / PMA</td>
<td>18.0 ± 2.0</td>
</tr>
</tbody>
</table>

* Motile trypomastigote forms were counted in Mφ monolayers after the indicated periods in culture, in the presence or absence of 0.5 ng/ml PMA. Significant differences (compared with unstimulated CD4 T cells) are italicized.
were clearly distinct from CD4+ T cells in the ability to help trypanosome growth.

Since activated CD4+ T cells clustered around infected Mφ in culture, we tested the need for direct T/Mφ cell contact for exacerbation of parasite replication (Fig. 2B). Infected Mφ monolayers and CD4+ T cells were cultured in the same compartment or separated by a cell-impermeable membrane in culture inserts. Splenic CD4+ T cells contained endogenous accessory cells to allow full activation when separated from Mφ. Separation of CD4+ T cells and Mφ markedly reduced parasite growth compared with that in unseparated cocultures (Fig. 2B). These results demonstrate the need for direct cell contact between activated CD4+ T cells and Mφ to potentiate the growth of *T. cruzi*.

**Modulation of CD4+ T cell activation by Fas-induced death regulates parasite replication in Mφ monolayers**

Activation of CD4+ T cells from *T. cruzi*-infected mice with anti-TCR, but not with anti-Ly-6, results in both AICD by apoptosis and down-regulated proliferation (14, 17). We then investigated the role of CD4+ T cell AICD in exacerbation of parasite growth. Following coculture with infected Mφ monolayers in the presence of different stimuli, AICD was evaluated in recovered CD4+ T cells. Addition of anti-TCR killed a substantial proportion of the T cells, while addition of anti-Ly-6 did not result in T cell death and, instead, increased T cell viability after 1 day in culture (Fig. 3A), presumably because of lymphokine-mediated rescue from spontaneous cell death. The Fas/FasL death pathway has been implicated in mediating CD4+ T cell AICD (16). Addition of an agonist anti-Fas mAb killed CD4+ T cells, and when combined with anti-Ly-6, anti-Fas abrogated the protective effect of anti-Ly-6, leading to cell death (Fig. 3A). Susceptibility to both anti-TCR- and anti-Fas-mediated death was restricted to CD4+ T cells from infected, but not from control, mice (not shown). The same results were observed in a repeat experiment. The data indicate that AICD is preserved in CD4+ T cell interaction with infected Mφ and confirm that the Ly-6 activation pathway is deficient in inducing AICD. We then explored this differential behavior by evaluating the role of Fas-induced death in the control of parasite replication (Fig. 3, B and C). Contrasting with the exacerbated trypanosome growth elicited by TCR activation, Ly-6 activation of CD4+ T cells from infected mice did not result in appreciable enhancement of parasite replication in Mφ (Fig. 3, B and C) compared with that in unstimulated T cells. Strikingly, when Ly-6 activation was combined with prior treatment with anti-Fas, a marked enhancement of parasite replication was seen, similar to that with TCR activation (Fig. 3, B and C). The experiment was repeated with identical results. Treatment with a control hamster IgG mAb instead of anti-Fas did not result in enhanced parasite growth (not shown). In the absence of T cells, neither anti-Fas nor anti-Ly-6 mAbs had any effect on Mφ viability or parasite replication. Both killing and activation of host CD4+ T cells are required for exacerbating parasite growth, since treatment with anti-Fas alone was far less effective (Fig. 3, B and C) despite inducing comparable T cell killing in the presence or the absence of anti-Ly-6 (see Fig. 3A).

To confirm that Fas-mediated AICD is involved in exacerbation of *T. cruzi* growth, a neutralizing anti-FasL mAb, specific for B6 mouse FasL allotype (22), was used to block CD4+ T cell AICD. In several experiments, anti-FasL mAb consistently blocked 50 to 60% of AICD in CD4+ T cells from *T. cruzi*-infected B6 mice, while an isotype control mAb had no effect (Fig. 4A). Anti-FasL Ab had little or no effect on proliferation or viability of CD4+ T cells from control B6 mice, but consistently increased the otherwise depressed anti-TCR;CD3 proliferative responses of CD4+ T cells from infected mice (not shown). When added to cocultures of infected B6 Mφ and TCR-activated CD4+ T cells from infected B6 mice, anti-FasL mAb, but not an isotype control, markedly reduced the growth of *T. cruzi* trypanomastigotes in culture (Fig. 4, B and C) in a manner consistent with the blockade of AICD achieved in CD4+ T cells (Fig. 4A). Blockade of parasite replication by anti-FasL was confirmed in two additional experiments. On the other hand, anti-FasL mAb had no effect on trypanomastigote replication in Mφ cultured in the absence of T cells (data not shown). Together, these results indicate the involvement of CD4+ T cell AICD in up-regulation of parasite replication in Mφ monolayers.

