INFECTION AND IMMUNITY, June 2006, p. 3170–3179 Vol. 74, No. 6
0019-9567/06/$08.00+0 doi:10.1128/IAI.01648-05
Copyright © 2006, American Society for Microbiology. All Rights Reserved.

# Macrophage Migration Inhibitory Factor Contributes to Host Defense against Acute *Trypanosoma cruzi* Infection

José L. Reyes,[1] Luis I. Terrazas,[1] Bertha Espinoza,[2] David Cruz-Robles,[3] Virgilia Soto,[3] Irma Rivera-Montoya,[1] Lorena Gómez-García,[1,3] Heidi Snider,[4] Abhay R. Satoskar,[4] and Miriam Rodríguez-Sosa[1]*

*Unidad de Biomedicina, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México (UNAM), Tlalnepantla,[1] and Instituto de Investigaciones Biomédicas, UNAM,[2] and Instituto Nacional de Cardiología Ignacio Chávez,[3] Mexico D.F., México, and Department of Microbiology, The Ohio State University, Columbus, Ohio[4]*

Received 7 October 2005/Returned for modification 12 December 2005/Accepted 15 March 2006

**Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that is involved in the host defense against several pathogens. Here we used MIF$^{-/-}$ mice to determine the role of endogenous MIF in the regulation of the host immune response against *Trypanosoma cruzi* infection. MIF$^{-/-}$ mice displayed high levels of blood and tissue parasitemia, developed severe heart and skeletal muscle immunopathology, and succumbed to *T. cruzi* infection faster than MIF$^{+/+}$ mice. The enhanced susceptibility of MIF$^{-/-}$ mice to *T. cruzi* was associated with reduced levels of proinflammatory cytokines, such as tumor necrosis factor alpha, interleukin-12 (IL-12), IL-18, gamma interferon (IFN-γ), and IL-1β, in their sera and reduced production of IL-12, IFN-γ, and IL-4 by spleen cells during the early phase of infection. At all time points, antigen-stimulated splenocytes from MIF$^{+/+}$ and MIF$^{-/-}$ mice produced comparable levels of IL-10. MIF$^{-/-}$ mice also produced significantly less Th1-associated antigen-specific immunoglobulin G2a (IgG2a) throughout the infection, but both groups produced comparable levels of Th2-associated IgG1. Lastly, inflamed hearts from *T. cruzi*-infected MIF$^{-/-}$ mice expressed increased transcripts for IFN-γ, but fewer for IL-12 p35, IL-12 p40, IL-23, and inducible nitric oxide synthase, compared to MIF$^{+/+}$ mice. Taken together, our findings show that MIF plays a role in controlling acute *T. cruzi* infection.**

Chagas' disease is caused by the flagellate protozoon *Trypanosoma cruzi*, which is transmitted by the feces of blood-sucking insect vectors (*Triatoma*). The parasite plays a fundamental role in inducing immunopathology and tissue damage in organs such as the heart, esophagus, and colon by sequentially inducing inflammatory responses, cellular lesions, and fibrosis (60). Host resistance in experimental acute *T. cruzi* infection is dependent on both innate and acquired immune responses mediated by macrophages, natural killer (NK) cells, CD4$^+$ T cells, CD8$^+$ T cells, and B cells (17, 31, 33). Proinflammatory cytokines, such as interleukin-12 (IL-12), tumor necrosis factor alpha (TNF-α), and gamma interferon (IFN-γ), also play a critical role in protective immunity against *T. cruzi* (40, 44, 48, 59), since genetically engineered mice lacking any of these cytokines fail to control parasitemia and rapidly succumb to infection. IL-12 mediates immunity to *T. cruzi* infection by enhancing IFN-γ production from NK cells, which is required for the induction of NO-dependent macrophage microbicidal activity as well as the subsequent induction of a protective Th1 response (21, 24, 30, 40). On the other hand, the anti-inflammatory cytokines IL-4 and IL-10 mediate susceptibility to *T. cruzi*, but IL-10 is also required for preventing immune hyperactivity and organ immunopathology (22, 23).

