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Impairment of Monocytic Function during *Trypanosoma cruzi* Infection

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During acute infection, *Trypanosoma cruzi*, the etiologic agent of Chagas' disease, causes immunosuppression by mechanisms that are not fully delineated. Since mononuclear phagocytes are major target cells in trypanosomiasis, we investigated monocytic function during acute *T. cruzi* infection. A series of human monocyte and macrophage hybridomas, which represent clonal expansions of subpopulations of human macrophages and possess many normal monocytic functions, were successfully infected with *T. cruzi*. Clones 63 and 53, chosen for stability in long-term culture, were studied extensively after infection with *T. cruzi*. Following infection of clone 63, the trypomastigote did not transform into the amastigote multiplicative form, suggesting that clone 63 did not support the entire *T. cruzi* life cycle. The typical life cycle was completed in clone 53, and thus, clone 53 was used in subsequent studies. Following infection, clone 53 lost expression of class II antigens compared with uninfected cells (DR of 2.2% versus 29.3% and mean channel fluorescence intensity [mean channel] of 4.1 versus 30.5, DQ of 2.3% versus 15.6% and mean channel of 5.4 versus 11.4, and DP of 6.3% versus 27.2% and mean channel of 10.3 versus 33.4). The expression of Class I antigens (87.9% versus 82.8%; mean channel, 20 versus 120) and the adhesion molecules LFA-1 (72.9% versus 28.7%; mean channel, 50.7 versus 23.7) and LFA-3 (10.8% versus 0.7%; mean channel, 20.7 versus 15.1) was increased in infected cells compared with that in uninfected cells. Production of interleukin-1 alpha was decreased and interleukin-6 production was increased in infected clone 53 compared with those in the uninfected cells, while production of tumor necrosis factor alpha was increased. Finally, infected clone 53 showed a decreased ability to present antigen to major histocompatibility complex-matched T cells, which may relate to the absence of Class II antigens or to aberrant cytokine production. These data suggest that (i) only selected subpopulations of human macrophages support *T. cruzi* replication and (ii) at least in some subpopulations, macrophage dysfunction may contribute to the altered immune function observed in Chagas' disease.

The protozoan *Trypanosoma cruzi*, the causative agent of Chagas' disease, is an obligate intracellular parasite of humans and many other mammals. Chagas' disease is widely distributed in Central and South America. In infected laboratory animals and humans, immunosuppression occurs in the acute and chronic phases of the disease (21). Infection can lead to anergy to both parasitic and nonparasitic antigens and decreased responses to mitogens. Both humoral and cell-mediated immune responses may be impaired (5, 8, 21). Presently, knowledge regarding the mechanisms of acute-phase immunosuppression is limited. Since the monocyte is a target cell in *T. cruzi* infection and monocytes play a major role in regulating immune responses, monocyte dysfunction may contribute to immunosuppression. To test this hypothesis, we utilized a series of human monocyte and macrophage hybridomas. These hybridomas represent clonal expansions of subpopulations of human monocytes and macrophages and possess many normal monocytic functions, including antigen presentation, class II antigen expression, and cytokine production (15). We have previously used these cell lines to study monocytic function during acute and chronic human immunodeficiency virus type 1 and influenza virus infection (14). Using this model system, we evaluated monocytic function during acute *T. cruzi* infection in vitro.

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MATERIALS AND METHODS

Generation of human macrophage hybridomas. Human macrophage hybridomas were generated as previously described (15). In brief, monocytes obtained from healthy blood donors from the Mount Sinai Blood Bank were allowed to mature into macrophages in Teflon bag cultures. The macrophages were fused with an hypoxanthine-guanine phosphoribosyltransferase-deficient promonocytic line, U-937. Several clones, including clones 30, 38, 39, 43, 53, 62, and 63, were capable of being infected with *T. cruzi*. However, clones 53 and 63 were chosen for further study because of their stability in long-term culture.

Infection of human macrophage hybridomas with *T. cruzi*. Macrophage hybridomas were infected with *T. cruzi* by methods previously described (9). The parasites were obtained from the blood of Crl-CD-1 (ICR) Swiss mice (Charles River Laboratory, Portage, Mich.) who were infected intraperitoneally with 10^5 organisms of the Tulahuen strain of *T. cruzi* 2 weeks before use in these studies. The parasites were purified by anion-exchange centrifugation (350 × *g*, 20°C for 45 min) over Ficoll-Hypaque followed by chromatography through DEAE cellulose. After two washings with RPMI 1640 (GIBCO, Grand Island, N.Y.) containing 100 IU of penicillin and 100 μg of streptomycin per ml, the parasites were suspended in the same medium supplemented with 10% fetal calf serum (GIBCO). The suspension consisted of 100% trypomastigotes (>99% viability as determined by trypan blue staining). For infection of the hybridomas, *T. cruzi* trypomastigotes were placed in coculture at 37°C for 2 h at a parasite-to-cell ratio of 3:1. Unbound and uninternalized organisms were removed by

washing with phosphate-buffered saline (PBS). To determine the efficiency of this method of infection, the cells were fixed with methanol and stained with Giesma. The number of parasite-containing cells per culture was determined by light microscopy. Utilization of this approach resulted in 100% infection of the hybridoma cells. These cells remained viable for at least 72 h after infection, as assessed by trypan blue and propidium iodide staining.

