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Intercellular Adhesion Molecule 1 Deficiency Leads to Impaired Recruitment of T Lymphocytes and Enhanced Host Susceptibility to Infection with *Trypanosoma cruzi*¹

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In this study, we investigated the involvement of Th1 cytokines in the expression of cell adhesion molecules (CAM) and recruitment of inflammatory cells to the heart of mice infected with *Trypanosoma cruzi*. Our results show that endogenously produced IFN-γ is essential to induce optimal expression of VCAM-1 and ICAM-1 on the cardiac vascular endothelium of infected mice. Furthermore, the influx of inflammatory cells into the cardiac tissue was impaired in Th1 cytokine-deficient infected mice, paralleling the intensity of VCAM-1 and ICAM-1 expression on the vascular endothelium. Consistent with the importance of ICAM-1 in host resistance, ICAM-1 knockout (KO) mice were highly susceptible to *T. cruzi* infection, as assessed by mortality rate, parasitemia, and heart tissue parasitism. The enhanced parasitism was associated with a decrease in the numbers of CD4⁺ and CD8⁺ T lymphocytes in the heart tissue of ICAM-1 KO mice. Additionally, ICAM-1 KO mice mounted an unimpaired IFN-γ response and IFN-γ-dependent production of reactive nitrogen intermediates and parasite-specific IgG2a. Supporting the participation of ICAM-1 in cell migration during *T. cruzi* infection, the entrance of adoptively transferred PBL from *T. cruzi*-infected wild-type C57BL/6 mice into the cardiac tissue of ICAM-1 KO mice was significantly abrogated. Therefore, we favor the hypothesis that ICAM-1 plays a crucial role in T lymphocyte recruitment to the cardiac tissue and host susceptibility during *T. cruzi* infection. *The Journal of Immunology*, 2004, 173: 463–470.

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² Abbreviations used in this paper: MIG, monokine induced by IFN-γ; CAM, cell adhesion molecule; IHS, immunohistochemistry; IP-10, IFN-γ-inducible protein 10; KO, knockout.
and CXCL10/IFN-α-inducible protein 10 (IP-10), which are important in mediating the migration of activated CD4+ and CD8+ αβ T lymphocytes (5, 13, 14, 25).

In T. cruzi-infected animals, expression of cellular adhesion molecules (CAMs) such as ICAM-1 and VCAM-1 is induced in the cardiac tissue, whereas a high percentage of peripheral blood T lymphocytes become highly positive for LFA-1, ICAM-1, and VLA-4 during the early acute and chronic phase (14, 26, 27). However, the crucial elements controlling the expression of these CAMs and their importance in cell recruitment and host resistance to infection have been poorly investigated. ICAM-1, but not VCAM-1, is constitutively expressed at low levels by endothelial cells and is up-regulated by inflammatory cytokines such as IL-1, TNF-α, IFN-γ, and platelet-derived growth factor, as well as microbial products, such as bacterial LPS (28). Once activated, leukocytes express VLA-4 and LFA-1 that present high affinity for their respective ligands VCAM-1 and ICAM-1 expressed on activated vascular endothelium, leading to firm attachment of circulating leukocytes to vascular endothelium and subsequent diapedesis into inflamed tissue (28). The CAMs are also important molecules involved in the interaction of lymphocytes and APCs and therefore induction and maintenance of T cell-mediated immune responses (29).

The purpose of our study was to better comprehend the importance of endogenous IFN-γ in inducing expression of CAMs on circulating leukocytes and heart tissue from infected mice and to evaluate the role of ICAM-1 in the process of lymphocyte recruitment as well as host resistance to T. cruzi infection. Our results suggest that endogenous IFN-γ is crucial for optimal expression of CAMs on cardiac vascular endothelium and that ICAM-1 is effectively involved in the resistance to T. cruzi infection, contributing to leukocytes migration to cardiac tissue of T. cruzi-infected mice and, consequently, in the control of parasite replication.

Materials and Methods

Animals

Female C57BL/6, IFN-γ-deficient (IFN-γ knockout (KO)), and IL-12 KO mice were maintained under standard conditions with environmental barriers in the animal house from Oswaldo Cruz Foundation (Rio de Janeiro, Brazil). The KO mice, all in the C57BL/6 genetic background, were provided by the Laboratory of Gnotobiology-Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais (Belo Horizonte, Brazil) and School of Medicine of Ribeirão Preto, USP. ICAM-1 KO mice were purchased from Jax Mice Laboratory (Bar Harbor, ME).

