INFECTION AND IMMUNITY, Mar. 1997, p. 971–977 Vol. 65, No. 3
0019-9567/97/$04.00+0

Infection with *Trypanosoma cruzi* Selectively Upregulates B7-2 Molecules on Macrophages and Enhances Their Costimulatory Activity

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Received 19 August 1996/Returned for modification 25 September 1996/Accepted 3 December 1996

T-cell-mediated immune responses are essential for protection against infection with the protozoan *Trypanosoma cruzi*. In this study, we investigated the influence of infection of murine macrophages with *T. cruzi* on costimulatory signals for T lymphocytes provided by these cells. We demonstrate that bone marrow-derived macrophages (BMMph) selectively and strongly upregulate expression of B7-2 molecules after infection, while the expression of other costimulatory molecules such as B7-1, intercellular adhesion molecule 1, lymphocyte function-associated antigen 3, and heat-stable antigen is not significantly affected. Infection by live trypanosomes was required. As a consequence of the strong B7-2 upregulation, the infected macrophages are able to induce proliferation of splenic $CD4^+$ T cells in the presence of anti-CD3 antibodies with much higher efficiency than uninfected macrophages. Costimulation could be inhibited by an antibody to B7-2. Furthermore, costimulatory activity for established T-cell clones of Th1 and Th2 phenotype was also strongly enhanced in BMMph by infection with *T. cruzi*. Th1 cells stimulated either via anti-CD3 antibodies or via specific antigen proliferated with higher efficiency in the presence of infected macrophages than in the presence of uninfected cells. BMMph stimulated with gamma interferon (IFN-γ), expressing elevated levels of B7-2 molecules, are also able to enhance Th1 cell proliferation, which was highest, using macrophages which were infected and in parallel were stimulated with IFN-γ. Noteworthy, for cloned Th2 cells, the mechanism of costimulation differed, because costimulation of Th2 cells was not dependent on B7-2 upregulation but was due to secretion of interleukin-1α. These findings demonstrate that infection of macrophages with *T. cruzi* transforms the macrophage into a potent costimulatory cell based on the induction of two different costimulatory activities.

The protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas' disease, infects humans and mammals primarily in Latin America. The course of infection and the pathology are varied and complex. Although the precise mechanisms of the antiparasitic response remain to be characterized, it is clear that T-cell-mediated effector functions are essential for protection against the disease and the control of parasite growth and dissemination.

Both $CD4^+$ and $CD8^+$ T cells contribute to resistance to *T. cruzi*. Mice treated with anti-CD4 (3, 37, 39) or anti-CD8 (44) monoclonal antibodies (MAbs) become highly susceptible to *T. cruzi* and show an increase in parasite growth and mortality. Similar results were obtained by infecting gene knockout mice which lacked CD4 or CD8 molecules (38) or β_2-microglobulin (45). One important mechanism by which T cells contribute to the eradication of *T. cruzi* might be through the secretion of lymphokines. Gamma interferon (IFN-γ) seems to play a central role in the control of infection. Passive administration of IFN-γ results in an increased clearance of the parasite and a reduced mortality of infected mice (34), while anti-IFN-γ MAb augmented the susceptibility to acute *T. cruzi* infection (32). Also, BALB.Xid mice produce higher levels of IFN-γ than susceptible normal BALB/c mice and are relatively resistant to infection (29). Obviously, the initiation of a potent T-cell response is a critical step in the outcome of the infection.

Activation of resting T cells for induction of effector functions requires two signals provided by the antigen (Ag)-presenting cells (APC) (30). Signal 1 is delivered via the clonally distributed T-cell receptor (TCR), which recognizes specific Ag presented on major histocompatibility complex (MHC) molecules. Signal 2 is provided by several integral membrane proteins. By binding their cognate ligands on the APC-like lymphocyte function-associated antigen 3 (LFA-3) (28), intercellular adhesion molecule 1 (ICAM-1), ICAM-2, and ICAM-3 (10, 11, 48), or B7-1/2 (12, 23), coreceptor molecules such as CD2, LFA-1, and CD28 play important roles in T-cell adhesion and in the initiation of signal transduction events. The ability of the APC to deliver the second signal to resting T cells is inducible primarily by infectious agents or their constituents. Lipopolysaccharide, a component of the cell wall of gram-negative bacteria, is a potent inducer of costimulatory activity on murine peritoneal macrophages (24) and bone marrow-derived macrophages (BMMph) (20). Expression of costimulatory molecules is also inducible by exposure to *Listeria monocytogenes* (20). Furthermore, infection by certain parasites enables the macrophage to function as a potent costimulatory cell. *Leishmania major*-parasitized macrophages augment Th2 type T-cell activation due to their increased capacity to produce interleukin-1 (IL-1) (9). In contrast, parasites not only enhance T-cell activation but also modulate the accessory function of the APC in a negative way. *L. major*- or *L. amazonensis*-infected macrophages show a diminished Ag presentation capacity (15, 25, 33). Infection of BMMph by the parasite *L. donovani* results in the failure to trigger expression of the costimulatory molecules B7-1 and heat-stable antigen (HSA). Furthermore, infection inhibits the macrophage response to normal regulatory signals like lipopolysaccharide (20). Similar results are described in reference 41; in this study, B7-1 expression was decreased, while the expression

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of ICAM-1 was marginally increased in BALB/c BMMph after infection with *L. donovani*, resulting in the inhibition of delayed-type hypersensitivity-mediating functions of Th cells from BALB/c mice.

