# CCR5 Plays a Critical Role in the Development of Myocarditis and Host Protection in Mice Infected with *Trypanosoma cruzi*

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**The pathogenesis of myocarditis during *Trypanosoma cruzi* infection is poorly understood. We investigated the role played by chemokine receptor 5 (CCR5) in the influx of T cells to the cardiac tissue of *T. cruzi*–infected mice. mRNA and protein for the CCR5 ligands CCL3, CCL4, and CCL5 were detected in the hearts of infected mice in association with $CD4^+$ and $CD8^+$ T cells. There was a high level of CCR5 expression on $CD8^+$ T cells in the hearts of infected mice. Moreover, CCR5 expression on $CD8^+$ T cells was positively modulated by *T. cruzi* infection. CCR5-deficient mice infected with *T. cruzi* experienced a dramatically inhibited migration of T cells to the heart and were also more susceptible to infection. These results suggest that CCR5 and its ligands play a central role in the control of T cell influx in *T. cruzi*–infected mice. Knowledge of the mechanisms that trigger and control the migration of cells to the heart in patients with Chagas disease may help in the design of drugs that prevent myocarditis and protect against the development of severe disease.**

*Trypanosoma cruzi* is an obligatory intracellular protozoan parasite that causes Chagas disease, an important public-health problem in Latin America. In studies of infection, there is significant blood parasitemia, tissue parasitism, and tissue inflammation and a great degree of lethality, all of which are dependent on the strain of the parasite, the dose of the inoculum, and the species being studied. Control of the infection is eventually achieved. Several studies have reported an essential role for $CD4^+$ [1–3] and $CD8^+$ [3, 4] T cells and for interferon (IFN)–$\gamma$ and tumor necrosis factor (TNF)–$\alpha$ [5–8] in the control of acute infection. However, it is still not known how these types of cells migrate to sites of tissue infection and interact with inflammatory mediators at these sites to mediate the control of parasite replication.

We recently reported that *T. cruzi*–infected macrophages and cardiomyocytes expressed mRNA for a range of chemokines, including CCL3 (macrophage inflammatory protein [MIP]–1$\alpha$), CCL4 (MIP-1$\beta$), and CCL5 (RANTES) [9, 10]. In these studies, not only were these chemokines expressed, but *T. cruzi*–infected macrophages and cardiomyocytes also expressed NO, to mediate NO-dependent killing in vitro [9–13]. Moreover, we showed that mRNA for these chemokines was also expressed during acute infection in mice and that cytokines (i.e., TNF-$\alpha$ and IFN-$\gamma$) known to participate in the control of infection modulated the expression of chemokines [14–16]. Given that chemokines are well known for their ability to induce the migration of leukocytes and the activation of immune cells, these previous studies suggested that, in addition to facilitating the production of NO, the expression of chemokines could drive the migration of inflammatory and immune cells to sites of parasite infection.

The biological activities of chemokines are mediated by their interactions with cell-surface receptors that belong to the structurally related 7-transmembrane-

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domain superfamily of proteins [17]. CCR5 mediates the activity of the chemokines CCL3, CCL4, and CCL5, whose mRNA is found in vitro after infection of macrophages and cardiomyocytes with *T. cruzi* [9, 10] and in vivo after infection of macrophages and cardiomyocytes with the Colombiana strain [15]. As such, these chemokines may have a relevant role in driving the selective migration of $CCR5^+$ immune cells toward affected tissues. Here, we confirm the expression of CCL3, CCL4, and CCL5 in mice infected with the Y strain of *T. cruzi*. Moreover, using CCR5-deficient ($CCR5^{-/-}$) mice, we evaluate the role played by the interaction between CCR5 and its ligands in protection against *T. cruzi* infection and in the development and maintenance of myocarditis.

## MATERIALS AND METHODS

***Mice.*** A breeding pair of mice with disruption of the CCR5 gene [18] was obtained from Jackson Laboratories. Breeding stock backcrossed on C57BL/6 was obtained, and the genotype of CCR5 in mice was determined by polymerase chain reaction (PCR) of DNA [19]. Female C57BL/6 wild-type *(wt)* and $CCR5^{-/-}$ mice, 6–8 weeks old, were bred and maintained in microisolator cages. All procedures were in accordance with international guidelines for the use of animals and received prior approval by the animal ethics committee of the School of Medicine of Ribeirão Preto.

***Parasites and experimental infection.*** The Y strain of *T. cruzi* was used in all experiments. For in vitro experiments, trypomastigote forms were grown and purified from a fibroblast cell line (LLC-$MK_2$). In all experiments, the mice were inoculated intraperitoneally with $10^3$ blood-derived trypomastigote forms, which had been obtained from previously infected mice.

***Total RNA extraction and cDNA preparation by reverse-transcription (RT) PCR.*** Total RNA was isolated from the cardiac tissue of mice by use of Trizol LS (Life Technologies) reagent, in accordance with the manufacturer's instructions. Briefly, the samples were homogenized, and 0.2 mL of chloroform (Sigma) was added to each 1 mL of Trizol LS reagent. The samples were then centrifuged at 12,000 *g* for 15 min at 4°C, and the aqueous phase was transferred to a clean tube. The same volume of isopropyl alcohol was added, and the samples were mixed in a vortex and incubated for 15 min at −20°C, to precipitate the RNA from the aqueous phase. After further centrifugation, the RNA pellets were washed in 75% ethanol, and the samples were suspended in water at a concentration of 0.5 $\mu$g of RNA/$\mu$L. cDNA was synthesized by use of Superscript II reverse transcriptase (Gibco).

