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CD40 Ligation Prevents *Trypanosoma cruzi* Infection through Interleukin-12 Upregulation

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Because of the critical role of the CD40-CD40 ligand (CD40L) pathway in the induction and effector phases of immune responses, we investigated the effects of CD40 ligation on the control of *Trypanosoma cruzi* infection. First, we observed that supernatants of murine spleen cells stimulated by CD40L-transfected 3T3 fibroblasts (3T3-CD40L transfectants) prevent the infection of mouse peritoneal macrophages (MPM) by *T. cruzi*. This phenomenon depends on de novo production of nitric oxide (NO) as it is prevented by the addition of *N*-nitro-L-arginine methyl ester, a NO synthase inhibitor. NO production requires interleukin (IL)-12-mediated gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α) synthesis as demonstrated by inhibition experiments using neutralizing anti-IL-12, anti-IFN-γ, and anti-TNF-α monoclonal antibodies (MAb). We found that an activating anti-CD40 MAb also directly stimulates IFN-γ-activated MPM to produce NO and thereby to control *T. cruzi* infection. To determine the in vivo relevance of these in vitro findings, mice were injected with 3T3-CD40L transfectants or 3T3 control fibroblasts at the time of *T. cruzi* inoculation. We observed that in vivo CD40 ligation dramatically reduced both parasitemia and the mortality rate of *T. cruzi*-infected mice. A reduced parasitemia was still observed when the injection of 3T3-CD40L transfectants was delayed 8 days postinfection. It was abolished by injection of anti-IL-12 MAb. Taken together, these data establish that CD40 ligation facilitates the control of *T. cruzi* infection through a cascade involving IL-12, IFN-γ, and NO.

CD40 is a cell surface receptor expressed by various cells (B lymphocytes, dendritic cells, hematopoietic progenitors, endothelial cells, and epithelial cells) including monocytes and macrophages (56). Interaction of CD40 with its CD40 ligand (CD40L) (4, 22) triggers a pleiotropic pathway involved in both humoral and cellular immunity. By exerting potent biological activities on $CD4^+$ T cells and antigen-presenting cells such as dendritic cells and macrophages (49), this pathway plays a major role in anti-infective host defense (21). Indeed, CD40-CD40L interactions result in the secretion of multiple cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-10, IL-12, gamma interferon (IFN-γ), and tumor necrosis factor alpha (TNF-α) by immunocompetent cells. In particular, IL-12 has emerged as a potent immunoregulatory cytokine involved in the control of intracellular infections (44, 54, 55).

Trypanosoma cruzi is a hemoflagellate protozoan parasite with intracellular multiplication. It infects humans as well as domestic and wild mammals and is the etiological agent of Chagas' disease (51). Experimental infection of BALB/c mice mimics the human disease and allows the study of host defense mechanisms. It displays an acute phase characterized by high parasitemia, followed by a chronic phase during which parasites become undetectable in peripheral blood while persisting in tissues. Various cytokines are implicated in the control of *T. cruzi* infection in mice including IL-12, IFN-γ, and TNF-α (1–3, 8, 25, 42, 45, 47, 53). IFN-γ and TNF-α have been shown to induce nitric oxide (NO) synthesis (13, 33), which in turn plays a crucial role in the control of *T. cruzi* infection in mice both in vitro and in vivo (1, 19, 24, 34, 35, 37–40, 57). The present work was undertaken to analyze the effect of CD40 ligation on cell infection in vitro and to investigate whether a CD40L stimulation was able to protect mice against *T. cruzi* infection.

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MATERIALS AND METHODS

SC and mouse peritoneal macrophages. Spleens were harvested from male BALB/c mice (6 to 8 weeks old) purchased from Bantin & Kingman Universal (Hull, United Kingdom) and maintained in our animal facilities on standard laboratory chow. Suspensions of erythrocyte-free spleen cells (SC) were obtained by spleen dilaceration and treatment for 30 s with distilled sterile water. SC (10^7 cells/ml) were then suspended in RPMI 1640 medium (GIBCO, Grand Island, N.Y.). They were plated in a 24-well cell culture plate (Nunc, Roskilde, Denmark) and incubated in a 5% CO_2 and water-saturated atmosphere.