In the work presented here, we have investigated the influence of infection with *T. cruzi* on the costimulatory capacity of BMMph for T-cell activation. We show that under the influence of infectious *T. cruzi*, the macrophages selectively upregulate the B7-2 molecule and become very efficient APC with a high potency to induce a T-cell-dependent immune response.

MATERIALS AND METHODS

Mice. BALB/c and C57BL/6 mice, originally obtained from Charles River, Kisslegg, Germany, were bred in our animal facilities. They were used as bone marrow donors between 4 and 8 weeks of age.

Parasites. The Tehuantepec strain of *T. cruzi* (36), which is macrophagotropic, was used in all experiments. The trypomastigotes were maintained in tissue culture by infection of a monolayer of the glioblastoma cell line 86HG39 (5), grown in Iscove's modified Dulbecco's medium (IMDM; Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum (FCS) (Life Technologies, Eggenstein, Germany). Parasites leaving their host cells 10 days later were collected within the supernatant by centrifugation at $100 \times g$ to separate them from cell debris and then pelleted by centrifugation at $500 \times g$. *T. cruzi* were resuspended in IMDM supplemented with 5% FCS and used for infection of macrophages. In some experiments, the parasites were killed by exposure to UV irradiation for 10 min. Total *T. cruzi* lysate from cultivated trypomastigotes was prepared by five freeze-thaw cycles and sonication followed by three freeze-thaw cycles.

Cytokines, antibodies, and reagents. Rat recombinant IFN-γ (rIFN-γ) was purchased from Life Technologies. Human recombinant IL-2 was a gift of EuroCetus GmbH, Frankfurt am Main, Germany.

Hybridoma clones M5/114.15.2 (anti-I-$A^{b,d,q}$, I-$E^{d,k}$, rat immunoglobulin G2b [IgG2b]) (4), FD441.8 (anti-CD11a, rat IgG2b) (42), YN1/1.7.4 (anti-CD54, rat IgG2a) (43), J11d (anti-HSA, rat IgM) (8), and GK1.5 (anti-CD4, rat IgG2b) (49) were obtained from the American Type Culture Collection, Rockville, Md. All MAbs were used as concentrated supernatants. Anti-B7-1 (1G10, rat IgG2a) (31), anti-B7-2 (GL-1, rat IgG2a) (17), and a rat IgG2a isotype control were purchased from Pharmingen, Hamburg, Germany. Clone 145-2C11 (anti-CD3ε, hamster IgG) (21) was the gift of J. Bluestone, Ben May Institute, Chicago, Ill. Serum-free supernatant was purified by protein G chromatography. Clone 37.51 (anti-CD28, hamster IgG) (16) was the gift of J. P. Allison, University of California at Berkeley.

T cells. The Th1 cell clones 12B9 and 9B11, specific for ovalbumin in combination with I-A^b MHC class II molecules (13), were the gift of A. B. Reske-Kunz, Clinical Research Group on Allergy, Mainz, Germany. T cells were restimulated with specific Ag and syngeneic spleen cells at intervals of about 30 days. They were used for the experiments described beginning 3 weeks after restimulation. The Th2 cell clone D10.G4.1 (19), reacting with conalbumin in the context of I-A^k molecules and also specific for the H-2^b alloantigen, was the gift of C. A. Janeway, Jr., Yale University, New Haven, Conn.

For preparation of $CD4^+$ T lymphocytes, whole spleen cells were treated with Gey's solution for 1 min to lyse erythrocytes. Cells (10^8) were incubated for 30 min at 4°C with MAb GK1.5 (anti-CD4). Cells were washed three times and incubated with goat anti-rat IgG-coated magnetic Dynabeads M-450 (Dynal, Hamburg, Germany) for 20 min at room temperature. The bead-to-cell ratio was 4:1. Positive selection was performed on a magnetic separator. Selected cells showed no proliferative response in the presence of anti-CD3 MAb, indicating negligible contamination with accessory cells.

Generation of BMMph and infection with *T. cruzi*. Bone marrow stem cells, prepared as described earlier (35), were plated at 2.5×10^6/10 ml in untreated petri dishes (Greiner, Nürtingen, Germany) in IMDM supplemented with 2 mM L-glutamine, 100 IU of penicillin per ml, 100 μg of streptomycin per ml (all from Life Technologies), 5×10^{-5} M 2-mercaptoethanol, 5% heat-inactivated horse serum (Boehringer, Mannheim, Germany), 10% heat-inactivated FCS, and 30% L929-Sup as a source of macrophage colony-stimulating factor activity (47). On days 4 and 6, cultures were fed with fresh medium. For assays, BMMph were used on days 8 to 12 of culture. These cells were essentially free of T cells. In some experiments, aliquots of BMMph were treated with rat rIFN-γ (100 U/ml) 48 h before cell harvest.