***Chemokine mRNA detection.*** The expression of mRNA for chemokines (CCL3, CCL4, and CCL5) and $\beta$-actin was analyzed by RT-PCR, which was performed with *Taq* polymerase (Gibco) in a PTC-100 thermal cycler (MJ Research). The reaction conditions were 35 cycles of 1 min at 94°C, 1 min at 54°C, and 2 min at 72°C, with a final extension step of 7 min at 72°C. For each set of primers, a negative sample (water) was run in parallel. The PCR products were separated by acrylamide gel electrophoresis and stained with silver nitrate. The primer sequences and PCR product sizes have been published elsewhere [9].

***Spleen-cell cultures.*** Suspensions of splenocytes from uninfected mice were washed in Hanks' balanced salt solution (HBSS) and treated with lysis buffer (9 parts 0.16 mol/L ammonium chloride and 1 part 0.17 mol/L Tris-HCl [pH 7.5]) for 4 min. The erythrocyte-free cells were then washed 3 times in HBSS and adjusted to $3 \times 10^6$ cells/mL of RPMI 1640 (Flow Laboratories) supplemented with 10% fetal calf serum (Hyclone), 2-mercaptoethanol ($5 \times 10^{-5}$ mol/L), L-glutamine (2 mmol/L), and antibiotics (all from Sigma). The cell suspension was distributed (1 mL/well) in 24-well tissue-culture plates (Corning) and cultured for 40 h at 37°C in a humidified 5% $CO_2$ atmosphere, in the presence or absence of trypomastigote forms of *T. cruzi* (5 parasites/cell) or concanavalin A (ConA; 2 $\mu$g/mL).

***Isolation of inflammatory cells from cardiac tissue of* T. cruzi*–infected mice.*** To isolate mononuclear cells from myocardia, 15–20 mouse hearts removed 17 days after infection were washed (to remove blood clots), pooled, minced with scissors into 1–2-mm fragments, extensively washed, and subjected to enzymatic digestion with a solution containing 0.05% trypsin (Gibco) and 0.01% collagenase type II (Worthington) for 10 min at 37°C. The cell suspension was spun, the supernatant was removed, and the pellet was suspended in 2 mL of 100% Percoll (Pharmacia). Aliquots (2 mL) of Percoll (70% and 40%) were added to the top of the cell suspension, and the suspension was centrifuged at 1500 *g* for 30 min. The leukocytes (between the 70% and 40% fractions) were removed and washed with RPMI 1640 (5%), and their phenotypes were evaluated by flow cytometry.

***Flow-cytometry analysis.*** Both inflammatory cells obtained from the cardiac tissue of *T. cruzi*–infected mice and splenocytes were stained with fluorescein isothiocyanate (FITC)– or phosphatidylethanolamine-labeled antibodies against CD3, CD4, CD8, CCR5 (Pharmingen), or CCR3 (Santa Cruz Biotechnology) molecules. Viable cells ($3 \times 10^5$) were analyzed by use of a flow cytometer and CellQuest software (version 3.3; Becton Dickinson).

***Histological evaluation.*** Groups of 3–4 mice were killed on different days after infection with *T. cruzi*. Hearts were fixed in neutral 10% formalin, embedded in paraffin, sectioned, stained with hematoxylin-eosin, and examined by light microscopy.

***Immunohistochemical analysis.*** Cardiac tissues of mice were removed on days 17 and 20 after infection with *T. cruzi*, placed in compound-embedding medium (Sakura Finetek),