Mouse peritoneal macrophages (MPM) were harvested from male BALB/c mice by washing their peritoneal cavities with chilled Hank's balanced salt solution without Ca^{2+} and Mg^{2+} (pH 7.4; GIBCO) (38). They were allowed to adhere (2×10^5 cells/well) on round sterile coverslips (Thermanox, 13-mm diameter; Miles Scientific, Naperville, Ill.) in 24-well microplates for 2 h at 37°C in a 5% CO_2 atmosphere. Nonadherent cells were removed by washing.

SC and MPM were cultured in RPMI 1640 medium supplemented with *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (25 mM), glutamine (25 mM), fetal calf serum (10%), penicillin (100 IU/ml), and streptomycin (100 μg/ml; GIBCO).

Infection of MPM and mice with *T. cruzi*. For in vitro experiments, *T. cruzi* trypomastigotes (Tehuantepec strain) were obtained from infected fibroblasts as previously described (14). Trypomastigotes (10^6 parasites/well) were added to MPM in a 5:1 parasite-to-cell ratio. After 24 h, cultures were washed to remove free parasites, and MPM were further incubated for 24 h. Then, cells were fixed with methanol and stained with Giemsa stain. The percentage of infected MPM and the mean number of amastigotes per infected MPM were recorded after microscopical examination of at least 200 cells per well. A parasitic index was calculated by multiplying the percentage of infected MPM times the mean number of amastigotes per infected MPM (58).

For in vivo experiments, BALB/c mice were inoculated intraperitoneally with 100 blood-form trypomastigotes in 0.2 ml of Alsever's solution. Parasitemia was monitored by counting trypomastigotes in blood samples collected by tail incision every 2 days and every day around the peak of parasitemia. Survival rates were determined daily.

CD40L-transfected fibroblasts. 3T3 fibroblasts transfected with the gene encoding CD40L (3T3-CD40L transfectants) were obtained as follows. The 5′ region of the mCD40L, including the Kozack sequence, the leader sequence, and the first 136 codons, was amplified by reverse transcriptase-PCR of mRNA derived from activated EL4 T cells. The primers used for amplification were based on the published sequence of the mCD40L cDNA (4). The 460-bp downstream region of mCD40L was obtained by *Bam*HI and *Hin*dIII digestion of the pHβAPr-1-neo-mCD40L-mCD8α plasmid (kindly provided by P. Lane, Basel Institute for Immunology, Basel, Switzerland) (31). The complete mCD40L cDNA was then cloned into the pCI-neo vector (Promega, Leiden, The Netherlands). Flow cytometry analysis of pCI-neo-mCD40L-transfected COS-7 cells, by using a biotinylated anti-mCD40L antibody (Pharmingen, San Diego, Calif.), showed that mCD40L was correctly assembled and expressed on the cell surface. Plasmid pCI-neo-mCD40L was used for the lipofection (LipoTaxi; Stratagene, Westburg, The Netherlands) of NIH 3T3 cells (American Type Culture Collection, Rockville, Md.). mCD40L-expressing cells were selected on the basis of growth in the presence of G418 sulfate (2 mg/ml, final concentration; Alexis Corporation, San Diego, Calif.) and flow cytometry. For a negative control in the different assay systems used, a 3T3 cell line transfected with empty pCI vector DNA (3T3 control fibroblasts) was selected.

3T3-CD40L transfectants and 3T3 control fibroblasts were grown in Dulbeco's modified Eagle medium (GIBCO) supplemented with fetal calf serum (5%), penicillin (100 IU/ml), and streptomycin (100 μg/ml), and for transfected cells, with G418 sulfate (2 mg/ml). They were harvested after trypsin-EDTA treatment (GIBCO) and irradiated at 30 Gy (Mark I-68A irradiator; J.L. Shepherd and Associates, San Fernando, Calif.) to prevent further cell replication. They were used to induce in vitro CD40 triggering on SC. 3T3-CD40L transfectants (5×10^4) were seeded together with 5×10^6 SC per well (24-well culture plate) in 500 μl of culture medium.