For infection, adherent macrophages were incubated with live or UV-irradiated *T. cruzi* in the ratio of 1:10 for 48 h. After rinsing with warm medium to remove nonphagocytosed parasites, infected macrophages were used for experiments. Infection of macrophages was routinely monitored by parallel treatment with *T. cruzi* of an identical macrophage sample adhered to a glass coverslip, which was subsequently stained by using the acridine orange-ethidium bromide staining system (7). Seventy to 90% of the adherent macrophages were infected with the parasite. For some experiments, macrophages (5×10^6) were stimulated with *T. cruzi* lysate in an amount equivalent to 5×10^7 live trypomastigotes.

Flow cytometry analysis. BMMph (10^6) were preincubated with 50 μl of rabbit serum at 4°C for 30 min to block Fc receptor binding of antibody. Cells were sedimented and then resuspended with 50 μl of the indicated MAb. After 30 min at 4°C, cells were washed twice with staining buffer (phosphate-buffered saline [PBS] supplemented with 2% FCS and 10 mM NaN_3) and were incubated for 30 min with 50 μl of DTAF (dichlorotriazinyl-aminofluorescein)-conjugated rabbit anti-rat IgG, $F(ab')_2$ fragment (Dianova, Hamburg, Germany), diluted 1:50. Following two washing steps, BMMph were fixed with 0.5 ml of 0.7% paraformaldehyde in PBS. Cells (10^4) of each sample were analyzed with a FACScan (Becton Dickinson, Heidelberg, Germany).

T-cell stimulation assays. For stimulation of the T cells via the TCR-CD3 complex, wells of flat-bottom microtiter plates (Greiner) were incubated with 50 μl of various doses of purified MAb 145-2C11 for 4 h at room temperature. The wells were then washed three times with PBS to remove unbound antibody. Plates were used immediately or, in the case of coupling, a second MAb (50 μl of anti-CD28 MAb) in various dilutions was added. After 4 h of incubation at room temperature, wells were washed three times. Cloned T cells (2×10^4/well) or $CD4^+$ T cells from spleen (5×10^4/well) were added alone or in combination with irradiated (20 Gy) accessory BMMph (5×10^3/well) in a final volume of 200 μl of IMDM supplemented with 5×10^{-5} M 2-mercaptoethanol, 100 IU of penicillin per ml, 100 μg of streptomycin per ml, 2 mM L-glutamine, and 5% heat-inactivated FCS. In some experiments, MAb, cytokines, or supernatants from BMMph in indicated concentrations were added to the cultures. Proliferation of T cells was assessed by addition of [^{3}H]thymidine([^{3}H]TdR, 3.7 kBq/well; Amersham Buchler, Braunschweig, Germany) on day 2 of culture for T-cell clones and day 3 for $CD4^+$ T cells from the spleen. After a further 18 h of incubation, incorporation of labeled nucleotides was determined by liquid scintillation counting.

For Ag-specific stimulation, irradiated BMMph (20 Gy) were cocultivated in various cell numbers with 2×10^4 9B11 T cells in the presence or absence of 100 μg of ovalbumin (Sigma, Deisenhofen, Germany) per ml. Proliferation was determined as described above.

All cultures were set up in triplicate. The standard error was less than 10%. Results are representative of three to five experiments. Statistical evaluation of differences between means of different groups were determined by Student's *t* test.

RESULTS

Selective upregulation of B7-2 molecules on macrophages infected with *T. cruzi*. To study the influence of the infection of BMMph with the parasite *T. cruzi* on the regulation of immunologically important surface molecules, the expression of MHC class II molecules, LFA-1, ICAM-1, B7-1, B7-2, and HSA was investigated. As shown in Fig. 1, BMMph expressed MHC class II molecules, B7-1, and HSA at very low levels, while LFA-1 and ICAM-1 were present at higher levels. Infection of the macrophages with *T. cruzi* did not significantly alter expression of all of these molecules. Interestingly, after infection, a selective and strong upregulation of B7-2 molecules on the surface of the BMMph was found. Not only was the percentage of B7-2-positive cells strongly enhanced but also the mean fluorescence intensity increased (Fig. 2). Noteworthy, the upregulation of B7-2 expression induced by infection with *T. cruzi* was much stronger than that induced by IFN-γ (Fig. 2). There was synergy with IFN-γ since addition of IFN-γ during the last 24 h of the 2 days of infection leads to an even greater expression of B7-2 molecules.

Interestingly, this strong upregulation of B7-2 expression was seen only when live parasites were used for infection of the macrophages. UV-irradiated trypanosomes slightly enhanced B7-2 expression, while whole lysates of *T. cruzi*, heat-killed parasites (Table 1), or paraformaldehyde-fixed parasites (data not shown) were not functional in this respect.