Parasites and experimental infection

The Colombian strain of T. cruzi was maintained by serial passages from mouse to mouse. C57BL/6 and different KO mice were infected i.p. with 5000 blood trypomastigote forms of T. cruzi. The levels of parasitemia were evaluated using 5 μl of blood in an optical microscope. All experimental procedures were conducted according to the institutional guidelines for animal ethics of the Oswaldo Cruz Foundation.

Reagents and mAbs

Specific polyclonal Ab recognizing T. cruzi Ags was a gift from Dr. R. Teixeira de Pinho (Oswaldo Cruz Institute-Fiocruz, Rio de Janeiro, Brazil). Specific polyclonal Ab recognizing mouse fibroinectin was purchased from Life Technologies (Gaithersburg, MD). mAbs purified anti-VLA-4 (CD49d, α4 chain, clone 9C10-MF4R.B), anti-VLA-4-biotin (CD49d, α4 chain, clone 9C10-MF4R.B), anti-VCAM-1-biotin (CD106, clone 51-10C9), and anti-ICAM-1-biotin (CD54, clone 3E2) were purchased from Becton Dickinson (San Diego, CA). Purified anti-F4/80 Ag (clone F4/80) Ab was purchased from Caltag Laboratories (Burlingame, CA). Purified mAbs anti-CD8α (clone 53-6.7) and anti-CD4 (GK1.5) were produced in our laboratory after hybridoma expansion. Biotinylated Abs recognizing rabbit Ig and peroxidase-streptavidin complex were purchased from Amersham (Little Chalfont, U.K.). Biotinylated Abs recognizing rat Ig was obtained from DakoCytomation (Glostrup, Denmark). Appropriate controls were prepared by replacing primary Abs with purified rat Ig or normal rabbit serum. mAb anti-mouse IFN-γ (clone R46A2), polyclonal rabbit serum specific for IFN-γ, and rIFN-γ were purchased from Genzyme (Cambridge, MA). Nonconjugated goat anti-mouse IgG1 or IgG2a and peroxidase-conjugated anti-goat IgG were obtained from Southern Biotechnology Associates (Birmingham, AL). PKH-26 red fluorescent cell linker (PKH-26GL) was purchased from Sigma-Aldrich (St. Louis, MO).

Histological evaluation

Half of the heart of three to five T. cruzi-infected mice of each group was fixed in neutral 10% Formalin, embedded in paraffin, sectioned, stained with H&E, and examined by light microscopy to evaluate the intensity of inflammation, the presence of amastigote nests, and tissue damage. The other half was included in JUNG tissue freezing medium (Leica Instruments, Nussloch, Germany), frozen, and stored in liquid nitrogen to characterize the inflammatory cells, T. cruzi Ags, fibrosis, and activation of vascular endothelium by immunohistochemistry (IHS).

Immunohistochemistry

For HIS, serial cryostat sections were mounted on poly-L-lysine-covered glass slides and fixed for 10 min in cold acetone, washed in PBS, and incubated for 30 min in a wet chamber at room temperature with PBS containing 0.1% sodium azide and normal goat serum (Sigma-Aldrich) diluted 1:20 to reduce nonspecific binding. They were then incubated for 40 min with anti-CD4, anti-CD8, anti-VLA-4, anti-T. cruzi polyclonal rabbit serum, anti-ICAM-1-biotin, or anti-VCAM-1-biotin according to a previous titration assay. Biotinylated secondary Abs for anti-CD4, anti-CD8, and anti-VLA-4 was goat anti-rat Ab and for anti-T. cruzi was donkey anti-rabbit Ab. The sensitivity was improved with streptavidin-peroxidase. The reaction was visualized by incubating the section with 9-amino-3-ethylcarbazole (Sigma-Aldrich) in the presence of hydrogen peroxide. The material was counterstained with Mayer’s hematoxylin. Three sections were counted for each animal and the individual data were determined as the mean result of the three sections (5 μm distance between each section). The quantification of inflammatory cells and T. cruzi-positive areas after immunostaining was counting in 50 microscope fields (10 × 25 magnification) per section. For characterization of VCAM-1 expression, the numbers of VCAM-1 expressing and nonexpressing VCAM-1 blood vessels were counted and the data were expressed as percentage. The expression of ICAM-1 in heart tissue sections was also evaluated using a digital morphometric evaluation. Images were digitized using a color digital video camera (CoolSnap cf; Media Cybernetics, Carlsbad, CA) adapted to an AX-70 microscope (Olympus, Tokyo, Japan), with motorized xyz stage (Media Cybernetics). The images were analyzed using the Image-Pro Plus Program (Media Cybernetics), so that the areas expressing and nonexpressing ICAM-1 molecule were integrated. The ICAM-1 expression was determined as percentage reaction. Ten fields per section in four sections per heart, were analyzed. The percentage of area expressing ICAM-1 was measured in 10–20 fields and expressed as percentage area per 6 mm2.