Surface antigen staining. Clone 53 was stained for the expression of surface antigens as previously described (15), with various primary monoclonal antibodies to class I antigens (anti-DP, anti-DQ, anti-DR, anti-LFA-1, and anti-LFA-3]) or isotypic controls, followed by fluorescein isothiocyanate (FITC)-conjugated $F(ab)'_2$ goat anti-mouse immunoglobulin G (Tago, Burlingame, Calif.). Stained cells were analyzed by flow cytometry, with gating on live cells. VG2 (anti-DR) was kindly provided by Shu Man Fu. B7/21 (anti-DP) was a gift of Steven Burakoff. TS1/22 (anti-LFA-1-alpha), TS2/9 (anti-LFA-3), W6/32 (anti-class I), and genox 3.53 (anti-DQ) were obtained from the American Type Culture Collection (Rockville, Md.).

Antigen-specific proliferation assay. Both uninfected clone 53 and clone 53 infected with *T. cruzi* ($53_{T.\ cruzi}$) were used to present tetanus toxoid (TT) to TT-specific T cells obtained from two major histocompatibility complex (MHC)-matched (DR2+) volunteers immunized with TT, by utilizing a previously described method (14). T cells were isolated from the volunteer's peripheral blood mononuclear cells by rosetting with neuramidase-treated sheep erythrocytes followed by separation by Ficoll-Hypaque. After erythrocyte lysis, the T cells were washed with sterile PBS and treated with 5 mM leucine methyl ester (Sigma) for 40 min at 25°C to deplete monocytes. T cells treated in this manner failed to respond to TT in the absence of added antigen-presenting cells (APC), indicating that monocyte depletion was complete. Clones 53 and $53_{T.\ cruzi}$ (24 h after infection) were irradiated at a dose of 6,000 rads (cesium source), which renders the cells nonproliferative but does not affect *T. cruzi*, which is radio resistant. Cells were cultured in RPMI 1640 (GIBCO) supplemented with 10% human agamma globulinemic serum, 1% penicillin or streptomycin, and 2 mM L-glutamine (GIBCO), henceforth referred to as complete medium (CM). Responder T cells (10^5) from TT-boosted DR2+ individuals were cocultured with 10^5, 10^4, or 10^3 irradiated clone 53 or $53_{T.\ cruzi}$ cells in 0.2 ml of medium in the presence or absence of TT (40 μg/ml), in triplicate round-bottom microtiter wells (Linbro, Oxnard, Calif.). Three concentrations of TT (40, 4, and 0.4 μg/ml) were used in our experiments. In all of the studies, 40 μg/ml was found to produce the highest proliferative responses. Control cultures included the addition of autologous monocytes, which restored a normal TT proliferative response. The cocultures were incubated at 37°C in 5% CO_2 for 5 days. At 18 h prior to harvesting, 1 μCi of [^{3}H]thymidine (ICN, Irvine, Calif.) was added to each well. The cocultures were harvested onto glass fiber filters, and incorporated radiolabel was determined by scintillation counting.

Cytokine analysis. Interleukin-1 alpha (IL-1α), IL-1β, IL-6, and tumor necrosis factor (TNF) are well-defined accessory and proinflammatory cytokines produced by monocytes. They were chosen for analysis because of their roles as costimulatory factors for T cells.

(i) IL-1α and IL-1β. Constitutive and induced (16 h of treatment with 10 μg of lipopolysaccharide [LPS; Sigma] per ml) cytokine production by clones 53 and $53_{T.\ cruzi}$ was determined by assaying culture supernatants. IL-1α and IL-1β production were determined by a competitive enzyme-linked immunosorbent assay (ELISA; ARI Systems, Paint Branch, Md.).

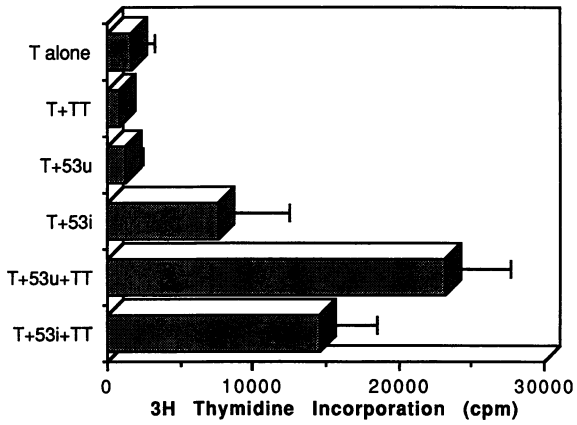

FIG. 1. Clone $53_{T.\ cruzi}$ is less capable of presenting TT to primed responder T cells. TT-primed T cells (T+TT) were incubated with irradiated uninfected clone 53 (53u) or $53_{T.\ cruzi}$ (53i) in the presence of TT (40 μg/ml) for 5 days. Proliferation was determined by [^{3}H]thymidine incorporation. Clone $53_{T.\ cruzi}$ shows a marked decrease in its ability to induce T-cell proliferation compared with that of the uninfected cells. These results are representative of an experiment for one DR-2+-matched donor, which was repeated five times. Similar results were obtained from T cells from another DR2+-matched individual. Error bars indicate standard errors of the means.