Migration inhibitory factor (MIF) is a pleiotropic cytokine produced by activated T cells (5, 13), macrophages (8), and the pituitary gland (4). While MIF is involved in the pathogenesis of several inflammatory diseases, such as chronic colitis (14, 47), rheumatoid arthritis (41), and atherosclerosis (29), it is also a key effector molecule in modulating protective immunity against bacteria (10, 28, 46, 54), protozoan parasites (26, 37, 55), and helminths (51, 56). The protective role of MIF during microbial infection has been attributed to its ability to initiate an innate immune response by inducing TNF-α production (7, 11, 16), up-regulating Toll-like receptor 4 (TLR4) expression (52), and enhancing macrophage microbicidal activity (26). While some previous studies had suggested that MIF plays a critical role in T-cell activation and cytokine production, we and others have found that MIF-deficient T cells proliferate as efficiently as wild-type (WT) T cells following in vitro antigenic or mitogenic stimulation and produce significant amounts of Th1/Th2-associated cytokines comparable to those in WT mice, indicating that a lack of MIF has no clear effect on T-cell activity (51, 55).

The present study was undertaken to examine the in vivo role of MIF in determining the outcome of experimental Chagas' disease caused by *T. cruzi*. Our results demonstrate that MIF plays an important role in the host defense against acute *T. cruzi* infection by favoring production of the proinflammatory cytokines IL-12, IL-18, TNF-α, IL-1β, and IFN-γ during the early phase of infection. Moreover, they also show that increased susceptibility of MIF$^{-/-}$ mice to *T. cruzi* is not due to poor T-cell activation or a compensatory increase in the production of anti-inflammatory cytokines.

* Corresponding author. Mailing address: Unidad de Biomedicina, FES-Iztacala, UNAM, Av. De los Barrios #1, Los Reyes Iztacala, 54090 Tlalnepantla, Edo. de México, Mexico. Phone: (5255) 5623-1291, ext. 438. Fax: (5255) 5623-1225. E-mail: rodriguezm@campus.iztacala.unam.mx.

## MATERIALS AND METHODS

**Mice.** Eight- to 10-week-old male BALB/c mice were purchased from Harlan (México City, México) and maintained in a pathogen-free environment at our

TABLE 1. Oligonucleotide primers used for gene expression analysis by RT-PCR

| Target | Primer direction, sequence (5′–3′)[a] | Product size (bp) | No. of cycles | Reference |
|---|---|---|---|---|
| IFN-γ | F, TGG ACC TGT GGG TTG TTG ACC TCA AAC TTGGC<br>R, TGC ATC TTG GCT TTG CAG CTC TTC CTC ATG GC | 243 | 30 | 45 |
| CCL5 | F, GTG CCC ACG TCA AGG AGT AT<br>R, GGG AGG CGT ATA CAG GGT CA | 260 | 30 | 15 |
| β-Actin | F, GTG GGC CGC TCT AGG CAC CAA<br>R, CTC TTT GAT TGC ACG CAC GAT TTC | 536 | 30 | 27 |
| GAPDH | F, TCG GTG TGA ACG GAT TTG GC<br>R, CTC TTG CTC AGT GTC CTT GC | 1,035 | 25 | 38 |

[a] F, forward primer; R, reverse primer.

animal facility in accordance with institutional and national guidelines. $MIF^{-/-}$ mice were developed as described previously and backcrossed for >10 generations to a BALB/c genetic background (7).