ICAM-1 mRNA detection

ICAM-1 and β-actin mRNA expression was analyzed by RT-PCR. PCRs were performed with Taq polymerase (Life Technologies) in a PTC-100 thermal cycler (MJ Research, Watertown, MA). The reaction conditions were 30 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 primers sequences and PCR product sizes have been published elsewhere (30).

Quantification of IFN-γ and parasite-specific IgG1 and IgG2a

IFN-γ was assayed by ELISA using a rat anti-IFN-γ mAb R46A2 and a polyclonal rabbit serum specific for the cytokine. IFN-γ levels were calculated by reference to a standard curve constructed with recombinant cytokine. Sensitivity of this method was 100 pg/ml. The OD was read with a microplate reader set to 405 nm. To detect anti-T. cruzi-specific IgG1 and IgG2a, the plates (Nalge Nunc International, Rochester, NY) were coated overnight at 4°C with 10 μg/ml T. cruzi Ag in PBS. Serum from individual animals was diluted 1/100 PBS. Titers were corrected for the dilution and incubated for 1 h at 37°C, after blocking with 1% albumin in PBS for 2 h at 37°C. One hundred microliters of 1/1000 nonconjugated goat anti-mouse IgG1 or IgG2a diluted in PBST was added to each well and the plate was incubated for 1 h at 37°C. For the detection of anti-IgG1 and IgG2a Abs, a peroxidase-conjugated anti-goat IgG (1/5000) was incubated for 1 h at 37°C. This assay was developed using the APTS substrate (Sigma-Aldrich), and the reaction was stopped with 20 μl of 20% sulfuric acid solution.
NO quantification

Plasma samples from control and infected C57BL/6 and ICAM-1 KO mice were collected from the retro-orbital plexus 18 to 25 days after T. cruzi infection. Nitrate was reduced to nitrite with nitrate reductase as previously described by Schmidt et al. (31), and the concentration of nitrite was determined by the Griess method. The nitrite concentration in plasma was assayed in a microplate by mixing 50 µl of culture supernatant or plasma with 50 µl of Griess reagent (32). The A540 was read 10 min later, and the NO\textsuperscript{2−} concentration was determined by reference to a standard curve of 0.78–200 µM NaNO\textsubscript{2}.

Adoptive cell transfer experiments

To test the participation of ICAM-1 expressed on endothelial cell of T. cruzi-infected mice in the cell migration into the cardiac tissue, mononuclear cells were isolated from peripheral blood of acutely T. cruzi-infected C57BL/6 mice and adoptive cell transfer experiments were performed as previously described (33). Briefly, mononuclear cells were isolated from peripheral blood of control or T. cruzi-infected mice (28 days postinfection) using Ficoll-Hypaque separation and labeled with PKH-26GL (Sigma-Aldrich) according to the manufacturer’s instructions. Two million cells (>98% PKH-26GL\textsuperscript{+}) were i.v. transferred into normal and acutely T. cruzi-infected C57BL/6 and ICAM-1 KO mice. Three days later, the animals were sacrificed, the cardiac tissue was removed and embedded in JUNG tissue-freezing medium (Leica Instruments, Deerfield, IL), and serial 5-µm-thick sections were prepared, fixed in cold acetone, and mounted with PBS-glycerin. Using a fluorescence microscope (Leitz Wetzlar, Germany), the PKH-26GL-positive cells present in 100 microscopic fields (×400) were determined.