(ii) IL-6. The IL-6-dependent murine B9 plasmacytoma cell line (a generous gift of L. May) was used to determine IL-6 production by clones 53 and $53_{T.\ cruzi}$ (16). Test supernatants (16 h of treatment with 10 μg of LPS per ml) were added to 5×10^3 B9 cells, which were then cultured in CM for 3 days. [^{3}H]thymidine (1 μCi) was added to each well during the last 16 h of culture. The cells were harvested onto glass fiber filters, and incorporated radiolabel was determined by scintillation counting. Standard IL-6 curves were obtained for each assay by using known quantities of recombinant IL-6 (kindly provided by E. Siden). The specificity of the bioassay was determined with a polyclonal anti-IL-6 antibody (Amgen, Thousand Oaks, Calif.) which completely inhibited proliferation by the B9 cell line in response to IL-6 (22).

TNF. The L929 cell line was utilized to determine TNF production by clones 53 and $53_{T.\ cruzi}$ (2). The L929 fibroblast line is a TNF-sensitive line that lyses in the presence of TNF and actinomycin D but does not distinguish between TNF-α and TNF-β. L929 cells were plated onto 96-well flat-bottom microtiter plates and cultured until confluent. Hybridoma-derived culture supernatants at various times after infection were then added to triplicate wells in the presence of actinomycin D (4 μg/ml), and the plates were incubated for 18 h at 37°C. Standard curves were generated by using known concentrations of recombinant TNF-α. The wells were washed once with PBS, and 0.05% crystal violet in 20% ethanol was added to each well for 10 min. The crystal violet was removed with tap water. Methanol (100%) was added to each well to elute the stain from the cells. The plates were then read with a microtiter plate reader (Genetic Systems, Seattle, Wash.) at A_{570} or A_{630}. TNF-α levels from clones 53 and $53_{T.\ cruzi}$ were also determined by a direct binding ELISA (Endogen, Boston, Mass.).

RESULTS

***T. cruzi* can infect and complete its life cycle in most but not all human macrophage hybridomas.** To determine whether