**Parasites and infections.** The Mexican *Trypanosoma cruzi* isolate Ninoa (nonpathogenic) was obtained from a human patient in the acute phase of disease and used in part of this work (43). This isolate belongs to the *T. cruzi* I lineage and was maintained by sequential culture in liver infusion tryptose medium (Difco, Detroit, MI), *Triatoma pallidipennis* infection, and murine passages as described previously (18). Mice were inoculated by subcutaneous injection ($1 \times 10^3$ metacyclic trypomastigotes) into the hind footpad. The Mexican *T. cruzi* isolate Querétaro (pathogenic) was obtained from a human patient in the acute phase of disease and was maintained by sequential murine passages. This isolate belongs to the *T. cruzi* I lineage (6). Blood trypomastigotes were obtained from previously infected mice, counted, and adjusted to $5 \times 10^4$/ml in sterile phosphate-buffered saline (PBS), and 100 μl was injected intraperitoneally (i.p.) into male $MIF^{-/-}$ and $MIF^{+/+}$ mice. Parasitemia was determined by using hemocytometer counts of parasites in blood diluted 1:10 in PBS with 3.8% sodium citrate.

***Trypanosoma cruzi* lysate antigen.** Briefly, epimastigotes of *T. cruzi* that had been maintained by sequential culture in liver infusion tryptose medium were isolated, washed three times in PBS, and centrifuged at $1 \times 10^4$ rpm for 15 min. Protease inhibitors were added (0.1 to 2 μg/ml aprotinin, 0.5 to 2 mM EDTA, 1 to 5 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin, 50 μg/ml TLCK [*N*α-*p*-tosyl-L-lysine chloromethyl ketone] [all from Sigma, St. Louis, MO]), and parasites were then sonicated six times for 10 s each time at 50 W, using a Sonic Dimembrator 300 instrument (Fisher). Parasite destruction was determined using a microscope. Parasite lysates were then centrifuged at $1 \times 10^4$ rpm for 30 min to separate out the soluble fraction, which was stored at −70°C until use. The protein concentration was determined by the Lowry method (35).

**Cell preparations, culture conditions, and cytokine assays.** Spleen cells were obtained and cultured as previously described (51). Briefly, single-cell suspensions were prepared in complete RPMI 1640 supplemented with 10% fetal bovine serum, 100 units of penicillin-streptomycin, 2 mM glutamine, 25 mM HEPES buffer, and 1% nonessential amino acids (all from GIBCO BRL, Grand Island, NY). Erythrocytes were lysed, and viable cells were adjusted to $3 \times 10^6$ cells/ml. Cell suspensions (100 μl/well) were plated in 96-well flat-bottomed culture plates (Costar, Cambridge, MA) and stimulated with *T. cruzi* lysate antigen (25 μg/ml) for 72 h at 37°C. Supernatants from these cell cultures and sera obtained from infected $MIF^{+/+}$ and $MIF^{-/-}$ mice were analyzed to determine the production of IL-18, IL-12, IFN-γ, IL-4, IL-10 (all from Pharmingen, San Diego, CA), IL-1β, and TNF-α (Peprotech, México) by enzyme-linked immunosorbent assays (ELISAs) performed according to the manufacturers' instructions.

**Antibody ELISAs.** Peripheral blood was collected at 5-day intervals from tail snips of *T. cruzi*-infected $MIF^{-/-}$ and $MIF^{+/+}$ BALB/c mice. Blood was centrifuged at 200 × *g*, and sera were collected and tested for *T. cruzi* lysate-specific immunoglobulin G1 (IgG1) and IgG2a contents. Briefly, 96-well polystyrene ELISA plates (Corning, Corning, NY) were coated with 100 μl/well (5 μg/ml) of *T. cruzi* lysate antigen diluted in PBS, pH 9.0, and incubated overnight at 4°C. Plates were washed using PBS with 0.05% Tween 20 (PBS-Tween 20; Sigma, St. Louis, MO) and were blocked with PBS supplemented with 1% bovine serum albumin for 1 h at 37°C. Serially diluted serum samples (1/100 starting dilution in PBS-Tween 20) were added to the plates in duplicate and incubated for 2 h at 37°C. Bound antibodies were detected by incubation with either horseradish peroxidase-conjugated rat anti-mouse IgG1 or horseradish peroxidase-conjugated rat anti-mouse IgG2a (diluted 1/5,000 in 25% bovine serum and 75% PBS; Zymed, San Francisco, CA). After incubation for 1 h, the plates were washed, developed with ABTS solution (Zymed), and read on a microplate reader at 405 nm (Multiskan Ascent; Thermo Labsystems). Results are expressed as mean end-point titers ± standard errors (SE).