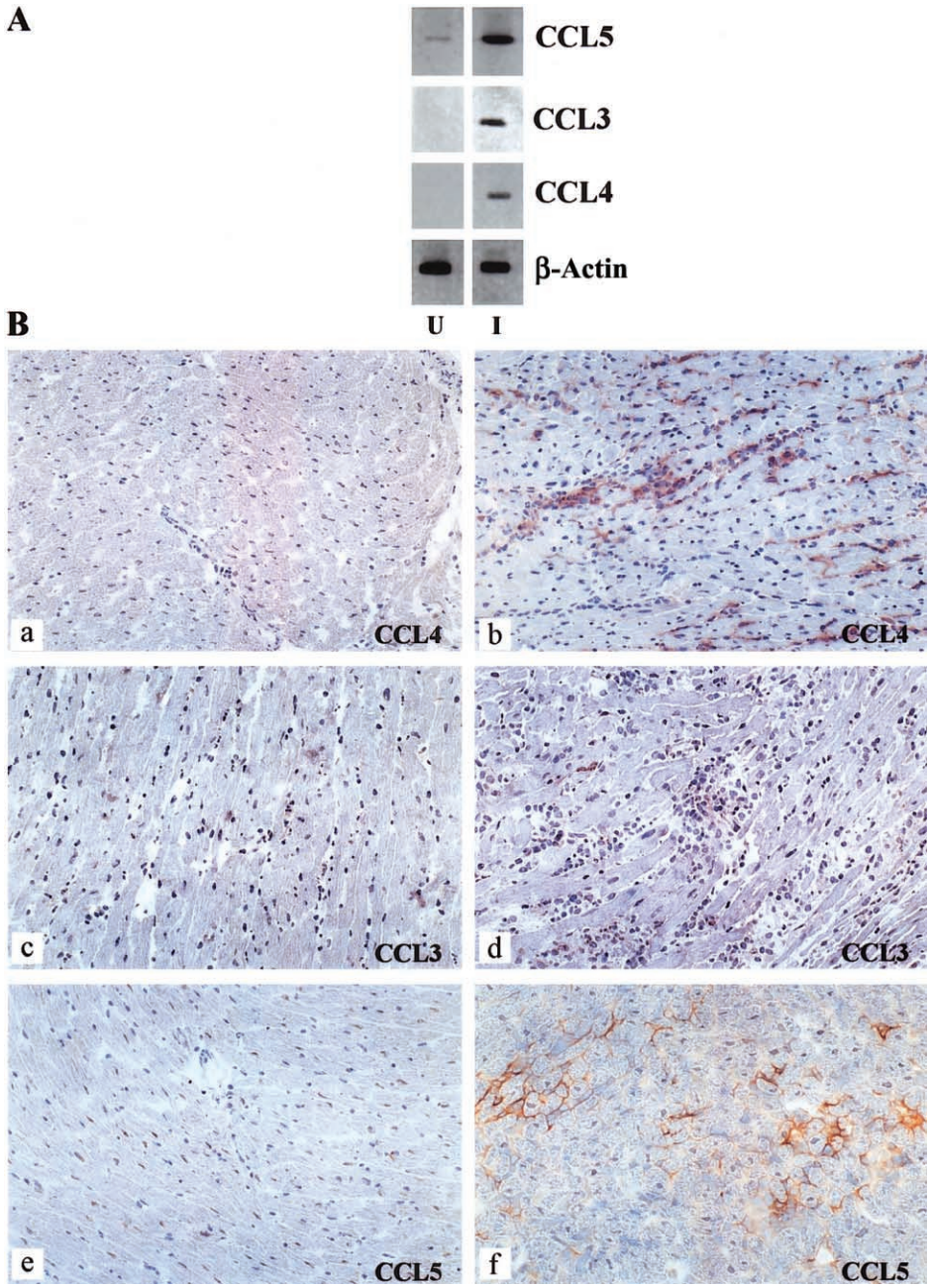


**Figure 1.** Expression of CCL3, CCL4, and CCL5 in the myocardia of *Trypanosoma cruzi*–infected mice. *A,* Total myocardial RNA was extracted from uninfected (U) and infected (I) mice on day 15 after infection, and expression of CCL3, CCL4, CCL5, and *β*-actin was assessed by reverse-transcription polymerase chain reaction (PCR). The PCR products were electrophoresed in polyacrylamide gels and stained with silver nitrate. *B,* Hearts of uninfected *(a, c,* and *e)* and infected *(b, d,* and *f)* mice on day 20 after infection with 1000 trypomastigote forms of *T. cruzi* were prepared for immunohistochemical analysis, and the presence of CCL4 *(a* and *b),* CCL3 *(c* and *d),* and CCL5 *(e* and *f)* were evaluated by use of the immunoperoxidase method (see Materials and Methods). The presence of the chemokines was revealed by use of diaminobenzidine tetrahydrochloride as the substratum for the peroxidase, which generated a brown coloration. Results shown are representative of 3 different experiments. Original magnification for all micrographs, ×200.

snap-frozen by use of liquid nitrogen, and stored at −70°C. Ten-micrometer sections were prepared in a cryostat, collected on polylysine-coated slides, fixed with cold acetone, and allowed to air dry.

The endogenous peroxidase activity was blocked by use of 3% hydrogen peroxide for 20 min, and nonspecific binding was blocked by use of goat serum (diluted 1:200 in PBS) for 45 min. The slides were washed and incubated overnight with