For in vivo experiments, mice were intravenously injected with 10^6 3T3-CD40L transfectants in 150 μl of phosphate-buffered saline (PBS). 3T3 control fibroblasts and PBS were used as controls. To assess the kinetics of IL-12 secretion, blood samples (100 μl/mouse) were taken from uninfected mice by retroorbital puncture on days 0, 1, 4, 7, and 10 postinjection. Individual blood samples were centrifuged (10 min, $800 \times g$), and plasma samples were stored at −70°C until use. For in vivo neutralization of IL-12, anti-IL-12 monoclonal antibody (MAb) (see below, 1.3 mg) was injected intraperitoneally on the day of infection (day 0) and then on days 2 and 4 postinfection (p.i.).

MAbs and reagents. Neutralizing anti-IFN-γ MAb (R4-6A2; immunoglobulin G1 [IgG1]) and anti-TNF-α MAb (MP6-XT3; IgG1) were purchased from Pharmingen. The anti-IL-12 MAb (C17.8; IgG2a) used in the in vitro experiments was purchased from Genzyme (Cambridge, Mass.). Corresponding control isotype-matched antibodies R3-34 (IgG1) and R35-95 (IgG2a) were purchased from Pharmingen. The hybridoma FGK45, producing an IgG2aκ specific for murine CD40, was kindly provided by A. Rolink (Basel Institute for Immunology, Basel, Switzerland) (43). The hybridoma cells were cultured in standard conditions in RPMI 1640 containing 1% bovine serum. The rat MAb was purified by affinity chromatography with a mouse anti-rat kappa MAb immobilized on Sepharose beads and 3.5 M $MgCl_2$ as elution buffer. Eluted antibodies were extensively dialyzed against PBS and filter sterilized. A nonrelated rat MAb was purified similarly and used as a negative control. Another anti-CD40 MAb (3/23; IgG2a) was from Pharmingen. The in vitro working concentration for all MAbs was 10 μg/ml except for anti-CD40 MAb (20 μg/ml). For in vivo experiments, ascitic MAb anti-IL-12 (C17.8; IgG2a) was used (kindly provided by V. Flamand, Université Libre de Bruxelles, Brussels, Belgium). The ascitic IgG2a antibodies used in vivo as a control were a kind gift from H. Bazin (Université Catholique de Louvain, Brussels, Belgium). FGK45 anti-CD40 MAb was also tested in vivo for its ability to protect mice against *T. cruzi* infection. For this purpose, we used high doses of FGK45 compared with the one used by others (46): eight mice were injected intravenously with 200 μg of FGK45 MAb at the time of *T. cruzi* inoculation, and four of them received again 100 μg of MAb at days 1 and 4 p.i. Two control groups of five mice were injected with isotype-matched control MAb or PBS.

N-nitro-L-arginine methyl ester (NAME, 5 mM; Sigma Chemical Co., St. Louis, Mo.) was used as the competitive inhibitor of NO synthase.

Recombinant murine IFN-γ (rIFN-γ; 10 U/ml) was kindly supplied by A. Billiau and H. Herremans (Rega Institute, Leuven, Belgium).

The concentration of endotoxin in all the reagents and media was below 80 pg/ml according to the colorimetric limulus amoebocyte lysate assay (detection limit, 1 pg/ml) (Coatest endotoxin; Chromogenix, Mölndal, Sweden).

Cytokine determinations and nitrite assay. Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Genzyme for determination of IL-12 (p40 and p70), IFN-γ, and TNF-α. The lower limits of detection of these assays were, respectively, 15, 30, 20 and 35 pg/ml. NO production by MPM was assayed by measuring nitrite, its stable degradation product, by the Griess reaction (20). Supernatants (50 μl) from cultured MPM were harvested after 24 h and mixed with 50 μl of Griess solution (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride, 2% H_3PO_4). The absorbance was measured at 540 nm in a microplate ELISA reader (Spectracount Microplate Photometer; Packard, Meriden, Conn.). Sodium nitrite ($NaNO_2$) diluted in culture medium was used as a standard. The detection limit of the assay was 2.5 μM.