**Histopathology.** Hearts and skeletal muscle from legs were fixed overnight in formaldehyde and embedded in paraffin blocks, after which 5-μm-thick transverse sections were mounted on slides and subsequently stained with hematoxylin and eosin. Using an Olympus BX51 microscope (Olympus American, Melville, NY) equipped with a digital video camera, 25 individual fields were evaluated per sample for each tissue, covering at least 80% of the total area of the section. The severity of inflammation was evaluated as the number of positive inflammatory cells per field, and the numbers of amastigote nests were counted in the same sections by two pathologists.

**RT-PCR analysis.** Total RNA was isolated from the hearts of $MIF^{+/+}$ and $MIF^{-/-}$ *T. cruzi*-infected mice at 22 days postinfection by the TRIzol method (GIBCO BRL) according to the manufacturer's instructions. cDNA samples were amplified by SuperScript One-Step reverse transcription-PCR (RT-PCR) for 25 to 30 cycles, using Platinum *Taq* polymerase (Invitrogen) and specific primers (the sequences of the primers used are given in Table 1). After amplification, PCR products were separated by electrophoresis on 1.5% agarose gels containing SYBR green I, a nucleic acid gel stain used at 1,000× (Amresco), and were visualized with the FLA-5000 chemiluminescence detection system (Fujifilm). The data were normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and analyzed using the Multi Gauge Image program. Statistical analysis was performed using Student's *t* test. All reactions were repeated two times to ensure reproducibility.

**Relative gene expression by real-time quantitative PCR.** IL-23 (p19), IL-12 p35, IL-12 p40, and inducible nitric oxide synthase (iNOS) mRNA expression in the hearts of $MIF^{+/+}$ and $MIF^{-/-}$ mice at 22 days post-*T. cruzi* infection was determined by RT, followed by analysis of relative gene expression by real-time quantitative PCR and the $2 - \Delta\Delta C_T$ method (34). Briefly, total RNA of heart cells was extracted using TRIzol reagent (GIBCO BRL) according to the manufacturer's instructions. RT-PCR was performed using an Access RT-PCR system kit according to the manufacturer's protocol (Promega). The p19, p35, p40, and iNOS primers were labeled with SYBR green I (1×) (Amresco) and processed as described elsewhere (49). Real-time PCR was performed on a Rotor Gene RG 3000 instrument (Corbett Research, Australia). As a control, GAPDH housekeeping gene expression was evaluated using the same PCR protocol as that for p19, p35, p40, and iNOS. The specificities of PCRs for p19, p35, p40, and iNOS were verified by the absence of a signal in no-template controls for the heart samples. The cycle threshold ($C_T$) was used to reflect the relative p19, p35, p40, and iNOS mRNA expression levels and to calculate the change in gene expression between infected $MIF^{-/-}$ and $MIF^{+/+}$ mice. Data evaluation and processing were performed according to the methods of Ponchel et al. (49). The sequences of the primers used were as follows: for IL-23 (p19), 5′-TGC TGG ATT GCA GAG CAG TAA-3′ and 5′-CTG GAG GAG TTG GCT GAG TC-3′ (3); for IL-12 (p35), 5′-GCA AGA GAC ACA GTC CTG GG-3′ and 5′-TGC ATC AGC TCA TCG ATG GC-3′ (61); for IL-12 (p40), 5′-GGA GAC CCT GCC CAT TGA ACT-3′ and 5′-CAA CGT TGC ATC CTA GGA TCG-3′ (3); and for iNOS, 5′-CTG GAG GAG CTC CTG CCT CATG-3′ and 5′-GCA GCA TCC CCT CTG ATG GTG-3′ (63).

**Statistical analysis.** Comparisons between the $MIF^{+/+}$ and $MIF^{-/-}$ groups were made by using Student's unpaired *t* test. *P* values of $<0.05$ were considered significant. For survival assays, a log-rank test was used with the GraphPad computer program (GraphPad, San Diego, CA). The statistical significance of serum titers was determined by using the nonparametric Mann-Whitney U-Wilcoxon rank sum test.