Finally, since IFN-γ is critically involved in the control of parasite replication in Mφ, it was of interest to investigate the effect of Fas-mediated death in IFN-γ production by CD4+ T cells from *T. cruzi*-infected mice. To avoid the coincidence of IFN-γ secretion and the onset of AICD (25), T cells were first treated overnight with either anti-Fas or a control hamster mAb and subsequently stimulated with anti-TCR, and resulting supernatants were assayed for IFN-γ content (Fig. 5). Prior overnight treatment with anti-Fas results in killing of 25 to 30% of CD4+ T cells from infected hosts.
but eliminated 70.6% of the IFN-γ produced upon subsequent TCR activation (Fig. 5). Killing with anti-Fas also eliminated 67.0% of IFN-γ production upon Ly-6 activation (not shown). These results indicate that although they are a minor population, Fas-susceptible CD4+ T cells comprise the majority of IFN-γ-producing cells or contain most of IFN-γ high producer CD4+ T cells in T. cruzi-infected hosts.

Discussion

A functional link between suppression of host T cell responses and escape of T. cruzi from immune attack had been proposed in early investigations of cell-mediated immunity in this parasitic infection (26). However, common mechanisms merging these two aspects of experimental Chagas disease remain poorly characterized. We have previously described CD4+ T cell AICD in the course of T. cruzi infection with metacyclic trypomastigotes (17) and identified AICD as a major cause of CD4+ T cell unresponsiveness in vitro (14). In the present investigation we developed an in vitro system

(see Fig. 3A), but eliminated 70.6% of the IFN-γ produced upon subsequent TCR activation (Fig. 5). Killing with anti-Fas also eliminated 67.0% of IFN-γ production upon Ly-6 activation (not shown). These results indicate that although they are a minor population, Fas-susceptible CD4+ T cells comprise the majority of IFN-γ-producing cells or contain most of IFN-γ high producer CD4+ T cells in T. cruzi-infected hosts.

Discussion

A functional link between suppression of host T cell responses and escape of T. cruzi from immune attack had been proposed in early investigations of cell-mediated immunity in this parasitic infection (26). However, common mechanisms merging these two aspects of experimental Chagas disease remain poorly characterized. We have previously described CD4+ T cell AICD in the course of T. cruzi infection with metacyclic trypomastigotes (17) and identified AICD as a major cause of CD4+ T cell unresponsiveness in vitro (14). In the present investigation we developed an in vitro system
of infected host-derived CD4+ T cells cocultured with T. cruzi-infected Md. Assessment of extracellular accumulation of infective trypomastigotes in culture was used to evaluate the roles of T cell activation and AICD in modulation of parasite replication in Md. Activation of CD4+ T cells led to surprisingly deleterious effects, helping parasite replication and spreading in Md monolayers.

Extracellular trypomastigote accumulation strictly required the presence of adherent Md, since it was not seen in cultures of partially purified and activated CD4+ T cells (data not shown), even though these cultures initially contained a few motile trypomastigotes. Moreover, supernatants from activated CD4+ T cells did not potentiate trypomastigote accumulation, arguing against an unlikely effect of T cell products on extracellular trypomastigote replication. In fact, it is well established that growth of T. cruzi as trypomastigotes requires a suitable host cell for invasion and replication as amastigotes before trypomastigotes are released to the medium (23). In the absence of host cells, extracellular trypomastigotes do not divide and differentiate into noninfective epimastigotes forms capable of extracellular growth (23). The ability of activated CD4+ T cells to potentiate trypomastigote growth in Md was unexpected. It is known that IFN-γ is able to kill intracellular forms of T. cruzi through activation of inducible NO synthase and NO production (8, 9). Studies have demonstrated intense IFN-γ production in vivo (27) and by activated CD4+ T cells ex vivo (12) during acute T. cruzi infection. We have confirmed that CD4+ T cells from infection secrete large amounts of IFN-γ upon activation (not shown), and that their supernatants reduced trypomastigote growth in cocultured Md. Despite this antiparasite effector potentiality, TCR activation of intact CD4+ T cells exacerbated trypomastigote growth in cocultured Md. The potentiating effect on parasite replication required physical association with infected Md, as demonstrated in experiments of separation by a cell-impermeable membrane. A small, but discernible, deleterious effect was also seen in the absence of exogenous stimulation. This effect could reflect ongoing activation of CD4+ T cells by Md-presenting parasite Ags, but this possibility needs to be tested.

A critical role for AICD in exacerbation of parasite growth was established from several evidences. First, anti-TCR activation of CD4+ T cells potentiated parasite growth in culture, while anti-Ly-6 activation had little or no effect. We have previously demonstrated that, different from TCR activation, Ly-6 activation of CD4+ T cells from infected mice does not induce AICD (14). Similar results were obtained in the presence of cocultured Md. Possibly, Ly-6 activation delivers weaker activation signals to T cells, insufficient to induce AICD. In previous studies with T cell hybridomas, Ly-6 activation also failed to induce AICD to the same extent as direct TCR;CD3 engagement (28).