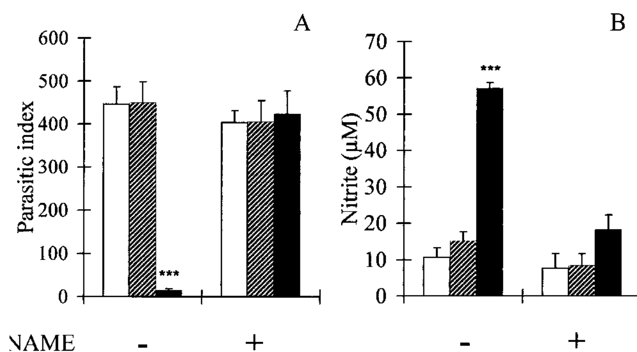

FIG. 1. Effect of supernatants from CD40L-activated SC cultures on *T. cruzi* infection of MPM. Supernatants harvested from SC (open bars), from SC incubated with 3T3 control fibroblasts (hatched bars), or from CD40L-activated SC (black bars) were added to MPM at the time of *T. cruzi* infection, in the presence or absence of NAME, and the parasitic index (A) and nitrite levels (B) were measured. Data are means ± standard deviations from three independent experiments performed in duplicate. ***, $P < 0.001$ compared to 3T3 control fibroblasts (Student's *t* test).

RESULTS

3T3-CD40L transfectants induce *T. cruzi* infection clearance through a NO-mediated IL-12-dependent pathway. The involvement of CD40 ligation in *T. cruzi* infection was first tested in vitro by using a two-step procedure. As *T. cruzi* readily infects 3T3 fibroblasts (data not shown), SC were first cocultured with either 3T3-CD40L transfectants, 3T3 control fibroblasts, or medium. Supernatants of these cocultures were then added to MPM cultured at the time of *T. cruzi* addition. The parasitic index was calculated on MPM, 48 h later. Supernatants from CD40L-activated SC clearly improved control of *T. cruzi* infection by MPM, whereas supernatants of SC alone or cocultured with 3T3 control fibroblasts did not exert a significant effect (Fig. 1A).

In light of the well-known protective role of NO in *T. cruzi* infection, NO levels were determined in culture supernatants of MPM. Supernatants from CD40L-activated SC induced a strong up-regulation of NO production by MPM. By contrast, the NO level was low when MPM were incubated with supernatants from SC-3T3 control fibroblast cultures or SC alone (Fig. 1B). The addition of NAME to MPM at the time of SC supernatant addition abolished the NO overproduction as well as their effect on the parasitic index (Fig. 1).

To identify the causative agents responsible for the induction of NO production by MPM, IFN-γ and TNF-α levels were measured in supernatants from CD40L-activated SC. As expected, IFN-γ and TNF-α synthesis was induced by 3T3-CD40L transfectants (data not shown). Furthermore, the ad-

TABLE 1. Nitrite production is dependent on IFN-γ and TNF-α production by CD40L-activated SC[a]

MAb added to CD40L-activated SC	Nitrite production (μM) by macrophages
Isotype control	60.7 ± 5.1
Anti-IFN-γ	11.7 ± 5.7***
Anti-TNF-α	12.9 ± 2.6***

[a] SC were activated with 3T3-CD40L transfectants in the presence of anti-IFN-γ or anti-TNF-α MAb. Supernatants were harvested after 48 h and transferred to MPM cultures at the time of *T. cruzi* infection. Nitrite levels were measured after 24 h. Data are means ± standard deviations of three independent experiments performed in duplicate. ***, $P < 0.001$ compared to isotype control (Student's *t* test).

TABLE 2. Production of IL-12 p40 and IL-12 p70 by SC incubated with 3T3-CD40L transfectants[a]

Treatment of SC	IL-12 p40 (pg/ml)	IL-12 p70 (pg/ml)
None	3,052 ± 1,079	<15
3T3	5,852 ± 3,201	<15
3T3-CD40L	34,266 ± 10,523*	63.8 ± 35.3*

[a] SC were incubated with either 3T3 control fibroblasts or 3T3-CD40L transfectants for 48 h. IL-12 p40 and IL-12 p70 levels were measured in culture supernatants. Data are means ± standard deviations from three (IL-12 p40) and five (IL-12 p70) independent experiments performed in duplicate. *, $P < 0.05$ compared to 3T3 control fibroblasts (Student's *t* test).