Statistical analysis

The results were expressed as means and SDs of the means. Student’s t test was used to analyze the statistical significance. Variance analysis was performed with INSTAT software (GraphPad, San Diego, CA) to compare the levels of parasitemia. The Kruskal-Wallis test was used to analyze the intensity of ICAM-1 expression and the survival rates. Values of p of 0.05 or less were considered to be significant.

Results

Tissue parasitemia and inflammatory cells in the cardiac tissue from C57BL/6, IFN-γ, and IL-12 KO mice infected with T. cruzi

Parasitemia was followed from days 10 to 15 after infection with blood trypomastigote forms of the Colombian strain of T. cruzi. IFN-γ KO and IL-12 KO mice showed increased parasitemia as compared with C57BL/6 mice (Fig. 1A). IHS was performed to quantify amastigote nests in the cardiac tissue at day 15 postinfection. IFN-γ and IL-12 KO mice show higher numbers of positive areas than C57BL/6 mice (Fig. 1B). In agreement with our previous results (9, 25), the inflammatory infiltrates were reduced in IFN-γ and IL-12 KO as compared with C57BL/6-infected mice (Fig. 1C). Consistently, IFN-γ-producing cells were rarely found in IL-12 KO mice, as compared with a high frequency in the cardiac tissue from C57BL/6 mice, and, as expected, no IFN-γ-producing cells were found in IFN-γ KO mice (Fig. 1D).

Phenotype of inflammatory cells in the cardiac tissue of animals infected with the Colombian strain of T. cruzi

Our results show that in the early acute phase (day 15 postinfection), the majority of leukocytes in the myocardium of C57BL/6 mice are CD4\textsuperscript{+} (Fig. 2A). Differently, the frequency of CD4\textsuperscript{+} T cells was extremely low in IL-12 KO and IFN-γ KO mice. The frequency of CD8\textsuperscript{+} T cells was low at 15 days postinfection in C57BL/6 mice, with even lower numbers of CD8\textsuperscript{+} T cells in IL-12-KO and IFN-γ-KO mice (Fig. 2A). When assessing VLA-4 expression in the heart tissue from infected mice, we observed decreased numbers of VLA-4\textsuperscript{+} cells in IL-12 KO and IFN-γ KO as compared with heart tissue from C57BL/6 mice (Fig. 2A). A higher number of F4/80\textsuperscript{+} cells (macrophages) was observed in the
cardiac tissue from infected C57BL/6 and IL-12 KO mice as compared with IFN-γ KO mice (data not shown).

Decreased expression of VCAM-1 and ICAM-1 in cardiac tissue of IFN-γ-deficient T. cruzi-infected mice

Expression of CAMs is predominantly involved in leukocyte trafficking and extravasation of inflammatory cells to target tissues (28). Therefore, we evaluated the in situ expression of CAMs in cardiac tissue from animals infected with the Colombian strain of T. cruzi. In C57BL/6 mice, we observed an increase in the number of VCAM-1-bearing blood vessels (69.1 ± 13.4%) comparing with IL-12 KO and IFN-γ KO mice (47 ± 12.8% and 21 ± 7.9%, respectively; Fig. 2A). The intensity of ICAM-1 assayed by IHS was also augment on endothelial cells, cardiomyocytes and mononuclear cells in infected C57BL/6 > IL-12 KO > IFN-γ KO mice compared with the control uninfected mice (Fig. 2B). The quantification of the red color indicating expression of ICAM-1 clearly shows higher expression of ICAM-1 in infected C57BL/6 mice compared with IL-12 KO, IFN-γ KO, and uninfected mice (Fig. 2C). Similar results were found when we assayed the expression of ICAM-1 by RT-PCR (Fig. 2, D and E) of heart tissue of mice at day 6 postinfection. It was remarkable that IFN-γ appears to be necessary for optimal VCAM-1 and ICAM-1 expression in the heart tissue from T. cruzi-infected mice. Our results also showed no major differences in the expression of fibronectin in the blood vessel walls when comparing infected and uninfected C57BL/6, IFN-γ KO, and IL-12 KO mice. In contrast, myocytes from C57BL/6 mice showed increased expression of fibronectin and with less degree in IL-12 KO and IFN-γ KO mice when comparing infected and uninfected animals (data not shown).