$CD4^+$ T cells freshly isolated from spleen and stimulated with anti-CD3 MAb proliferate with higher efficiency in the presence of *T. cruzi*-parasitized macrophages than in the presence of uninfected BMMph. To test whether the expression of B7-2 molecules had functional consequences, assays for the capacity of infected BMMph to costimulate T cells were performed. To test solely the costimulatory capacity of the BMMph, independent of the Ag-processing capacity and MHC class II expression, $CD4^+$ T cells freshly isolated from the spleen were stimulated by directly engaging the TCR-CD3 complex, using immobilized anti-CD3 MAb. To this end, the T

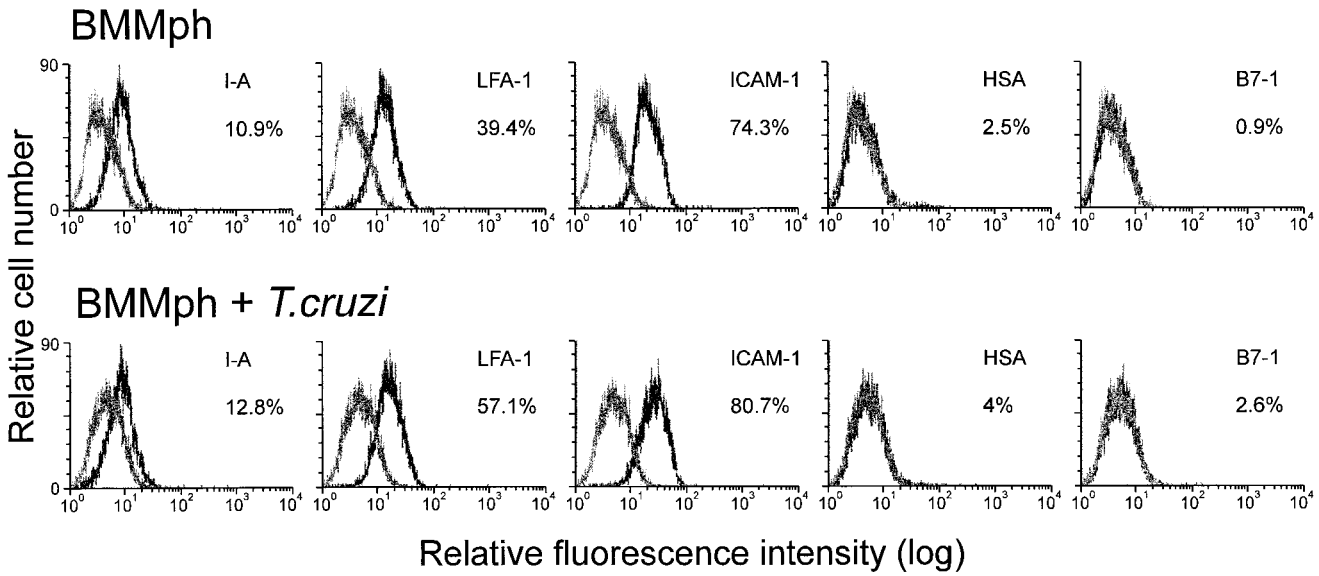

FIG. 1. Expression of MHC class II and costimulatory molecules on BMMph infected with *T. cruzi*. Ten-day-old BMMph from BALB/c mice were infected for 48 h with *T. cruzi* at a parasite-to-cell ratio of 10:1 or were left untreated; 85% of the cells were infected. Viability of all BMMph populations used was ≥98%. The macrophages were washed, incubated with rabbit serum, and stained with MAbs M5/114.15.2 (anti-I-A), FD441.8 (anti-LFA-1), YN1/1.7.4 (anti-ICAM-1), J11d (anti-HSA), and 1G10 (anti-B7-1) or an irrelevant MAb as a control. Bound MAbs were detected with a DTAF-conjugated rabbit anti-rat IgG $F(ab')_2$ fragment. Percent positive cells was calculated by subtracting percent positive cells of the control from percent positive cells showing specific MAb binding.

cells were cultured in plates coated with titrated concentrations of MAb 145-2C11 in the presence or absence of macrophages either infected with *T. cruzi* or uninfected. The T cells did not proliferate following stimulation with immobilized anti-CD3 MAb. Addition of macrophages induced T-cell proliferation, which was highest with BMMph parasitized by *T. cruzi* (Fig. 3). Anti-CD28 MAb served as a positive control for the costimulatory activity via the B7-2 ligand CD28. This antibody induced approximately the same proliferation as infected BMMph. To exclude the possibility that the proliferation observed was that of the parasite within the macrophages, T cells were incubated with BMMph in the absence of plate-bound anti-CD3 MAb; no proliferation was measured.

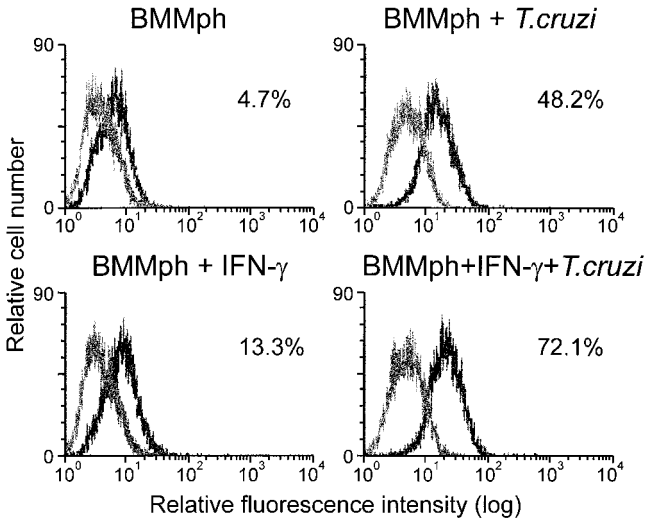

FIG. 2. Upregulation of B7-2 molecules on BMMph infected with *T. cruzi*. Fluorescence analysis studies were performed as described in the legend to Fig. 1. The infection rate of the macrophages was 75%. In addition to the macrophages infected or not, one aliquot of each macrophage population was stimulated with rat rIFN-γ (100 U/ml) for the last 24 h of culture. Viability of all macrophage populations was ≥95%. For detection of B7-2 expression, MAb GL1 was used. An irrelevant MAb served as a control (light line).