TABLE 3. Effect of activating anti-CD40 MAb on IFN-γ-activated MPM[a]

Treatment	Parasitic index		Nitrite (μM)	
	Isotype control	Anti-CD40 MAb	Isotype control	Anti-CD40 MAb
None	377 ± 53	118 ± 30**	23.6 ± 1.3	43.1 ± 4.4**
NAME	459 ± 81	428 ± 93	6.4 ± 3.6	7.7 ± 3.9
Anti-IL-12 MAb	355 ± 104	115 ± 11**	23.9 ± 3.0	41.5 ± 7.1**

[a] MPM were treated at the time of *T. cruzi* infection with 10 U of rIFN-γml in combination with isotype-matched control MAb or FGK45 anti-CD40 MAb in the presence or absence of NAME or neutralizing anti-IL-12 MAb. Parasitic index and nitrite levels were measured. Data are from three independent experiments performed in duplicate. **, $P < 0.01$ compared to isotype-matched control MAb (Student's *t* test).

dition of anti-IFN-γ and anti-TNF-α MAbs to CD40L-activated SC inhibited NO production by MPM, demonstrating that NO production was dependent upon the presence of these two cytokines (Table 1).

IL-12 is a major component of a complex biochemical pathway inducing IFN-γ synthesis and leading to NO production by macrophages. Accordingly, IL-12 p40 and IL-12 p70 (the bioactive heterodimer) levels were assayed in the supernatants from CD40L-activated SC collected after 48 h (Table 2). In contrast to supernatants from SC cultured in the presence of 3T3 control fibroblasts or medium alone, we found a significant increase of both IL-12 p40 and p70 when SC had been cocultured with 3T3-CD40L transfectants. The addition of neutralizing anti-IL-12 MAb to the culture of CD40L-activated SC inhibited the production of IFN-γ by SC (Fig. 2A). The supernatants also failed to induce NO production by MPM (Fig. 2B) and lost their clearing effect against *T. cruzi* infection (Fig. 2C).

Activating anti-CD40 MAb directly enhances parasite control by IFN-γ-activated macrophages. We also triggered the CD40-CD40L pathway by using agonistic anti-CD40 MAb (FGK45) to directly activate MPM in vitro. MPM were infected with *T. cruzi* trypomastigotes and treated with anti-CD40 MAb together with a suboptimal concentration of 10 U of rIFN-γ ml. FGK45 anti-CD40 MAb clearly augments NO production by IFN-γ-activated MPM (Table 3). Similar results were found with another anti-CD40 MAb (3/23, data not shown). This NO up-regulation correlated with an improved control of *T. cruzi* infection, which was blocked in the presence of NAME but still active in the presence of neutralizing anti-IL-12 MAb (Table 3).

Injection of 3T3-CD40L transfectants protects mice against *T. cruzi* infection. To evaluate in vivo the protective effect of CD40 stimulation on *T. cruzi* infection, mice were injected with 3T3-CD40L transfectants, 3T3 control fibroblasts, or PBS at the same time of *T. cruzi* inoculation. The injection of 3T3-CD40L transfectants reduced the peak of parasitemia (Fig. 3A) and considerably increased the survival rate of *T. cruzi*-infected mice (Fig. 3B). Most of the mice (10 of 15; $n = 3$) survived the acute phase of infection and entered the chronic phase. In contrast, only one of the infected mice survived after 3 weeks when the groups were injected either with 3T3 control fibroblasts (1 of 14; $n = 3$) or PBS (1 of 14; $n = 3$). These data indicate that a single injection of 3T3-CD40L transfectants protects most of the mice against fatal infection. However, injection of 400 μg of activating anti-CD40 MAb did not protect mice against *T. cruzi* infection (data not shown).