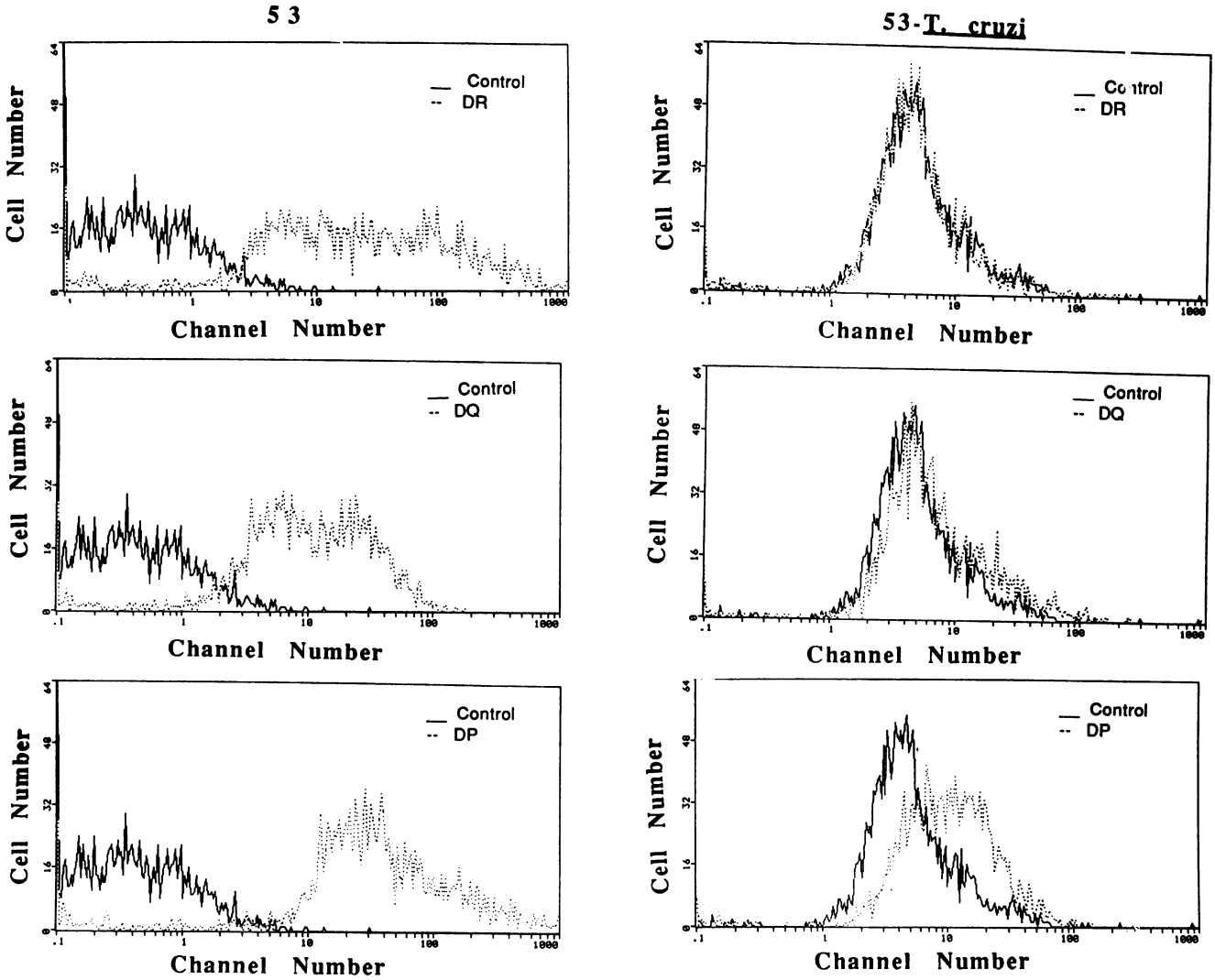

FIG. 2. Clone $53_{T.\ cruzi}$ fails to express class II antigens 48 h after infection. Clones 53 and $53_{T.\ cruzi}$ were stained with a panel of anti-class II MAbs followed by a FITC-conjugated $F(ab)'_2$ goat anti-mouse Ig and analyzed by flow cytometry. Clone $53_{T.\ cruzi}$ fails to express DR, DQ, and DP compared with the uninfected cells.

our findings for the hybridomas could be extrapolated to total monocyte populations, we analyzed class II antigen expression in peripheral monocytes infected with *T. cruzi*. Infection rates were variable, as were the effects on class II antigens, underscoring the difficulty of using the heterogeneous populations described above (data not shown). Because our hybridoma lines each represent a distinct monocyte subpopulation, our system is ideal for studying the specific deleterious effects of *T. cruzi* on monocyte function and surface antigen expression in permissive subpopulations.

One hundred percent of the clone 30, 38, 39, 43, 53, 62, and 63 cells were successfully infected with *T. cruzi*. The entire life cycle of *T. cruzi*, including transformation of the trypomastigote into the amastigote replicative form, was completed in six of the seven hybridomas that were infected (clones 30, 38, 39, 43, 53, and 62), resulting in cell rupture 96 h after infection. In six of the seven hybridoma cell lines, the typical intracellular, nonflagellated amastigote form of *T. cruzi* within the cytoplasm was observed 48 h after infection. In contrast, in clone 63 at 48 and 96 h after infection, transformation of the trypomastigote into the amastigote did not occur. Atypical trypomastigotes (no visible nucleus and a chromatin-like appearance) could be observed swimming in the cytoplasm of the infected clone 63 cells. Even though *T. cruzi* did not complete its life cycle in clone 63, there was replication of the atypical forms in the infected cells which caused rupture 96 h after infection, as observed in other infected clones. However, the morphology of *T. cruzi* cells following passage through clone 63 was atypical, with cells demonstrating a pseudoflagellated form. In contrast, following rupture of the other infected hybridoma lines 96 h after infection, typical trypomastigote forms and amastigote forms were observed free in culture. While clone 63 might be aberrant in this system, it has been used to successfully propagate human immunodeficiency virus type 1 and influenza virus (14). Therefore, the data presented here suggest that *T. cruzi* infection may be different in distinct monocyte subpopulations. Studies are presently underway to determine why the entire *T. cruzi* life cycle is not completed in clone 63.

Clone 53 infected with *T. cruzi* does not present TT to MHC-matched responder T cells. Since loss of antigen-specific