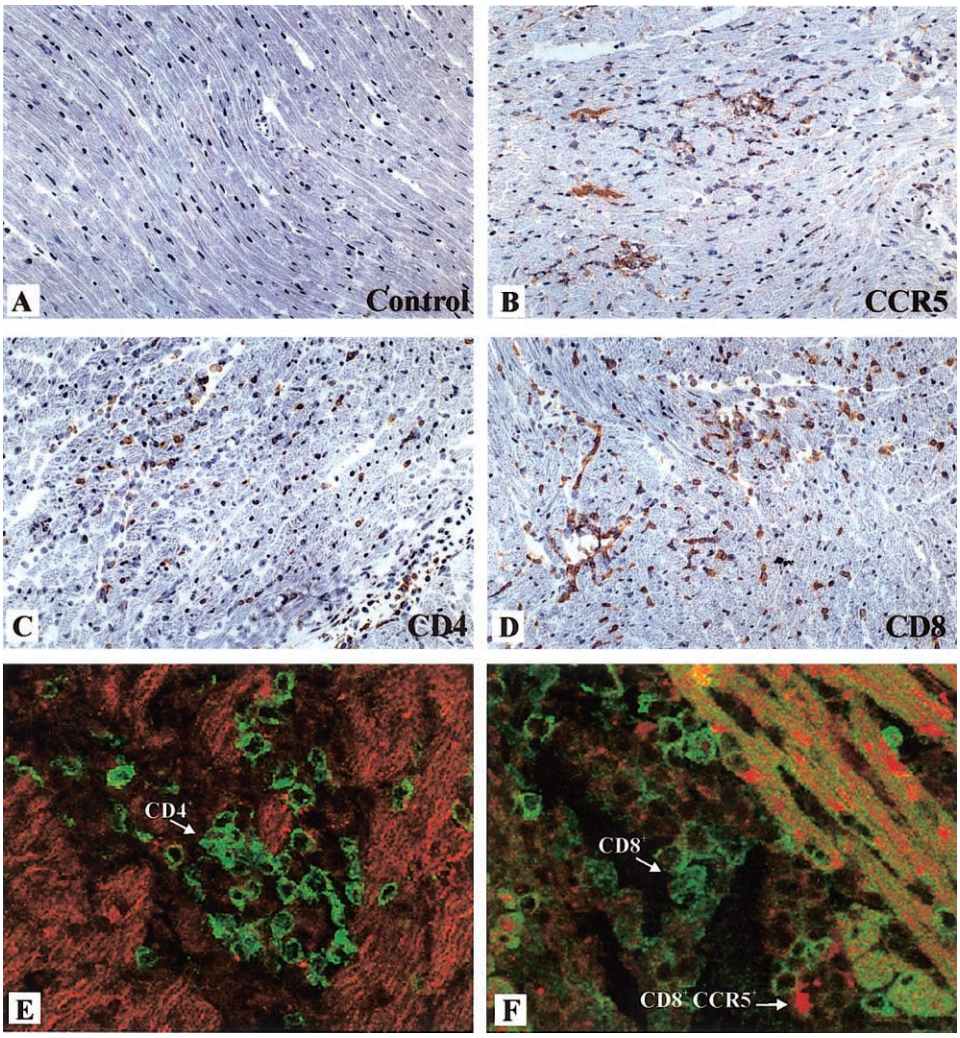


**Figure 2.** Expression of CCR5, CD8, and CD4 in the myocardia of uninfected or *Trypanosoma cruzi*–infected mice. Hearts of uninfected *(A)* or infected *(B–F)* mice on day 17 after infection with 1000 trypomastigote forms of *T. cruzi* were obtained and prepared for analysis by immunohistochemistry and immunofluorescence. Expression of CCR5 *(A* and *B),* CD4 *(C),* and CD8 *(D)* in myocardia was evaluated by use of the immunoperoxidase method (see Materials and Methods). Diaminobenzidine tetrahydrochloride was used as the substratum for the peroxidase, which generated a brown coloration. Panels *E* and *F* show the results of double immunofluorescence labeling, in which CD4 *(E)* and CD8 *(F)* were stained with fluorescein-labeled specific antibody and CCR5 *(E* and *F)* was stained with antibody conjugated with Texas red. Original magnification for all microphotographs, ×200.

goat anti–mouse MIP-1$\alpha$, MIP-1$\beta$, RANTES, or CCR5 (Santa Cruz Biotechnology) or with rat anti–mouse CD4 or CD8 (Pharmingen) diluted 100 times in PBS–1% bovine serum albumin (BSA). After extensive washes and incubation for 30 min with biotin-labeled rabbit anti–goat or goat anti–rat antibody (Pharmingen), the reaction product was detected by use of avidin-biotin–peroxidase complex (Vector Laboratories), the color of the reaction was developed by use of diaminobenzidine tetrahydrochloride (DAB; Sigma), and the reaction was counterstained with Mayer's hematoxylin. Controls were performed by incubation of cells with nonimmune goat or rat IgG.

***Immunofluorescent staining and confocal analysis.*** Cryostat sections obtained and fixed as described above were incubated overnight at 4°C with anti–CD16/CD32 monoclonal antibody (MAb; Pharmigen) in PBS–1% BSA and then were blocked with avidin-biotin. Sections were incubated with the appropriate dilutions of rat anti–mouse MAb against CD4 and CD8 (labeled with FITC) and biotinylated anti-CCR5 or normal rat IgG for 1 h. Immunostaining for CCR5 was detected by incubation with streptavidin–Texas red (Pharmigen) for 1 h. Between each incubation step, slides were washed 3 times in PBS and mounted in aqueous medium. The slides were analyzed by fluorescent microscopy (Leica), and the images were processed by use of Adobe Photoshop software (version 7.0).

***Statistical analysis.*** Results are expressed as the mean ± SE of the triplicate cultures or experiments. Statistical analysis