The strong costimulation of resting $CD4^+$ T cells by *T. cruzi*-infected BMMph depends on the expression of B7-2 molecules. To demonstrate that the strong proliferation of anti-CD3-activated $CD4^+$ T cells induced by *T. cruzi*-infected BMMph could be attributed to the high expression of B7-2 molecules, $CD4^+$ T cells were cultivated on anti-CD3 MAb-coated plates in the presence of infected or noninfected BMMph and a MAb against B7-2. Figure 4 shows that anti-B7-2 MAb inhibited the augmentative effect of *T. cruzi*-infected BMMph for T-cell proliferation down to the level induced by uninfected macrophages. A nonspecific IgG antibody used as a control did not alter T-cell proliferation. These results suggest that the high B7-2 expression was directly responsible for the augmented T-cell proliferation.

TABLE 1. Expression of B7-1/2 molecules on the surfaces of BMMph after infection with live or inactivated *T. cruzi*

Treatment of BMMph[b] with:	% Positive cells[a] expressing:	
	B7-1	B7-2
Medium	1.5	4.1
T. cruzi		
Live	0.5	52.0
UV irradiated	0.9	28.3
Heat killed	0.9	4.3
Lysates	0.4	6.3

[a] Calculated by subtracting percent positive cells of the control from percent positive cells with specific MAb binding.

[b] BMMph were stimulated for 48 h with live, UV-irradiated, or heat-killed *T. cruzi* parasites in a cell-to-parasite ratio of 1:10 or were left untreated. One aliquot of the macrophages was stimulated with the whole lysate of *T. cruzi* (equivalent to 5×10^7 parasites). Viability of all BMMph populations was ≥95%; 85 to 90% of the macrophages were infected.

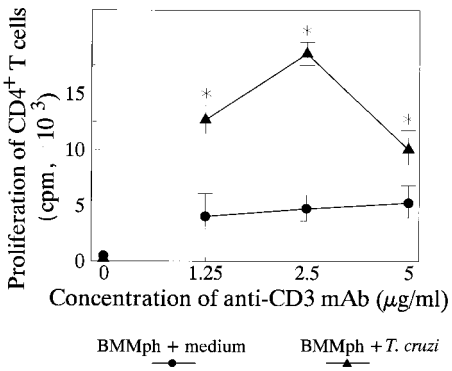

FIG. 3. $CD4^+$ T cells from spleen activated with anti-CD3 MAb were stimulated to high proliferation in the presence of *T. cruzi*-infected macrophages. Anti-CD3 MAb 145-2C11 was immobilized to microtiter wells in the indicated amounts. The wells were rinsed, and $CD4^+$ T cells (2×10^5/well) isolated by positive selection using a magnetic cell separation technique were added with or without gamma-irradiated (20 Gy) BMMph (5×10^3/well). BMMph were generated from BALB/c mice and were not infected or were infected with *T. cruzi* parasites in a cell-to-parasite ratio of 1:10 for 48 h (80% of the cells were infected; viability of the BMMph was ≥95%). For a control, T cells were stimulated via plate-bound anti-CD3 MAb plus anti-CD28 MAb 37.51 (10 μg/ml). Proliferation of the T cells was measured on day 4 of culture after a 20-h pulse with [^{3}H]TdR. Proliferation of anti-CD3-stimulated $CD4^+$ T cells in the absence of BMMph was ≤1.445 cpm. Levels of proliferation of anti-CD3-stimulated $CD4^+$ T cells in the presence of anti-CD28 MAb were 12,735 cpm (5 μg of anti-CD3 MAb per ml), 16,338 cpm (2.5 μg of anti-CD3 MAb per ml), and 7,806 cpm (1.25 μg of anti-CD3 MAb per ml). Background proliferation of T cells and macrophages was ≤152 cpm. The asterisks indicate statistically significant differences ($P < 0.001$) compared with uninfected macrophages.

***T. cruzi*-infected macrophages are efficient costimulators of cloned Th1 cells.** In the following experiments, we extended these results obtained with polyclonal resting $CD4^+$ T cells to established cloned T cells. Cloned Th1 cells activated via anti-CD3 MAb responded with higher proliferation to *T. cruzi*-infected BMMph than to uninfected BMMph in a dose-dependent fashion (Fig. 5). Macrophages stimulated with IFN-γ, expressing higher levels of B7-2 molecules than unstimulated and uninfected macrophages (Fig. 2), were more efficient in stimulating Th1 cells than uninfected macrophages. Furthermore, when the infected macrophages were stimulated for 24 h before cell harvest in addition with rIFN-γ, resulting in the highest B7-2 expression, proliferation of the T cells was even greater. Thus, the capacity of the macrophages to stimulate the Th1 cells correlated with their expression of B7-2 molecules (data shown in Fig. 5 are representative for the data obtained from two different cloned Th1 cells).