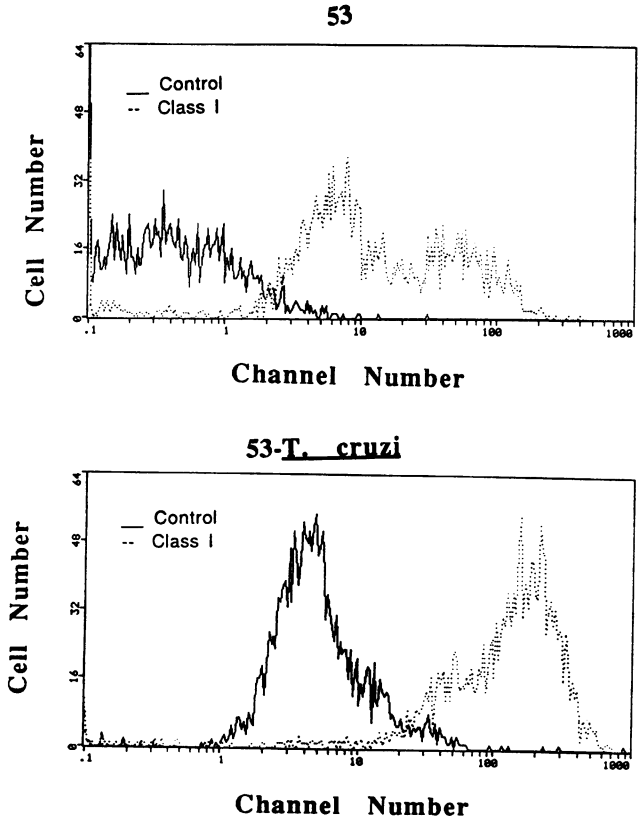

FIG. 3. Class I antigen expression is increased in clone $53_{T.\ cruzi}$ compared with that in clone 53. Clones $53_{T.\ cruzi}$ and 53 were stained with anti-class I MAbs followed by FITC-conjugated $F(ab)'_2$ goat anti-mouse Ig and analyzed by flow cytometry. In contrast class II antigen expression, Class I antigen expression is increased in infected cells compared with that in uninfected cells. These results are from a representative experiment repeated three times.

responses occurs during acute infection with *T. cruzi* (5, 21), we first assessed the ability of *T. cruzi*-infected macrophage hybridomas to present soluble protein antigen to primed T cells. Given the *T. cruzi* replication pattern observed in six of seven macrophage hybridoma cell lines, we assessed specific macrophage function in one cell line, clone 53, chosen for its stability in long-term culture. DR2+ APC-depleted TT-primed donor T cells and TT (40 μg/ml) were cocultured in the presence of various numbers of clone 53 or $53_{T.\ cruzi}$ cells. In the absence of APCs, no TT proliferative response was seen. Clone 53 was clearly able to restore a TT proliferative response in the APC-depleted responder T cells (Fig. 1). However, clone $53_{T.\ cruzi}$ was unable to restore TT-driven T-cell proliferation, suggesting that the ability to present antigens was compromised by infection. Clone $53_{T.\ cruzi}$ was used at 12 h postinfection to ensure that the hybridomas would remain viable during the critical initial 24 h required to process and present TT to responder T cells. Supernatant from clone $53_{T.\ cruzi}$ failed to affect the viability of either resting T cells, as determined by trypan blue and propidium staining, or phytohemagglutinin or alloantigen T-cell proliferation, as determined by thymidine incorporation, indicating that the inability to present TT is not due to the production of an inhibitory factor (data not shown). The decrease observed was even more remarkable given the consistent increase (eightfold) in background T-cell proliferation when clone $53_{T.\ cruzi}$ was cultured with T cells. Regardless of its cause, background counts subtracted from the TT response following coculture with clone $53_{T.\ cruzi}$ yielded a value reaching that of baseline thymidine incorporation, indicating a severe impairment in the line's ability to present antigen.

There are several possible explanations for the decrease in ability of clone $53_{T.\ cruzi}$ to present antigen, including loss of MHC class II antigen expression necessary for class II antigen-peptide complexes, loss of cell surface adhesion molecules necessary for cognate cellular interactions, and altered accessory cytokine production. Each possibility was analyzed.

Surface class II antigen expression is lost in *T. cruzi*-infected clone 53, with sparing of class I antigen and LFA expression. We stained clones 53 and $53_{T.\ cruzi}$ with specific monoclonal antibodies against class I and class II antigens and adhesion molecules 48 h after infection. Clone 53 constitutively expresses DR (29.3%; mean channel fluorescence intensity [mean channel], 30.5), DQ (15.6%; mean channel, 11.4), and DP (27.2%; mean channel, 33.4), as demonstrated by a peak channel shift compared with the isotype-matched control antibody (mean channel, 0.306) (Fig. 2). In contrast, clone $53_{T.\ cruzi}$ had decreased expression of all class II antigens DR (2.2%; mean channel, 4.1), DQ, (2.3%; mean channel, 5.4), and DP (6.3%; mean channel, 10.3) compared with that of the uninfected cells. Although autofluorescence was increased in the hybridoma cells following infection (mean channel, 4.1) (Fig. 2 to 4), this did not account for the absence of class II antigen expression (i.e., increased background did not obscure staining), since the initial mean channel fluorescence intensity of the class II antigens was comparable to that of LFA-1-alpha and LFA-3 but only class II antigen expression was affected. This loss of class II MHC could not be restored with gamma interferon treatment (100 U/ml for 48 h added 12 h after infection) (data not shown). Class II antigen expression was upregulated by similar treatment of uninfected clone 53. Furthermore, gamma interferon treatment did not affect the course of the *T. cruzi* infection in clone 53 (i.e., the kinetics of infection and lysis was unchanged). Class I antigen expression was increased following infection (87.9%, mean channel of 12.1; 82.9%, mean channel of 20.0) (Fig. 3), and expression of LFA-1 and LFA-3, which play a critical role in T-cell–macrophage interactions, appeared to be upregulated in clone $53_{T.\ cruzi}$ (72.9%, mean channel of 50.7; 10.8%, mean channel of 23.7) compared with the uninfected cells (28.7%, mean channel of 20.7; 0.7%, mean channel of 15.1). Thus, *T. cruzi* induced a decrease in class II antigen expression and an increase in class I and LFA antigen expression. This decrease could readily explain the decreased ability of the infected cells to present antigen to responder T cells.