We also tested the ability of 3T3-CD40L transfectants to modify the course of an established infection. For this purpose, mice were inoculated with *T. cruzi* and injected with 3T3-CD40L transfectants or 3T3 control fibroblasts 8 days later. Treatment by 3T3-CD40L transfectants reduced the peak of parasitemia to 4.0×10^6 parasite/ml versus 9.2×10^6 parasites/ml in mice injected with control transfectants ($P < 0.05$ between day 20 and 29 p.i., Mann-Whitney U test). In parallel, the lethality rate was slightly reduced (on day 41, 14% of control mice survived versus 40% of the treatment group); however, this difference did not reach significance (χ^2 analysis).

The protective effect of CD40 ligation in *T. cruzi*-infected mice is related to up-regulation of IL-12. To assess the role of IL-12 in the protective effect of CD40 ligation in vivo, we first

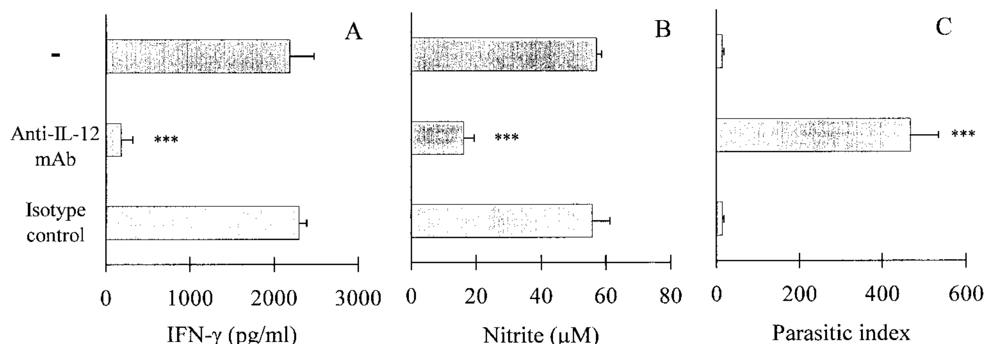

FIG. 2. Effect of neutralizing anti-IL-12 MAb added to CD40L-activated SC on their IFN-γ and NO production and parasitic index of *T. cruzi*-infected MPM. SC were activated with 3T3-CD40L transfectants in the presence of anti-IL-12 MAb, isotype-matched control, or medium for 48 h. IFN-γ levels were measured in culture supernatants after 48 h (A). Then, culture supernatants were transferred to *T. cruzi*-infected MPM. Nitrite levels (B) and parasitic index (C) were measured after 24 and 48 h, respectively. Data are means ± standard deviations from three independent experiments performed in duplicate. ***, $P < 0.001$ compared to data obtained with culture supernatants harvested from SC incubated with isotype-matched control (Student's *t* test).