To test whether the effect of *T. cruzi*-infected BMMph on TCR-mediated T-cell proliferation was also detectable in an Ag-specific assay system, we incubated the Th1 cell clone 9B11 with various doses of infected or uninfected BMMph and the specific Ag ovalbumin. In fact, Ag-specific T-cell proliferation induced by infected macrophages was much stronger than that induced by uninfected control cells (Fig. 6).

***T. cruzi*-infected macrophages are strong costimulators of cloned Th2 cells.** As shown in Fig. 7, anti-CD3-stimulated cloned Th2 cells proliferated with higher efficiency in the presence of *T. cruzi*-infected BMMph than in the presence of uninfected cells. Also, the expression of this costimulatory activity required infection with live parasites. Lysates of *T. cruzi* (Fig. 7) or heat-killed parasites (data not shown) did not promote costimulation of Th2 cells. Contrary to the results described above, the stimulation of the Th2 cells was not dependent on the expression of B7-2 induced on *T. cruzi*-infected macrophages because the anti-B7-2 antibody did not inhibit costimulation (data not shown). One factor responsible for the enhanced Th2 cell stimulation might be IL-1, since IL-1 has been shown to be produced by adherent spleen cells of *T. cruzi*-infected mice (46) and by BMMph infected in vitro with *T. cruzi* (our own unpublished results). We were able to inhibit the costimulatory activity of infected cells by using a MAb against IL-1α, resulting in a complete inhibition of T-cell proliferation (data not shown). This result shows that infected macrophages, by secreting IL-1α and expressing B7-2, deliver distinct costimulatory signals to different T-cell subsets.

DISCUSSION

Costimulatory molecules on infected APC are critical for the initiation and maintenance of an immune response against intracellular pathogens. Several pathogens, such as mycobacteria (40), *Listeria* (20), or *Leishmania* (9, 20, 41) have been shown to use various strategies for influencing the host immune response at the level of costimulatory signaling. The effects on T-cell activation range from inhibition of T-cell proliferation and cytokine production in the case of *L. donovani* (41) to strong augmentation of Th2 cell proliferation in the case of *L. major* (9).

In this study, we examined the effect of an infection with *T. cruzi* on the capacity of BMMph to deliver costimulatory signals to Th cells. We show that infection with *T. cruzi* dramatically enhances the costimulatory activity of macrophages both for resting T cells and for cloned Th cells. Two different mechanisms lead to this enhancement.

Most importantly, as a result of the infection, expression of B7-2 molecules was selectively enhanced within 24 to 48 h,

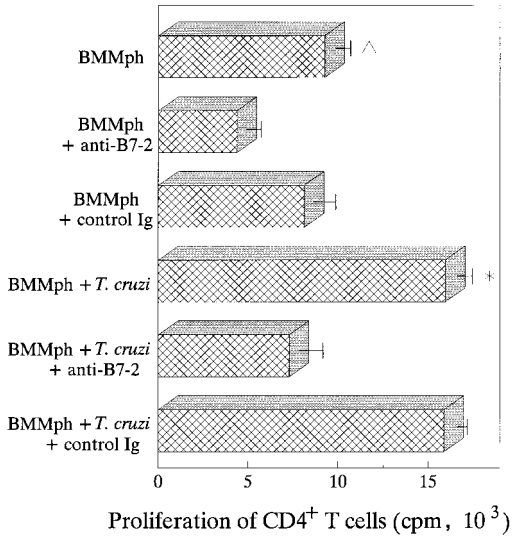

FIG. 4. Anti-B7-2 MAb neutralizes the augmentative effect of *T. cruzi*-infected BMMph on $CD4^+$ T-cell proliferation. $CD4^+$ T cells were cultured with plate-bound anti-CD3 MAb (1 μg/ml) in the presence of irradiated (20 Gy) BMMph (5×10^3) and MAb GL1 (anti-B7-2, 10 μg/ml) or a control rat IgG2a antibody. BMMph used were infected or not infected with *T. cruzi* (the cell-to-parasite ratio was 1:10, 85% of the cells were infected, and viability of all BMMph was ≥96%). During the last 20 h of a 96-h incubation period, [^{3}H]TdR was added to the assays. Proliferation of anti-CD3-activated T cells in the absence of BMMph was 1,955 cpm. Proliferation of T cells and macrophages in the absence of anti-CD3 MAb was ≤581 cpm. Background proliferation of APC and T cells alone was ≤129 cpm. The asterisk indicates statistically significant differences ($P < 0.01$) compared with uninfected macrophages or with infected macrophages in the presence of anti-B7-2 MAb. The triangle indicates statistically significant differences ($P < 0.03$) compared with uninfected macrophages in the presence of the control MAb.