Cytokine production by clone 53 infected with *T. cruzi* is aberrant. We next assessed cytokine production of both clone $53_{T.\ cruzi}$ and uninfected cells 24 and 48 h after infection (viability was maintained throughout this period). Induced (10 μg of LPS per ml for 16 h) production of IL-1α was decreased in infected clone 53 compared with that in uninfected cells, 10-fold at 24 h and 7-fold at 48 h (Fig. 5). There was no constitutive production of IL-1α from either the uninfected or infected cells. A decrease in IL-1β production was also observed in clone $53_{T.\ cruzi}$ at 24 and 48 h after infection. In contrast, both LPS-induced IL-6 and TNF production was increased in clone $53_{T.\ cruzi}$ compared with that in uninfected cells at both 24 and 48 h (Fig. 6 [24 h] and 7 [24 and 48 h]), respectively. There was no constitutive production of either IL-6 or TNF from either the uninfected or infected cells. *T. cruzi*-induced dysregulation of cytokine production may contribute to the decreased ability of infected cells to stimulate

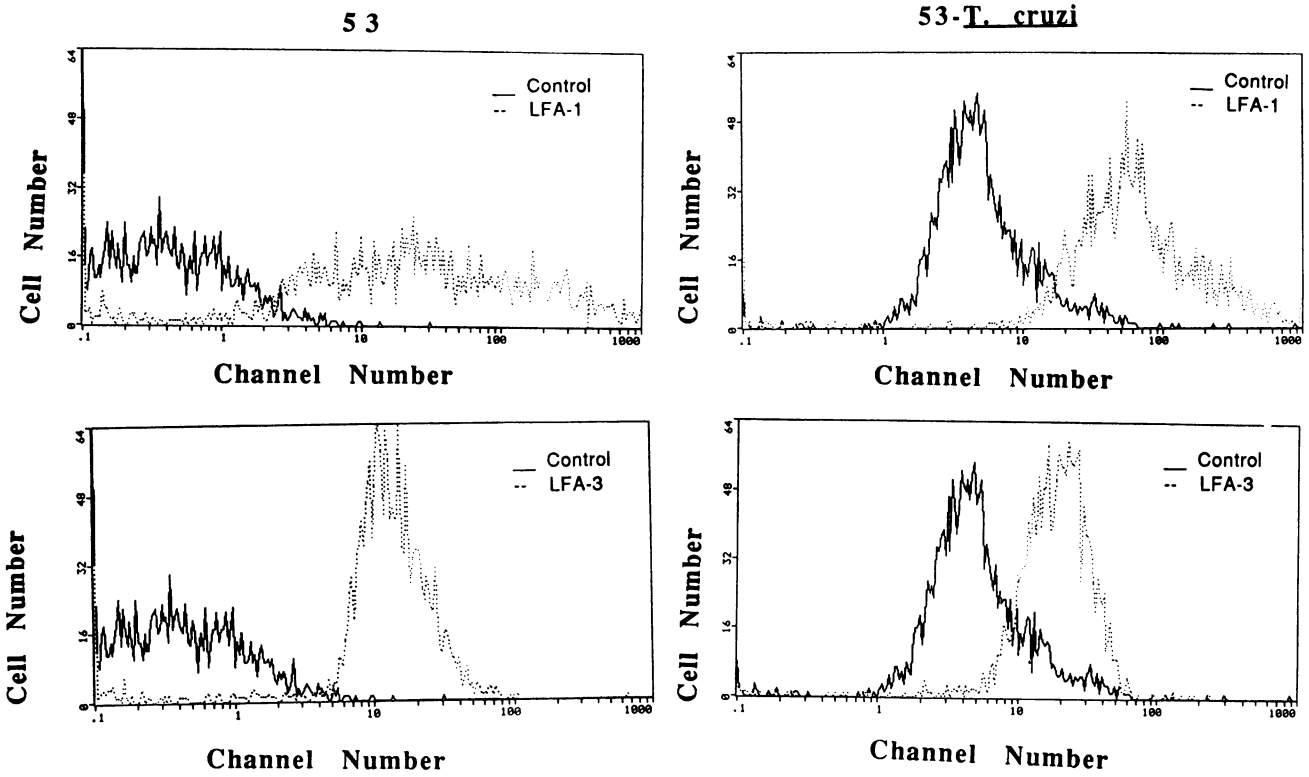

FIG. 4. Expression of LFA-1 and LFA-3 is increased in clone $53_{T.\ cruzi}$. Clones 53 and $53_{T.\ cruzi}$ were stained with anti-LFA-1 and anti-LFA-3 MAbs followed by FITC-conjugated F(ab)'$_2$ goat anti-mouse immunoglobulin, and analyzed by flow cytometry. There was increased expression of LFA-1 and LFA-3 in clone $53_{T.\ cruzi}$. These results are from a representative experiment repeated three times.