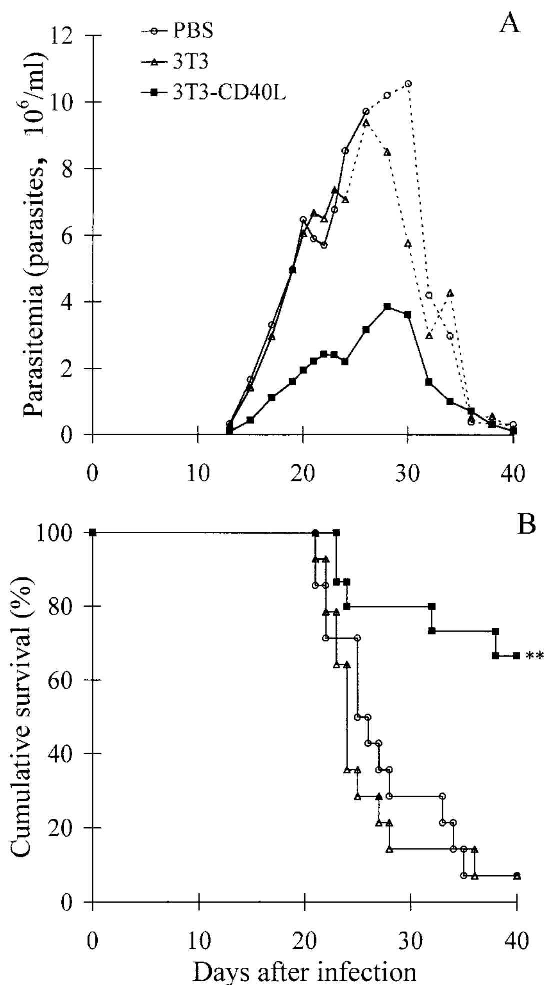

FIG. 3. Effect of injection of 3T3-CD40L transfectants in *T. cruzi*-infected mice. Three groups of mice were inoculated with *T. cruzi* and injected with either 3T3-CD40L transfectants, 3T3 control fibroblasts, or PBS. Parasitemia (A) and cumulative survival (B) are reported. Dotted lines represent the parasitemia when less than 50% of mice were still alive. Data were pooled from three independent experiments (n = 14 or 15 mice per group). For parasitemia, the difference between the experimental and control groups was significant ($P <$ 0.01, Mann-Whitney U test) for the period from day 15 to day 24 p.i. **, $P <$ 0.002 (χ^2 analysis).

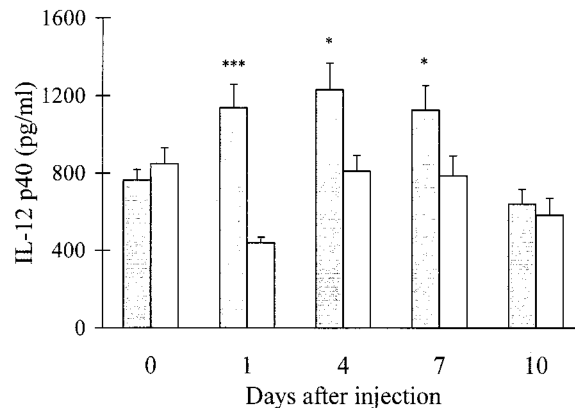

FIG. 4. Kinetics of IL-12 synthesis in serum of 3T3-CD40L transfectant-injected mice. IL-12 p40 levels were measured in blood samples obtained from mice injected with 3T3-CD40L transfectants (n = 10) (black bars) or 3T3 control fibroblasts (n = 8) (open bars). Data are means ± standard errors of the means. *, $P <$ 0.05; ***, $P <$ 0.01 compared to day 0 (Student's t test).

measured IL-12 p40 levels in the serum of 3T3-CD40L-injected mice. These experiments confirmed that CD40-CD40L interactions induce IL-12 synthesis in vivo (Fig. 4). We then evaluated the effect of IL-12 neutralization on the outcome of infection. For this, neutralizing anti-IL-12 MAb (or its isotype-matched control) was coinjected with 3T3-CD40L transfectants in *T. cruzi*-infected mice. Mortality follow-up showed that protection obtained with injection of 3T3-CD40L transfectants was prevented by IL-12 neutralization but not by injection of the isotype-matched control (Fig. 5). These data establish that CD40L-mediated protection in vivo depends on IL-12 release.

DISCUSSION

In the present study, we demonstrate that CD40 ligation leads to the control of *T. cruzi* infection through the induction of NO. Several studies have shown that CD40-CD40L interactions could result in NO production (32, 48, 50, 52). Our results indicate that this is achieved by inducing IL-12 production as well as by direct stimulation of IFN-γ-activated macrophages. In vitro, high levels of IL-12, IFN-γ, and TNF-α are produced by CD40L-activated SC. Supernatants from these SC cultures stimulate MPM, which become able to control *T. cruzi* infection through NO production. This NO production is inhibited when neutralizing anti-IL-12, anti-IFN-γ, or anti-TNF-α MAbs are added to the SC cultures with 3T3-CD40L transfectants. These data confirm that CD40-CD40L interactions among SC promote the synthesis of IL-12 (9, 27, 31), which in turn, induces IFN-γ secretion (10, 41, 44, 55). IFN-γ acts in synergy with TNF-α to stimulate the production of NO (17), resulting in parasite clearing. Finally, CD40 ligation also