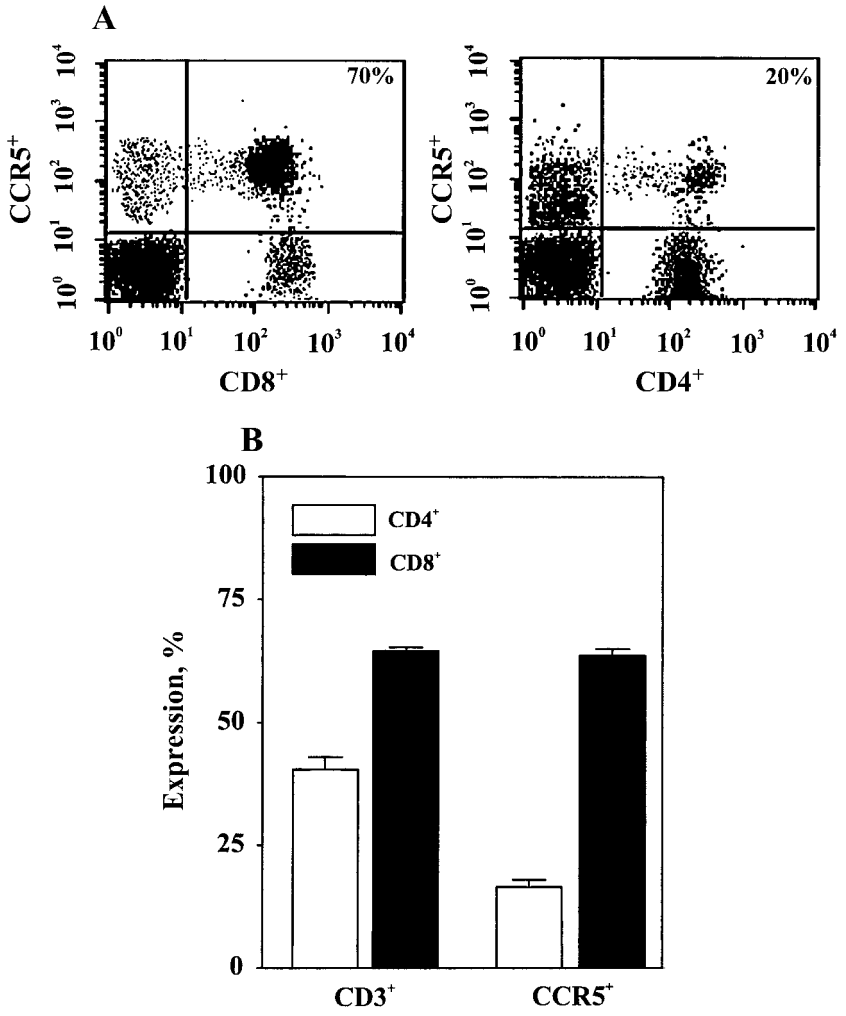


**Figure 3.** Predominance of $CCR5^+CD8^+$ T cells in the myocardia of *Trypanosoma cruzi*–infected mice. C57BL/6 mice were infected with 1000 trypomastigote forms; 17 days after infection, hearts were harvested and subjected to chemical (2750 U/mL of colagenase) and mechanical (shaking) dissociation of the cells. The lymphocytes were isolated by use of Percoll gradient; were identified by use of antibodies against CD4, CD8 (fluorescein isothiocyanate labeled), CD3, and CCR5 (phosphatidylethanolamine labeled); and were subjected to flow-cytometry analysis. *A,* An example of the dot plots obtained. *B,* Percentages of $CD4^+$ *(white bars)* and $CD8^+$ *(black bars)* T cells that expressed CD3 and CCR5. Each bar represents the mean ± SD of triplicate samples and is representative of 3 separate experiments.

was performed by use of analysis of variance, followed by the Student-Newman-Keuls test (InStat software; GraphPad). $P < .05$ was considered to be statistically significant.

## RESULTS

***Detection of CCL3, CCL4, and CCL5 in the myocardia of* T. cruzi*–infected mice.*** Detection of mRNA for CCL3, CCL4, and CCL5 in cardiomyocytes from mice infected with *T. cruzi* has been previously reported [10]. Here, we examined whether these chemokines were also expressed in the cardiac tissue of mice infected with *T. cruzi*. No detectable expression of CCL3 and CCL4 mRNA and only a very low expression of CCL5 mRNA were found in the myocardia of uninfected *wt* mice. Infection with *T. cruzi* resulted in significant mRNA for CCL3 and CCL4 and enhanced mRNA for CCL5 in the myocardia on day 15 after infection (figure 1*A*). Moreover, CCL3, CCL4, and CCL5 were also detected by immunohistochemistry in the myocardia of infected mice but not in the myocardia of uninfected mice (figure 1*B*). Presence of the chemokines was clearly associated with infiltrated cells in cardiac tissue. As shown in figure 1*B*, there was a mononuclear interstitial infiltrate in the myocardia of infected mice that was positive for the presence of CCL3, CCL4, and CCL5 chemokines. The staining was most intense in the cardiac tissue obtained on days 17–20 after infection. In the myocardia of uninfected mice, no

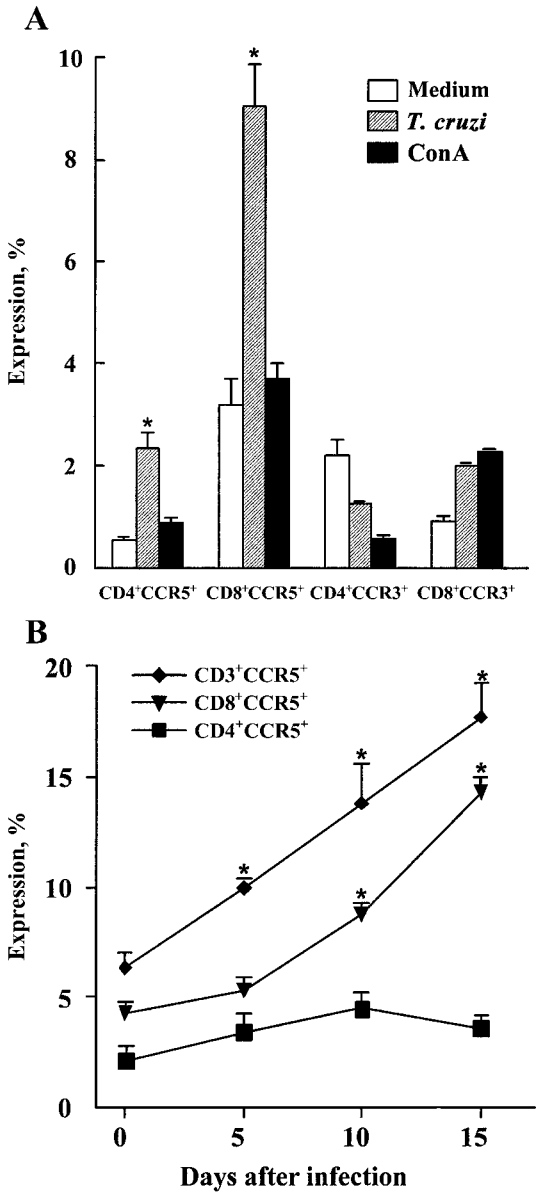


**Figure 4.** Induction of CCR5 expression in lymphocytes by trypomastigote forms of *Trypanosoma cruzi*. CCR5 expression was determined in *T. cruzi*–cultured T cells from C57BL/6 mice *(A)* and in T splenocytes from mice experiencing the acute phase of infection *(B)*. In panel *A*, the spleen cells were cultured with medium only, trypomastigote forms of *T. cruzi* (5 parasites/cell), or concanavalin A (ConA; 2 $\mu$g/mL). The cells were harvested; labeled with antibodies specific for CCR5, CCR3, CD4, and CD8; and subjected to flow-cytometry analysis. In panel *B*, CCR5 expression was determined by flow-cytometry analysis in T splenocytes from uninfected or infected mice on days 5, 10, and 15 after infection with 1000 trypomastigote forms of *T. cruzi*. Each point represents the mean ± SD of triplicate samples and is representative of 3 separate experiments. *$P < .05$, compared with the values obtained in the absence of *T. cruzi (A)* or with the values obtained for cells from uninfected mice *(B)*. Statistical analysis was performed by use of analysis of variance, followed by the Student-Newman-Keuls test.

inflammatory infiltrate was present, and no staining for CC chemokines was observed.