proliferation of antigen-primed responder T cells. Since the L929 fibroblast line does not distinguish between TNF-α and TNF-β, the TNF results were confirmed by TNF-α ELISA. Furthermore, lactate dehydrogenase values were equivalent in the supernatants from clones $53_{T.\ cruzi}$ and 53; so, the increases in IL-6 were not due to limited cell lysis.

DISCUSSION

T. cruzi elicits a variety of cellular and humoral immune defects during the acute, latent, and chronic phases of Chagas' disease. During the acute phase, immunosuppression is observed, with blunting of humoral and cellular responses to both parasitic and nonparasitic antigens (5, 8, 21). Specific immune defects which have been observed during acute infection include the development of suppressor macrophages (3) and suppressor T cells (19, 20) believed to mediate abnormalities in T-cell function; decreased expression of CD-3, CD-4, and CD-8 (18) and IL-2 receptors (9, 10), all of which are important contributors to lymphocyte activation; inhibition of IL-2 production and responsiveness of peripheral blood mononuclear cells to IL-2 (13); decrease in CD4 counts and increase in CD8 counts, leading to a decrease in the CD4-to-CD8 ratio; and neutrophil dysfunction. Furthermore, immunosuppression during the acute phase may play a role in the establishment of the chronic phase and contribute to the loss of tolerance and the autoimmune phenomena seen in Chagas' disease (7, 24).

Our laboratory has created a novel system to study *T. cruzi*-monocyte interactions through the infection of human monocyte and macrophage hybridomas (15). Study of discrete monocytic subpopulations can be performed by utilizing this system. Such a study is not possible with heterogeneous monocytes isolated from peripheral blood. Although the use of clonal populations of monocytes does not allow us to extrapolate to total populations, we have demonstrated that different cell lines can be infected with *T. cruzi* and can serve as useful

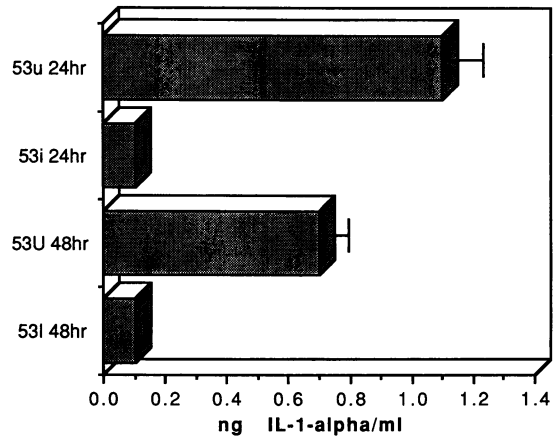

FIG. 5. LPS-induced IL-1α production from uninfected clone 53 (53u) and clone $53_{T.\ cruzi}$ (53i). IL-1α production was determined by a competitive ELISA 24 and 48 h after infection. LPS-induced IL-1α production was reduced in the infected cells at both times. The lowest level of detection for this kit is 0.1 ng/ml. Pooled results of five separate experiments are presented, with error bars representing the standard errors the means. Unactivated cells produced no IL-1α.

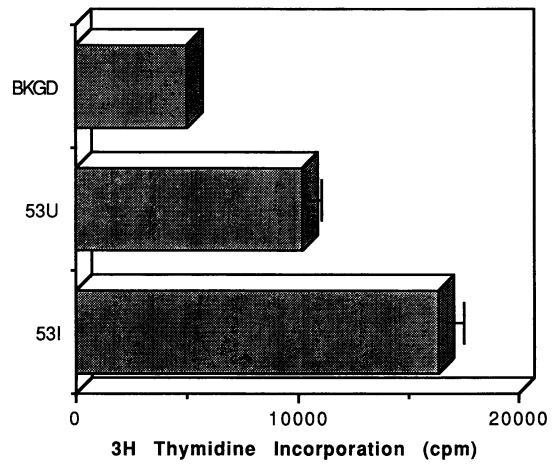

FIG. 6. LPS-induced IL-6 production from uninfected clone 53 (53U) and clone $53_{T.\ cruzi}$ (53I). IL-6 production was determined by the B9 bioassay 24 h after infection. In contrast to IL-1α production, LPS-induced IL-6 production was increased in the infected cells. Pooled results of five separate experiments are presented, with the error bars representing standard errors of the means. The background (BKGD) represents baseline proliferation of the unstimulated B-9 cells.