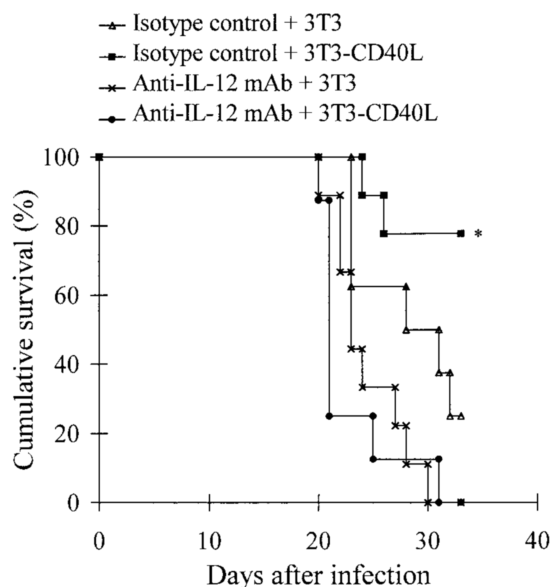

FIG. 5. Effect of injection of neutralizing anti-IL-12 MAb on *T. cruzi*-infected and 3T3-CD40L transfectant-treated mice. Mice (n = 10) were inoculated with *T. cruzi* trypomastigotes and injected with 3T3-CD40L transfectants or 3T3 control fibroblasts. Neutralizing anti-IL-12 MAb or isotype-matched control was injected intraperitoneally on the day of infection (day 0) and then on days 2 and 4 p.i. Cumulative survival was reported. *, $P <$ 0.05 (χ^2 analysis).

directly stimulates IFN-γ-activated MPM to produce NO and thereby to control *T. cruzi* infection. This cascade is probably operative in vivo as a single injection of 3T3-CD40L transfectants to mice at the time of *T. cruzi* inoculation exerts a clear-cut protective effect which is mediated by IL-12. We show also that a single injection of 3T3-CD40L transfectants even 8 days p.i. is still able to reduce parasitemia. This is in line with previous studies showing that treatment of *T. cruzi*-infected mice with anti-IL-12 MAb has an exacerbating effect on both parasitemia and mortality (3) while an exogenous supply of IL-12 protects mice (25).

Our results are also consistent with a recent study showing an IL-12-dependent protection in mice infected with *Leishmania* (a closely-related parasitic protozoa) and injected with anti-CD40-stimulating MAb (15). Furthermore, exacerbated *Leishmania* infection is observed when CD40-CD40L interactions are disrupted (6, 23, 26, 48). Likewise, a CD40-CD40L-mediated protective effect is also observed with other pathogens such as *Cryptosporidium parvum* (12) and *Pneumocystis carinii* (59). In contrast, this is not the case with other pathogens such as *Borrelia burgdorferi* (16), *Listeria monocytogenes* (21), *Mycobacterium tuberculosis* (7), and *Histoplasma capsulatum* (60). This discrepancy could be explained by differential abilities of infectious agents to induce IL-12 production by the host (7, 60). CD40-CD40L interaction would be determinant only when IL-12 production induced by pathogens is insufficient, as has been shown in the course of *Leishmania* infection (5).

3T3-CD40L transfectants are found to be more efficient than activating anti-CD40 MAb in improving parasite control, and this data is in agreement with previous observations obtained with B cells (30). This is most likely due to a more efficient cross-linking of CD40 molecules by CD40L expressed at high density at the fibroblast membrane compared with the agonistic anti-CD40 IgG MAb.

CD40L stimulating properties have already been used in the treatment of tumors, tumor regression being linked to restoration of major histocompatibility complex class I expression by tumor cells (18, 28, 36), IL-12 overproduction, or potentiation of host antigen-presenting cell functions. Moreover, CD40 engagement restores, at least in vitro, production of IL-12 by cells from human immunodeficiency virus (HIV)-infected patients and it stimulates macrophages to produce HIV-1-suppressive chemokines (11, 29). According to our results, activation of the immune system through CD40 ligation could also be considered a potent strategy for immunotherapy of parasitic diseases.

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