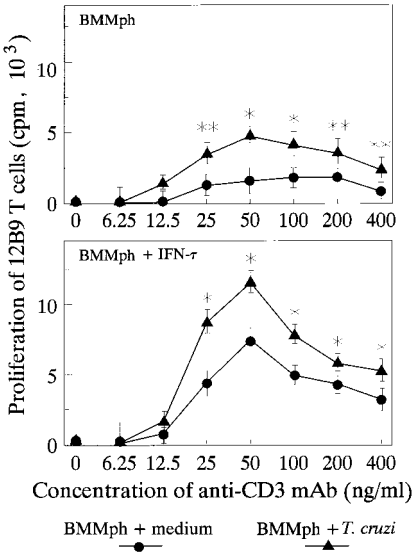

FIG. 5. BMMph infected with *T. cruzi* exerted a more profound costimulatory potential for Th1 cells compared to uninfected macrophages. MAb 145-2C11 was immobilized to microtiter plates in the indicated concentrations for 4 h at room temperature. After washing, 12B9 Th1 cells (2×10^4/well) and irradiated BMMph (5×10^3/well) were added. BMMph tested for their costimulatory function were untreated, were stimulated for 24 h with rat rIFN-γ (100 U/ml), were infected with *T. cruzi* (the cell-to-parasite ratio was 1:10; 79% of the cells were infected), or were infected with *T. cruzi* and in parallel stimulated with IFN-γ during the last 24 h of infection. T-cell proliferation was determined on day 3 by adding [^{3}H]TdR for the last 20 h of the culture. Proliferation of anti-CD3-stimulated T cells in the absence of BMMph was 193 cpm. Proliferation of T cells and macrophages in the absence of anti-CD3 MAb was ≤187 cpm. Background proliferation of BMMph and T cells alone was ≤34 cpm. The asterisks indicate statistically significant differences (*, $P < 0.05$; **, $P < 0.008$) compared with infected BMMph.

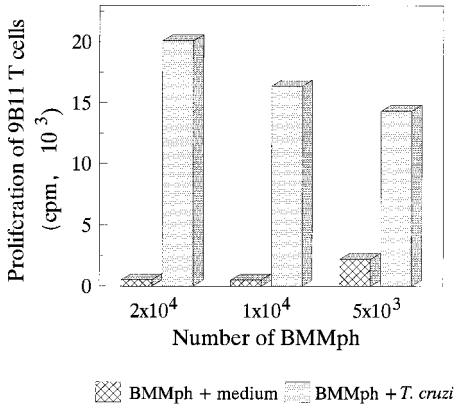

FIG. 6. Presentation of Ag by *T. cruzi*-infected BMMph is more efficient than presentation by uninfected BMMph. The cloned Th1 cells 9B11 (2×10^4/well) were stimulated, per well, with 10^4 or 5×10^3 gamma-irradiated (20 Gy) macrophages of C57BL/6 origin in the presence or absence of 100 μg of ovalbumin per ml. BMMph had been infected with *T. cruzi* for 48 h in a ratio of 1:10, 83% of the BMMph were infected. Viability of the macrophages was ≥94%. Proliferation of T cells was determined by adding [^{3}H]TdR on day 2 for 20 h. T-cell proliferation in the absence of antigen was ≤984 cpm. Background proliferation of APC and T cells was ≤294 cpm.

while other ligands for T cells, such as LFA-1, ICAM-1, B7-1, and HSA, were not significantly altered in their expression levels. That not all infected macrophages show upregulation of B7-2 molecules may be due to the fact that the macrophages used for the studies do not represent a homogeneous population and differ in reactivity to the stimuli. Alternatively, distinct cells may react with different kinetics depending on their activation state.

B7 molecules are essential costimulators for T-cell proliferation (12, 23). In in vivo studies, B7-2 has been shown to be the major CD28 costimulatory ligand active in clonal expansion of Ag-specific cells (reviewed in reference 6). Thus, B7-2 is the primary costimulatory molecule responsible for initiating T-cell responses. In agreement with this notion is our finding that the upregulated B7-2 molecules on parasitized BMMph are entirely responsible for the capacity of the BMMph to strongly costimulate resting $CD4^+$ T cells. This was shown by the strong inhibitory effects of the anti-B7-2 MAb. Furthermore, the studies using macrophages stimulated with IFN-γ, which express higher levels of B7-2 than unstimulated cells, demonstrate that these cells were better T-cell activators than unstimulated macrophages. BMMph, which were infected and stimulated in parallel with IFN-γ, not only expressed the highest levels of B7-2 but also induced the strongest T-cell proliferation. Therefore, this finding supports the necessity of B7-2 molecules for T-cell activation. That anti-B7-2 MAb also inhibited the low degree of costimulation by uninfected BMMph could be due to a low-level expression of B7-2 molecules on unstimulated BMMph, sufficient for the weak T-cell proliferation induced by these macrophages. Another explanation is that during the time of the assay, lymphokines like IFN-γ are produced by activated T cells which are able to upregulate B7-2 expression. Indeed, IFN-γ augments the level of B7-2 on BMMph.