Second, deliberate mimicking of AICD by killing with anti-Fas coupled to Ly-6 activation exacerbated trypomastigote replication in a manner comparable to the deleterious effect of anti-TCR. Different from control CD4+ T cells, which are resistant to killing by either anti-Fas or anti-TCR (data not shown), a proportion of CD4+ T cells from T. cruzi-infected mice (between 25–30%) are susceptible to death after ligation with anti-Fas mAb. Exacerbated parasite growth, however, required both Fas-mediated killing and Ly-6 activation. One possibility to explain these results could be that exacerbation of parasite growth results from summation of two opposing effects by distinct CD4+ T cell subpopulations. One could be a contact-dependent effect of Fas-resistant T cells, increasing parasite replication. The second effect would be AICD, which appears to kill Fas-susceptible T cells protective against intracellular parasite growth. However, alternative possibilities, such as release of parasite-helping mediators by Fas-stimulated cells, cannot be ruled out. The nature of the cell contact-dependent signal for parasite growth is unknown, but Fas-mediated Md killing is unlikely to play a role. Anti-Fas is not cytotoxic for Md and has no effect on parasite growth in Md alone (not shown). Moreover, Md killing should restrict, rather than potentiate, trypomastigote growth by reducing the number of target cells available for replication. On the other hand, several studies have demonstrated T cell-mediated Md activation (29–31) or deactivation (32) through cell contact-mediated signals such as membrane TNF-α (31) or CD40 ligand (31). Close physical association mediated by cell adhesion molecules could also be necessary for delivery of regulatory cytokines at high local concentrations. Cytokines such as TGF-β or IL-10, which regulate acute T. cruzi infection (33–35), could be involved. Further investigation is necessary to clarify the role of cell adhesion molecules, regulatory cytokines, and signal transduction pathways involved in exacerbation of parasite growth within Md monolayers.

Finally, additional evidence for the deleterious role of AICD on parasite replication was obtained by blockade of AICD with anti-Fasl mAb. While anti-Fas killing of CD4+ T cells potentiated parasite growth by Ly-6-activated T cells, blockade of TCR-mediated CD4+ T cell AICD by anti-Fasl reduced trypomastigote replication in Md. The extent of reduction in parasite growth mirrored the extent of protection from AICD achieved. On the other hand, addition of either anti-Fas or anti-Fasl to Md cultured alone had no effect on parasite replication (data not shown).

We observed that Fas-mediated killing of CD4+ T cells reduced subsequent IFN-γ production in a manner disproportionate to the amount of cell death. This result implies that Fas-susceptible cells contribute the majority of IFN-γ production in CD4+ T cells from T. cruzi-infected mice. The finding is perhaps not surprising, since susceptibility to Fas-mediated death is only acquired after chronic activation of T cells (36) and correlates with T cells bearing memory/activated phenotype in man (37). In fact, memory/activated T cells secrete severalfold more IFN-γ than naive T cells (38, 39). The onset of AICD could regulate parasite growth by selectively ablating those effector T cells showing intense and continued IFN-γ secretion. These results might have implications for infection in vivo. Infection with T. cruzi leads to intense polyclonal lymphocyte activation (40), which could result in induction of both Fas susceptibility and Fasl expression by T cells. We found increased Fas and Fasl expression by CD4+ T cells in the course of T. cruzi infection in vivo (M. F. Lopes, N. Giese, H. R. Morrison III, and G. A. DosReis, manuscript in preparation). Therefore, CD4+ T cells from infected mice are in many aspects similar to chronically activated T cells susceptible to propriocidal regulation by AICD (15, 41). Our results suggest that CD4+ T cells could play a dual role in host protection against T. cruzi, being able to either control or exacerbate the parasite load in tissues depending upon whether AICD is induced following encounters with infected Md. However, since AICD was studied in vitro with anti-TCR mAb, any extrapolation to infection in vivo must be interpreted with caution, waiting for studies with transgenic mice and defined T. cruzi Ags. Regarding involvement of distinct T cell subsets, our results are not incompatible with the suggested role of type 2 cytokines in exacerbating infection by metacyclic trypomastigotes (42). In fact, the increase in parasite burden we observe in vitro could have resulted from more than one mechanism, for example Th1 T cell AICD plus Th2-dependent down-regulation of Md activation. The culture system employed here could be useful in investigating molecular mechanisms of anti-parasite defense as well as down-regulatory signals involved in antimicrobial responses of host T lymphocyte subsets.
Acknowledgments

We thank Dr. Maria Bellio (Institut Pasteur, Paris, France) for providing the cell line producing anti-TCR mAb, and Dr. Ethan Shevach (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) for anti-CD4, anti-TCRy, and anti-Ly-6 mAbs.

References