***Phenotype of inflammatory cells in the cardiac tissue of* T. cruzi*–infected mice.*** Because CCL3, CCL4, and CCL5 bind to CCR5 on the surface of leukocytes, we examined whether the inflammatory cells in the myocardia of infected mice expressed CCR5. These experiments showed that there were very few $CD4^+$ and $CD8^+$ T cells in uninfected mice (figure 2*A*) and that the number of these cells increased markedly after infection (figure 2*C* and 2*D*). When we examined mouse cardiac tissue obtained during the acute phase of infection, we found that the inflammatory infiltrate was predominantly composed of $CD8^+$ T cells (figure 2*C* and 2*D*). The quantification of T cells infiltrating the cardiac tissue (figure 3*A*) revealed that the majority of $CD3^+$ T cells in the cardiac tissue of infected mice were also $CD8^+$ (figure 3*B*). Other cells found in cardiac tissue expressed CD11c (11%), CD14 (7%), and CD56 (20%). Expression of CCR5 was found to be positive in the majority of leukocytes in inflammatory infiltrate (figure 2*B*), indicating that a significant amount of $CD8^+$ T cells expressed CCR5. Indeed, double immunofluorescence labeling revealed that the majority of $CD8^+$ T cells (figure 2*F*), but not of $CD4^+$ T cells (figure 2*E*), expressed CCR5. These data were confirmed by flow-cytometry analysis, which showed that 70% of $CD8^+$ and 20% of $CD4^+$ T cells that were isolated from myocardia during acute infection expressed CCR5 (figure 3*A* and 3*B*).

***Inducement of enhanced expression of CCR5 in lymphocytes by infection with* T. cruzi.** Because there was a high expression of CCR5 in T cells found in cardiac tissue, we evaluated whether the parasites could modulate the expression of CCR5 in lymphocytes. Splenocytes were incubated with trypomastigote forms or with ConA, and the expression of CD4, CD8, CCR5, and CCR3 was evaluated. As shown in figure 4*A*, incubation with trypomastigote forms, but not with ConA, induced a significant enhancement of the expression of CCR5 in $CD4^+$ and $CD8^+$ T cells. Incubation of splenocytes with the parasites or ConA did not alter the expression of CCR3 (figure 4*A*). These results show that incubation of splenocytes with trypomastigote forms of *T. cruzi* for 40 h results in enhanced expression of CCR5 on the surface of murine T cells.

We found a linear increase in the expression of CCR5 on T cells after infection, on the basis that the increase was significant as soon as 5 days after infection (figure 4*B*). The increased expression of CCR5 was almost exclusively on $CD8^+$ T cells; expression on $CD4^+$ T cells was not significantly increased (figure 4*B*). These data indicate that *T. cruzi* is able to induce expression of CCR5 on $CD8^+$ T splenocytes.

***Greater susceptibility of CCR5$^{-/-}$ mice to infection with* T. cruzi*, which mediates the cell influx to cardiac tissue.*** To investigate the relevance of CCR5 to *T. cruzi* infection, $CCR5^{-/-}$ mice were infected with *T. cruzi*. The results showed a significantly

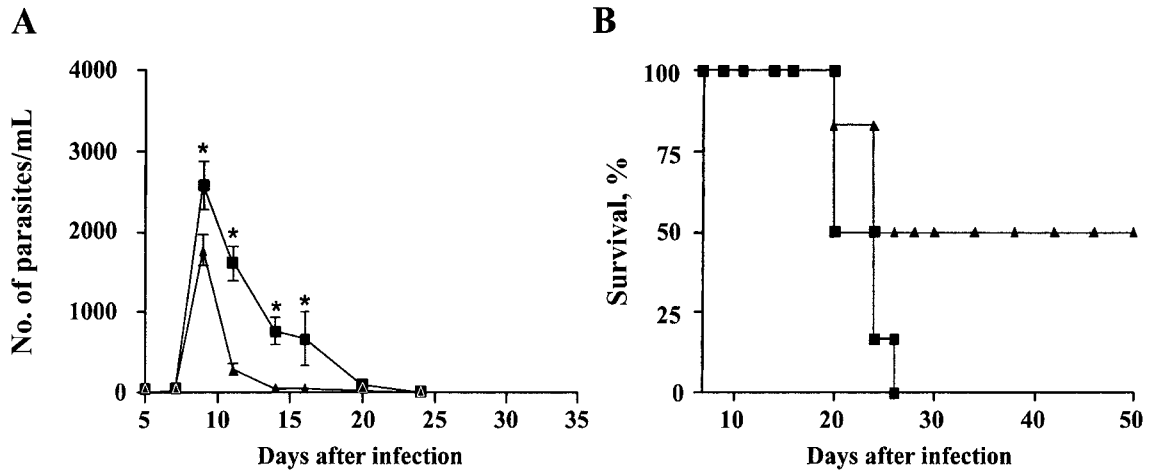


**Figure 5.** Natural course of *Trypanosoma cruzi* infection, as measured by parasitemia *(A)* and survival rate *(B)* of wild-type *(wt)* (▲) and CCR5-deficient ($CCR5^{-/-}$) (■) mice infected with 1000 trypomastigote forms of *T. cruzi* strain Y. $CCR5^{-/-}$ mice were found to be more susceptible to *T. cruzi* infection than *wt* mice. In panel *A,* the mean ± SD no. of parasites per microliter of blood for 1 of 4 representative experiments (10 mice/group) is shown. $*P < .05$, for the comparison of $CCR5^{-/-}$ vs. C57BL/6 *wt* mice infected with *T. cruzi.*