ICAM-1 deficiency enhances susceptibility of mice to infection with T. cruzi

To further investigate the role of ICAM-1 in mouse resistance to infection, ICAM-1 KO mice were infected with 5000 trypomastigote forms of the Colombian strain and parasitemia as well as mortality were monitored daily. ICAM-1 KO mice were more susceptible than C57BL/6 mice as indicated in the mortality (Fig. 3B) and parasitemia (Fig. 3A) curve. One hundred percent of ICAM-1 KO mice succumbed to infection by day 29 postinfection, whereas
Parasitemia (A) and survival (B) ICAM-1 KO and C57BL/6 mice infected with 5000 trypomastigote forms of the Colombian strain of *T. cruzi*. One and two asterisks indicate, respectively, that differences are statistically significant (*p < 0.001* and *p < 0.05*) when comparing ICAM-KO with C57BL/6-infected mice. Similar results were obtained in four other experiments.

100% of death in C57BL/6 was only observed at day 58 postinfection (Fig. 3A). The parasitemia curve presented the same tendency, being much higher in the ICAM-1 KO mice, reaching at day 21 postinfection levels at least 3-fold higher than those observed in C57BL/6 animals (Fig. 3B).

**ICAM-1 deficiency does not affect IFN-γ production and IFN-γ-mediated effector functions elicited during infection with *T. cruzi***

We next measured the IFN-γ levels in the serum of C57BL/6 and ICAM-1 KO mice. We found that the levels of IFN-γ were significantly higher in animals infected with *T. cruzi* compared to uninfected mice (Fig. 4A). However, no statistical difference was observed when comparing the IFN-γ levels obtained from C57BL/6 and ICAM-1 KO *T. cruzi*-infected mice. Similarly, the levels of nitrates (Fig. 4B) were higher in animals infected with *T. cruzi* compared to uninfected mice (*p < 0.05*). Infection with *T. cruzi* also elicited both parasite-specific IgG1 and IgG2a Ab isotypes in C57BL/6 and ICAM-1 KO mice (Fig. 4, bottom panels). Beside IgG2a, whose induction is highly dependent on IFN-γ synthesis, is increased in both C57BL/6 and ICAM-1 KO mice. No statistical differences were observed (*p > 0.1*) between parasite-specific IgG1 and IgG2a levels, comparing ICAM-1 KO with C57BL/6 mice infected with *T. cruzi*.

**ICAM-1 deficiency results in impaired T lymphocyte recruitment to the heart tissue from *T. cruzi*-infected mice***

The IHS results presented in Fig. 5 show that either C57BL/6 (A) or ICAM-1 KO (B) mice do not express detectable levels of ICAM-1 prior infection with *T. cruzi*. In contrast, at 15 days postinfection, heart tissue from C57BL/6 (Fig. 5C) but not ICAM-1 KO (Fig. 5D) mice infected with *T. cruzi* expressed high levels of ICAM-1, which was mainly localized on vascular endothelium, inflammatory cells, and cardiomyocytes. The inflammatory infiltrates in the heart tissue from C57BL/6 were consistently higher than those found in ICAM-1 KO mice infected with *T. cruzi* (Fig. 5, E and F, respectively). Fig. 5 also illustrates the less intense tissue parasitism in the heart tissue from C57BL/6 (G) as compared with ICAM-1 KO (H) mice on day 25 of infection. The frequency of CD4+ T cells in the heart tissue from infected ICAM-1 KO mice correspond to 47% (day 18 postinfection) and 51% (day 25 postinfection) of that found in the heart tissue from infected C57BL/6 mice (Fig. 5I). The same tendency was observed with regard to CD8+ T cells. Thus, in ICAM-1 KO the frequency of CD8+ T cells (Fig. 5J) was 50% (day 18 postinfection) and 40% (day 25 postinfection) of the ones observed in heart tissue from C57BL/6 mice infected with *T. cruzi*.