The mechanism by which upregulation of B7-2 molecules can occur is still unclear. The observation that this upregulation occurred only with BMMph infected with live trypanosomes and not with BMMph exposed to fixed or heat-inactivated parasites or to lysates of parasites shows that B7-2 upregulation depends on viable parasites. Similarly, macrophages which were treated with *L. major* lysates were unable to augment the proliferation of Th2 cells whereas living parasites were functional in this respect (9). In addition, the inability of heat-killed or fixed *T. cruzi* to upregulate B7-2 molecules or IL-1α production (data not shown) demonstrates that enhancement of expression of these costimulatory molecules is

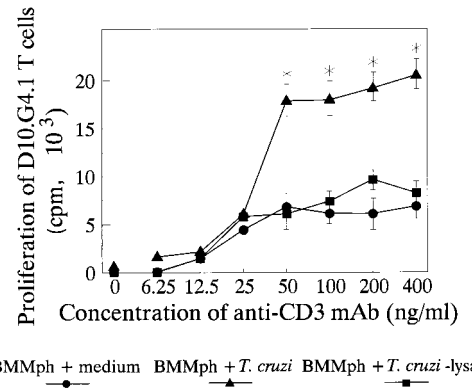

FIG. 7. *T. cruzi*-infected BMMph costimulate Th2 cell proliferation with higher efficiency than uninfected BMMph. D10.G4.1 T cells (2×10^4/well) were plated on anti-CD3-coated wells in the presence or absence of BMMph (5×10^3/well) infected with live *T. cruzi* parasites (cell-to-parasite ratio was 1:10; 89% of the macrophages were infected) or whole lysate generated by repeated freeze-thaw steps. Viability of the macrophages was ≥96%. Proliferation of T cells was determined by addition of [^{3}H]TdR for the last 20 h of the 68-h culture time. Background proliferation of APC and T cells was ≤295 cpm. The asterisks indicate significant differences ($P < 0.04$) compared with uninfected BMMph or BMMph incubated with *T. cruzi* lysate.

not the result of an uptake of trypanosomal particles, meaning that phagocytosis alone does not trigger the activation of the B7-2-specific gene. Although not shown here, we have found that soluble mediators appear not to be responsible for this effect. Cocultivation of infected and uninfected BMMph, separated via a membrane through which only cytokines can penetrate, or incubation with IL-1α or IL-12, both produced by infected BMMph (1, 14, 46), did not lead to B7-2 upregulation on uninfected BMMph. This result indicates that the upregulation of this important costimulatory molecule is an early cellular response of the infected cell itself. It is noteworthy in this respect that infection with *T. cruzi* was much more potent in inducing B7-2 than IFN-γ, known to be a strong upregulator of B7-2 expression (18). Moreover, there was a strong synergy between this cytokine and the infection.

Not only $CD4^+$ T cells freshly isolated from the spleen but also anti-CD3-activated cloned T cells of Th1 and Th2 phenotype proliferated with higher efficiency in the presence of *T. cruzi*-infected BMMph than in the presence of uninfected cells. In contrast to Th1 cells, which continue to require CD28 costimulation for activation (2), established Th2-mediated immune responses appear to become relatively independent of CD28 costimulation for their maintenance (27). A potential mechanism for this is suggested by the observation that CD28 costimulation is necessary for the induction of T-cell sensitivity to IL-4 (26). This possibility is supported by our results showing that the high B7-2 molecule expression was not responsible for the strong proliferative response of Th2 cells induced by *T. cruzi*-infected BMMph, as demonstrated by the inability of the anti-B7-2 MAb to affect the costimulatory capacity of infected BMMph for Th2 cells. For these cells, IL-1α is the molecule responsible for the enhanced proliferation of Th2 cells, induced by infected macrophages. Thus, these results support and extend earlier studies by Tarleton (46), showing that macrophages from *T. cruzi*-infected mice expressed IL-1 in the membrane-bound and soluble forms.

Taken together, the data that we have presented suggest two mechanisms by which *T. cruzi* infection enhances the stimulatory potential of BMMph for T cells. The upregulation of B7-2 molecules might be particularly important in the early immune response to *T. cruzi*, because stimulation of resting T cells is a critical step in an infection. The activation of naive T cells is mediated only by professional APC which deliver Ag-specific and costimulatory signals. One very potent APC is the dendritic cell, which expresses high levels of MHC molecules as well as a variety of costimulatory molecules, including B7. However, they are nonphagocytic. In contrary, macrophages are very potent phagocytic cells which are predominantly infected with *T. cruzi*. In their resting state, they express only few MHC class II molecules and do not express B7 molecules. Therefore, it will be of great benefit that the infection by trypanosomes renders the macrophage a potent stimulatory cell for resting T cells, being able to initiate an immune response. Later in the ongoing immune response, there will be further upregulation of costimulatory molecules by synergy with cytokines produced by activated T cells. In fact, we have found that B7-2 is abundantly expressed on many cell types during infection of mice with *T. cruzi* (unpublished observations). Since most of these cells are not infected with *T. cruzi*, this is probably a secondary phenomenon due to the production of cytokines like IFN-γ. When the immune response against the parasite has been established, further upregulation of B7-2 and IL-1 will augment and maintain the ongoing T-cell response.

ACKNOWLEDGMENTS

We thank J. Schottelius for providing *T. cruzi*, A. von Bonin for critically reading the manuscript, and S. Kraus for expert technical assistance.

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Editor: S. H. E. Kaufmann