increased parasitemia during the acute phase of infection in $CCR5^{-/-}$ mice (between days 7–15 after infection) (figure 5*A*). Moreover, although the mortality of *wt* mice was 50% on day 26 after infection, all $CCR5^{-/-}$ had died by that day ($P < .05$) (figure 5*B*). Interestingly, the intensity and kinetics of diffuse and focal inflammatory infiltrates were different in the cardiac tissue of $CCR5^{-/-}$ and *wt* mice infected with the parasite. Although, on day 20 after infection, significant inflammatory infiltrates were found in the myocardia of *wt* mice infected with *T. cruzi* (figure 6*C*), these were almost absent in the myocardia of $CCR5^{-/-}$ mice infected with *T. cruzi* (figure 6*D*). Instead, we found very large amounts of parasite nests (figure 6*D*), such that in several microscopy fields the amounts of parasites in the tissue were virtually uncountable. Also noticeable was the finding that the sizes of parasite nests in $CCR5^{-/-}$ mice were bigger than those in *wt* mice. In contrast, rare, isolated parasite nests were found in the cardiac tissue of *wt* mice (figure 6*C*). Absence of cell infiltrate was found in uninfected *wt* (figure 6*A*) and $CCR5^{-/-}$ (figure 6*B*) mice.

## DISCUSSION

It is now clear that IFN-γ– and TNF-α–activated macrophages control of the replication of *T. cruzi* in various hosts [20]. The mechanism by which macrophages are able to kill intracellular parasites has been shown to be dependent on NO [8–13, 20], although a recent study using *T. cruzi* strains Tulahuen and Brazil suggested that inducible NO synthase (iNOS) is not required to control parasite replication in vivo [21]. However, much less is known about the mechanisms governing the migration of leukocytes to sites of tissue infection, especially to hearts of infected hosts. More recently, a group of mediators, the chemokines, have been described in some detail and have been shown to participate in the recruitment of leukocytes in several models of inflammatory diseases [14, 22–24]. In the present study, we demonstrate an important role for CCR5-acting chemokines in the control of leukocyte migration and parasite replication during the acute phase of infection with the Y strain of *T. cruzi.* This strain induces a parasitemia that peaks around days 9–11 of infection and causes an intense inflammatory reaction in cardiac tissue that is more intense between days 13 and 20 [25].

Infection of macrophages [9] and cardiomyocytes [10] with *T. cruzi* trypomastigote forms is accompanied by the significant production of several chemokines, including CCL3, CCL4, and CCL5. Not only can the latter cells produce chemokines, but chemokines can also act on the cells to induce iNOS expression, NO expression, and the killing of parasites [9–13]. Thus, in the present study, it was important to ascertain whether chemokine expression would also occur in vivo after infection of mice with the Y strain of *T. cruzi.* Indeed, both mRNA and protein for CCL3, CCL4, and CCL5 could be found in the cardiac tissue of *T. cruzi*–infected mice, in close correlation with tissue inflammation; these results are in agreement with the findings of other studies [14–16]. Notably, these latter studies showed that the expression of chemokines was greatly under the control of IFN-γ and that the modulation of chemokine expression was accompanied by the modulation of the recruitment of leukocytes to sites of *T. cruzi*–induced inflammation [26]. Altogether, these studies and the present data demonstrate the close correlation between the expression of chemokines and the development of tissue inflammation after *T. cruzi* infection in vivo.