To finally determine the role of ICAM-1 expressed on cardiac vascular endothelium in the cell migration to the cardiac tissue, PBMC from infected wild-type C57BL/6 mice were purified, labeled with PKH-26GL, adoptively transferred to uninfected or infected C57BL/6 and ICAM-1 KO mice, and the cell migration into the heart tissue was evaluated 3 days later. The results show (Fig. 6) the presence of rare fluorescent inflammatory cells in the heart tissue while infected C57BL/6 and ICAM-1 KO mice received PKH26GL+ PBMC (>50% LFA-1+ and VLA-4+; >60% ICAM-1+). However, a significant increase in the percentage of stained cells is observed in the heart tissue of *T. cruzi*-infected mice. Finally, the cell migration to the heart tissue of infected C57BL/6 mice is significantly higher than that observed in infected ICAM-1 KO mice. These data indicated that ICAM-1 expression on the vascular endothelium of *T. cruzi*-infected mice favors the establishment of cardiac inflammatory reaction during the early acute phase of infection.

**Discussion***

This study was undertaken to investigate the role of endogenous IFN-γ in the expression of CAMs in the cardiac vascular endothelium and in the process of lymphocyte migration to the heart of mice infected with *T. cruzi*. To address this question, mice lacking functional genes for IFN-γ or IL-12 were infected with the Colombian strain of *T. cruzi* that causes an intense cardiomyopathy in mice (9, 14). Infection of IL-12 KO mice results in reduced IFN-γ production, showing an intermediate pattern of susceptibility to infection and pathology development compared with C57BL/6 and IFN-γ KO mice. In accordance with our previously published results (9, 25), we found severe reduction of T cell migration to the heart tissue of IFN-γ KO- and IL-12 KO-infected mice. The ability of IFN-γ to mediate myocarditis elicited by *T. cruzi* is attributed to its ability to stimulate the local production of CCL5/RANTES, CXCL9/MIG, and CXCL10/IP-10 (13, 14, 25); all involved in the...
recruitment of both activated CD4$^+$ and CD8$^+$ T lymphocytes (34, 35). Furthermore, in IFN-$\gamma$-deficient mice the production of CCL5/RANTES, CXCL9/MIG, and CXCL10/IP-10 is drastically reduced followed by a diminished recruitment of CD4$^+$ and CD8$^+$ T lymphocytes to the heart of infected mice (25). These results suggest that IFN-$\gamma$ produced into the heart tissue by the migrated inflammatory cells triggers a cascade of events that potentiate the chemokine production and NO synthesis, resulting in more T cell migration and in the control of parasites growth (25, 36), but that may also contribute to pathophysiology. However, since cardiomyocytes infected with $T. cruzi$ produce a marked amount of chemokines (37), one possibility that stems from these observations is that other molecules that are up-regulated by IFN-$\gamma$ are necessary to mediate T cell migration to the heart.

Our results show that IFN-$\gamma$ largely contributes to enhance the expression of ICAM-1 and VCAM-1 on the cardiac vascular endothelium from $T. cruzi$-infected mice, possibly mounting a traffic signal enabling inflammatory cells to migrate from the bloodstream to the site of infection and tissue injury. Furthermore, the induction and up-regulation of expression of VCAM-1, a VLA-4 ligand (28), and ICAM-1, a LFA-1 ligand (28), on cardiac vascular endothelium were associated with the establishment of the early acute $T. cruzi$-elicited myocarditis (27). Moreover, ICAM-1$^+$ and VCAM-1$^+$ blood vessels were associated with the presence of CD8$^+$-infiltrating T cells in the cardiac tissue of chagasic patients with severe cardiomyopathy (38). In addition, cardiomyocytes express ICAM-1 during the acute phase of experimental $T. cruzi$ infection (27, 30). Considering that ICAM-1-mediated interactions...
plays a crucial role in forming the immunological synapse (29, 39), ICAM-1− myocytes could be targets for LFA-1− cytotoxic T cells, contributing to heart dysfunction during chagasic myocarditis. Interestingly, in IFN-γ KO mice the remarkable decrease in the expression of VCAM-1 and ICAM-1 on cardiac vascular endothelium was associated with hampered migration of activated T cells to the heart tissue and elevation in the numbers of VLA-4+, LFA-1+, and ICAM-1−-activated T lymphocytes in peripheral blood (data not shown), paralleling inefficient parasite control. Altogether, these findings suggest that expression of ICAM-1 and VCAM-1 in the cardiac vascular endothelium of T. cruzi-infected mice contributes to the attachment of circulating activated T cells, leading to selective migration of inflammatory cells into the T. cruzi-infected myocardium.