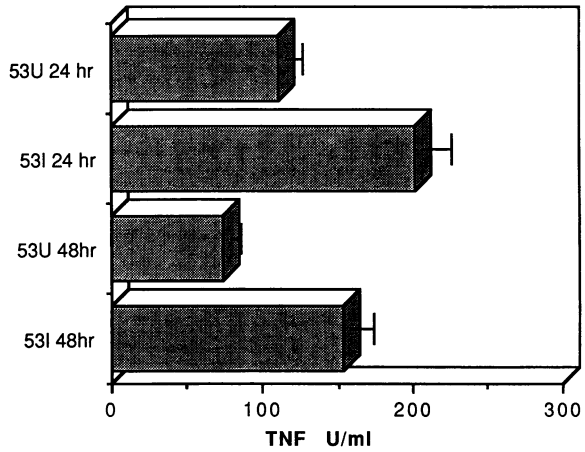

FIG. 7. LPS-induced TNF production from uninfected clone 53 (53U) and $53_{T.\ cruzi}$ (53I). TNF production was determined by the L929 bioassay 24 and 48 h after infection. Similar to that of IL-6, LPS-stimulated TNF production was increased after infection. Pooled results of five separate experiments are presented, with error bars representing standard errors of the means. There was no detectable baseline TNF.

model systems for studying specific aspects of monocyte function after *T. cruzi* infection. The complete life cycle of *T. cruzi* is fulfilled in most clones (clones 30, 38, 39, 43, 53, and 62), resulting in the transformation of the trypomastigote form to the replicative amastigote form. However, in clone 63, the *T. cruzi* life cycle is not completed. These data suggest that differential selectivity to infection exists in monocyte subpopulations. Interestingly, presentation of antigen is impaired in one clone in which the *T. cruzi* life cycle is completed. However, since only one clone is incapable of supporting the *T. cruzi* life cycle, this differential selectivity may be very limited or an artifact of our system. The ability of *T. cruzi*-infected clone 63 to present TT to responder MHC-matched T cells was also impaired even though the entire life cycle was not completed. The increase in background proliferation observed in our antigen presentation experiments (Fig. 1) may be the result of prior exposure of our volunteers to *T. cruzi* antigen, of the presence of T-cell clones in the volunteers which are cross-reactive to *T. cruzi* antigen, of an enhancement of an autologous MLR, or of the existence of a *T. cruzi* superantigen. There is no experimental evidence to support these possibilities. This inability to respond to TT may relate to the observed selective decrease in surface expression of class II antigens. Inducing a decrease in the class II antigen expression of APC may confer an advantage to *T. cruzi* by allowing the organism to escape recognition by the host's immune system.

Other intracellular pathogens are known to have deleterious effects on APC, contributing to broad immune dysfunction. Cluff et al. (4) demonstrated that infection of macrophages with *Listeria monocytogenes* and *Streptococcus pyogenes* dramatically reduced the processing and presentation of ovalbumin to MHC-matched T cells. Morrison et al. (12) and Sweetser et al. (17) observed the absence of presentation of hemagglutinin by macrophages to class II-restricted cytotoxic T cells during infection with influenza virus. Domanico et al. (6) demonstrated that infection with influenza virus completely blocked presentation of soluble antigen by macrophages. Adenovirus infection of APC resulted in the retention of class I antigens in the endoplasmic reticulum, reduces the transcription of class I mRNA by reducing the binding of NF-κB, and inhibits phosphorylation of class I antigens (11). The mechanisms underlying the inhibition of class II antigen expression induced by *T. cruzi* are currently being investigated.

Accessory cytokine production was also aberrant in *T. cruzi*-infected clone 53, with decreased production of IL-1α and increased production of IL-6 and TNF compared with that in uninfected cells. Aberrant cytokine production, especially of IL-1α, could contribute to an inability to stimulate antigen-primed T cells. Studies are presently underway to determine if aborting the infection with chemotherapy would normalize cytokine production. Our cytokine results differ somewhat from those of a previous study utilizing heterogeneous human and murine peripheral blood mononuclear cell populations (23). This may relate to biologic differences in various strains of *T. cruzi*. Van Voorhis (23) used a CL strain in his studies, while we utilized the Tulahuen strain (1). In this study, an increase in production of IL-1β was observed in peripheral blood mononuclear cells following coculture with *T. cruzi*. Similar to results in our system, an increase in both TNF-α and IL-6 production was also observed. The differences could reflect differences in the *T. cruzi* strain used or the combined responses of mixed populations of T cells, B cells, and monocytes to *T. cruzi* infection.

It is difficult to generalize the findings from a single cloned cell line to a large heterogeneous population of monocytes and macrophages in vivo. However, the findings for clone 53 with impaired antigen presentation and aberrant macrophage cytokine production as a result of the *T. cruzi* infection may represent an altered function in subpopulations of monocytes or macrophages which may contribute to the development of some of the immunologic abnormalities seen in Chagas' disease.

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