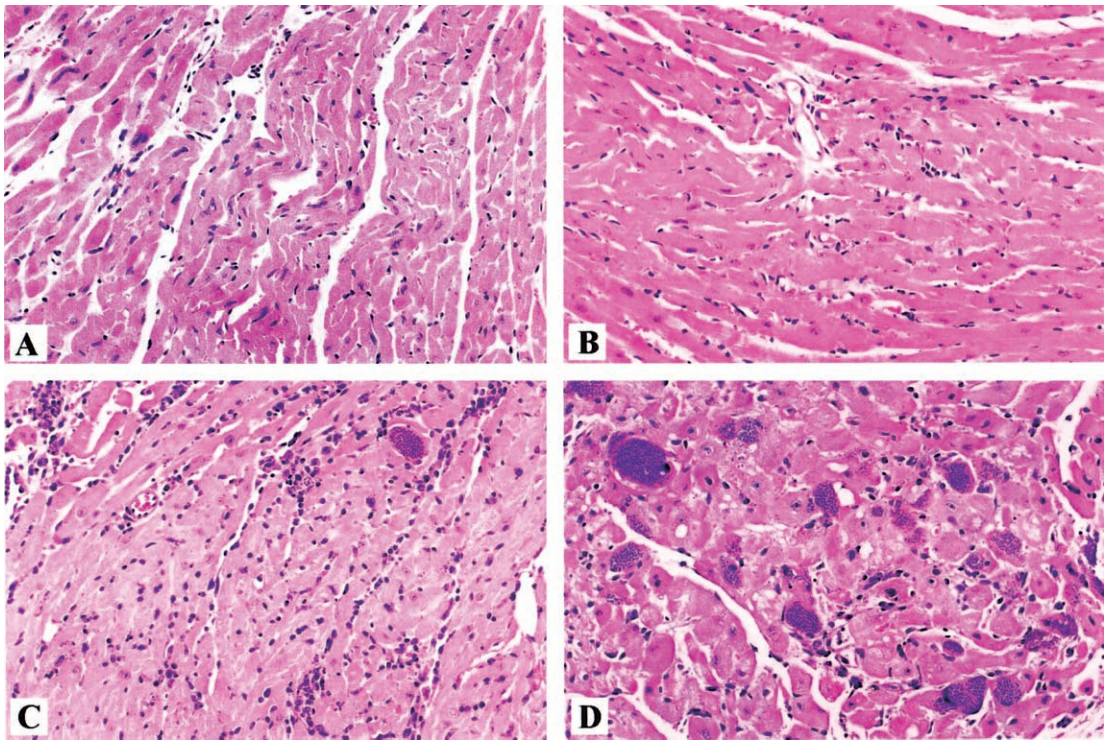


**Figure 6.** Inflammatory infiltrate and parasite nests in wild-type *(wt)* and CCR5-deficient ($CCR5^{-/-}$) mice. Histological sections of cardiac tissue from *wt (A* and *C)* and $CCR5^{-/-}$ *(B* and *D)* mice that were either uninfected *(A* and *B)* or infected *(C* and *D)* with 1000 trypomastigote forms of *Trypanosoma cruzi* were obtained and stained with hematoxylin-eosin. Hearts were harvested on day 20 after infection. Note the absence of inflammatory cells and the increased no. of parasite nests in the cardiac tissue of $CCR5^{-/-}$ mice. Original magnification for all microphotographs, ×200.

Not only was the expression of chemokines enhanced, but the expression of CCR5, a receptor for CCL3, CCL4, and CCL5, was also enhanced on infiltrating leukocytes in the hearts of *T. cruzi*–infected mice. More specifically, there was a marked increase in the number of $CD8^+$ T cells expressing CCR5, as assessed by both immunohistochemistry and flow-cytometry analysis. The number of $CD4^+$ T cells expressing CCR5 in the cardiac tissue of infected mice also increased, but to a lesser extent. Interestingly, the increased expression of CCR5 on tissue lymphocytes was mirrored by a significant increase in the expression of this receptor on circulating lymphocytes. Indeed, infection with *T. cruzi*, but not stimulation with ConA, also enhanced the expression of CCR5 on both $CD4^+$ and $CD8^+$ T cells cultivated in vitro. These results are in agreement with those of a recent study that showed that both $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells obtained from the peripheral blood of infected patients expressed CCR5 to a greater degree than did those obtained from uninfected individuals [27]. In the latter study, the enhanced CCR5 expression on T cells was lost only when heart failure supervened chronic Chagas disease. Thus, infection with *T. cruzi* was accompanied by a parasite-associated increase in the expression of CCR5 and migration of T cells to infected tissues, in which several CCR5-acting chemokines are produced. The increased expression of CCR5 could be due to the production of IFN-$\gamma$ [28, 29], TNF-$\alpha$ [8, 30], interleukin (IL)–10 [7, 31], and/or IL-12 [30, 32, 33] that occurs after the addition of trypomastigote forms of *T. cruzi* to cultures of splenocytes or after the infection of mice. Indeed, CCR5 expression is positively regulated by these cytokines [34–37].

The role of CCR5 in the control of *T. cruzi* infection and in the disease associated with the infection was evaluated in $CCR5^{-/-}$ mice. In these mice, a higher level of parasitemia and greater mortality was observed, compared with those in *T. cruzi*–infected *wt* mice. Moreover, the amount of parasites in the cardiac tissue of $CCR5^{-/-}$ mice was greatly enhanced. The reduced control of parasite replication was associated with a dramatic reduction of the migration of T cells and overall disease, indicating that CCR5 was crucial to the migration of lymphocytes to the hearts of infected mice. Importantly, there was no defect in the ability of macrophages from $CCR5^{-/-}$ mice to produce NO and IL-12 or to kill the parasite in vitro (data not shown). Similar to our results in intracellular adhesion molecule–1–deficient mice [38], the decreased number of lymphocytes in cardiac tissue was associated with decreased local production of IFN-$\gamma$ (data not shown) and an increased level of parasitemia, suggesting that there was a defect in the migration of cells capable of producing cytokines (such as IFN-$\gamma$) that activate the production of NO. Thus, the lack of CCR5

is accompanied by the inability of $CCR5^+$ T cells to migrate to the hearts of infected mice and the inability of the host to control parasite replication. Similarly, absence of CCR5 leads to increased susceptibility to *Cryptococcus neoformans* infection, because of the absence of the migration of cells to the brain [18]. On the contrary, a defective trafficking of leukocytes in $CCR5^{-/-}$ mice has been associated with (1) resistance to the development of cerebral malaria in mice infected with *Plasmodium berghei* [39] and (2) demyelination after infection with mouse hepatitis virus [40].

$CD8^+$ T cells compose the great majority of cells observed in the inflammatory infiltrate in the hearts of patients with chagasic cardiomyopathy [41–44]. These cells may play a role in the damage to the heart that is observed during the chronic phase of disease [41–44]. Indeed, cardiac lesions observed during the chronic phase of infection with *T. cruzi* have been associated with the local inflammatory response that is driven by host immune cells [45, 46]. It is not known, however, whether the $CD8^+$ T cells that infiltrate the hearts of patients with Chagas disease also express CCR5 or whether there is a functional relevance for the observed increase of CCR5 expression on the $CD8^+$ T cells that are obtained from the peripheral blood of patients with Chagas disease [27]. A role for CCR5 in human disease has also been suggested by a study that showed that a point mutation of the CCR5 promoter was significantly increased in asymptomatic patients, compared with that in patients with chagasic cardiomyopathy [47]. It is clear that further studies are necessary to understand the role played by CCR5 in the pathogenesis of Chagas disease.

In summary, our results demonstrate that CCR5-acting chemokines are expressed in the hearts of *T. cruzi*–infected mice and that CCR5 is most relevant to the control of the lymphocyte migration and parasite replication. Future studies should be conducted to evaluate whether CCR5 is also relevant to the pathophysiology of chronic infection and whether the blockade of CCR5 could be a viable target for the prevention of the migration of lymphocytes to inflamed cardiac tissue.

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