Another point that deserves attention is that increased expression of fibronectin, that is up-regulated by inflammatory cytokines as TNF-α and IFN-γ (40), in the heart tissue of T. cruzi-infected mice (14) and humans (41) is associated with inflammation, while the vanished expression of this extracellular matrix component is associated with down-modulation of inflammatory processes in IFN-γ-deficient mice. Interestingly, fibronectin is involved in adhesion and uptake of trypanastigotes by macrophages (42). Thus, our results point to the fact that IFN-γ deficiency may also contribute to parasitism enhancement down-modulating fibronectin-mediated parasite uptake.

We next infected mice genetically deficient in ICAM-1 to confirm the participation of ICAM-1 in the T cell influx to the heart tissue and the effector immune response associated with resistance to T. cruzi infection. We found that ICAM-1-deficient mice show a reduction in the number of CD4+ and CD8+ T lymphocytes in the heart tissue, which was associated with a local increase in parasitism. The ICAM-1 absence significantly hampers the migration of cells toward the target tissue, which could lead to a reduction of the local production of attractive factors for T cells, as chemoattractant cytokines, contributing for the decreased cellular recruitment and resulting in increased parasitism. In this context, LFA-1-ICAM-1 interactions are related to the preferential recruitment of Th1 cytokines producers toward inflamed cardiac tissue, as suggested in murine cardiac allografts (43). Thus, the increased parasitism in the heart tissue of ICAM-1 KO mice may result in the deficient migration of Th1 and β chemokine producers essential for the control of T. cruzi replication and cell recruitment (5, 9, 19, 25).

The presence of inflammatory cells in the cardiac tissue of ICAM-1-deficient mice on day 25 postinfection could be due to compensatory mechanisms of cellular migration, as observed in mice infected with visceral leishmaniasis and disseminated candidiasis (44, 45). Furthermore, it may indicate that although ICAM-1 is required for cell migration, the activated cell trafficking to and through the cardiac vascular endothelium during T. cruzi-elicited myocarditis could be mediated by ICAM-1-independent pathways, as previously described in other models (44, 45). Supporting this possibility, the cell migration into the cardiac tissue was only partially abrogated (34–51%) when activated blood leukocytes from T. cruzi-infected wild-type mice were adoptively transferred to infected ICAM-1 KO mice.

Consistent with the crucial role of ICAM-1-mediated interactions in cell recruitment and a minor participation of these interactions in effector immune functions, upon infection ICAM-1-deficient animals showed unimpaired IFN-γ responses as well as IFN-γ-mediated effector functions, such as the production of parasite-specific IgG2a Abs and nitrates. In accordance, minimal effects on the generation of specific T cell response and humoral immunity were observed in the absence of ICAM (46). However, we cannot discard the bare possibilities that other effects due to the deficiency of ICAM-1 renders the animals more susceptible to the infection, although the levels of NO in the sera, the main effector mechanism of parasite killing, were similar in wild-type and ICAM-1 KO T. cruzi-infected mice.

Together our results suggest that IFN-γ plays a role in eliciting ICAM-1 and VCAM-1 expression in cardiac tissue of mice during experimental infection with T. cruzi. This appears to be an important aspect of the ability of IFN-γ in promoting lymphocyte migration to the site of T. cruzi infection. Remarkably, our results show that during experimental T. cruzi infection, ICAM-1 is essential having a nonredundant role necessary for migration of CD4+ and CD8+ T lymphocytes to the heart. The deficit on lymphocyte migration to the heart was associated with a drastic increase in the numbers of amastigote nests, indicating the importance of local action of lymphocytes to control tissue parasitism. Finally, the studies presented here suggest that CAMs, particularly ICAM-1, should be considered as a potential immunotherapeutic target to prevent undesirable excessive T lymphocyte extravasations toward the cardiac tissue during infectious processes.

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References

FIGURE 6. Quantification of adoptively transferred PKH-26GL+ cells in the cardiac tissue of uninfected or infected C57BL/6 and ICAM-1 KO mice. PBMC from infected wild-type C57BL/6 mice (25 days postinfection) were purified, labeled with PKH-26GL, adoptively transferred to uninfected (□) or infected (■) C57BL/6 and ICAM-1 KO mice, and the migrated cells into the heart tissue were evaluated 3 days later in 100 microscopic fields (magnification, ×400). One asterisk indicates that the difference is statistically significant (p < 0.001) when comparing ICAM-1 KO and C57BL/6-infected mice.