## RESULTS

**MIF$^{-/-}$ mice develop high parasitemia levels and show increased mortality when infected with nonpathogenic and pathogenic strains of *T. cruzi*.** MIF is required for initial IL-12 and TNF-α production by macrophages in response to lipopolysaccharide-mediated septic shock and to *Salmonella* infection (9, 28, 36). To further investigate the role of MIF in immunity to *T. cruzi*, MIF$^{-/-}$ and MIF$^{+/+}$ mice were infected with two different strains of *T. cruzi*. One strain, Ninoa, which is nonpathogenic (according to its inability to produce tissue lesions and mortality), was injected into the footpad at a low dose of $1 \times 10^3$ metacyclic trypomastigotes obtained from the urine of the intermediate host *Triatoma*, and parasitemia was monitored. *T. cruzi*-infected MIF$^{-/-}$ mice developed significantly higher blood parasitemia levels than similarly infected MIF$^{+/+}$ mice, which contained nearly threefold fewer parasites in their blood by days 22 to 30 postinfection (p.i.) (Fig. 1A) ($P < 0.05$).

Independent experiments were performed using the pathogenic strain (Querétaro) of *T. cruzi*, with MIF$^{-/-}$ and MIF$^{+/+}$ mice being infected by i.p. inoculation of $5 \times 10^3$ blood trypomastigotes. Both groups developed blood parasitemia on day 10 p.i., but on day 16 p.i., MIF$^{-/-}$ mice displayed significantly greater levels of parasitemia, which peaked at day 27 p.i. and was positively detected until day 44 p.i. (Fig. 1B). In contrast, the maximum peak of parasitemia in MIF$^{+/+}$ mice was observed on day 22 p.i., and these mice controlled the infection by day 30. Furthermore, MIF$^{-/-}$ mice also started to succumb to infection as early as day 18 p.i., and just 30% of them survived to day 45. In contrast, 70% of MIF$^{+/+}$ mice survived throughout the infection (Fig. 1C).

**IL-12 and IFN-γ responses are impaired in MIF$^{-/-}$ mice during early *T. cruzi* infection.** IL-12 and IFN-γ have largely been associated with protection during acute *T. cruzi* infection (1, 24, 30, 33, 57). Antigen-presenting cells are the major source of innate IL-12 production in response to *T. cruzi* and its products (12). On the other hand, besides NK cells, T cells are a major source of IFN-γ (58). To determine whether the increased susceptibility and mortality of MIF$^{-/-}$ mice correlated with altered production of these cytokines, we compared IL-12 and IFN-γ levels in the sera of *T. cruzi*-infected MIF$^{+/+}$ and MIF$^{-/-}$ mice as early as 3 days p.i. until 30 days p.i. After infection with the pathogenic strain of *T. cruzi*, MIF$^{+/+}$ mice displayed rapid production of IL-12 and IFN-γ (Fig. 2A and B), which was sustained, or in some cases increased, for at least 3 weeks after infection. In contrast, MIF$^{-/-}$ mice displayed a significant impairment in the production of these cytokines early in the infection (Fig. 2A and B). As infection progressed, however, increased levels of IL-12 and IFN-γ were detected in the sera of MIF$^{-/-}$ mice. Consistent with these in vivo data, spleen cells from MIF$^{+/+}$ infected animals produced more IL-12 and IFN-γ in response to *T. cruzi* lysate antigens than did MIF$^{-/-}$ mice in early infections. Similarly, by day 22 p.i., both in vitro groups produced comparable levels of these cytokines (Fig. 2C and D).

**Proinflammatory cytokine production is altered in MIF$^{-/-}$ mice after *T. cruzi* infection.** Other potent proinflammatory cytokines, such as TNF-α, IL-1β, and IL-18, have been shown to play a role in determining the outcome of *T. cruzi* infection (25, 40, 44). The main sources of the early production of these

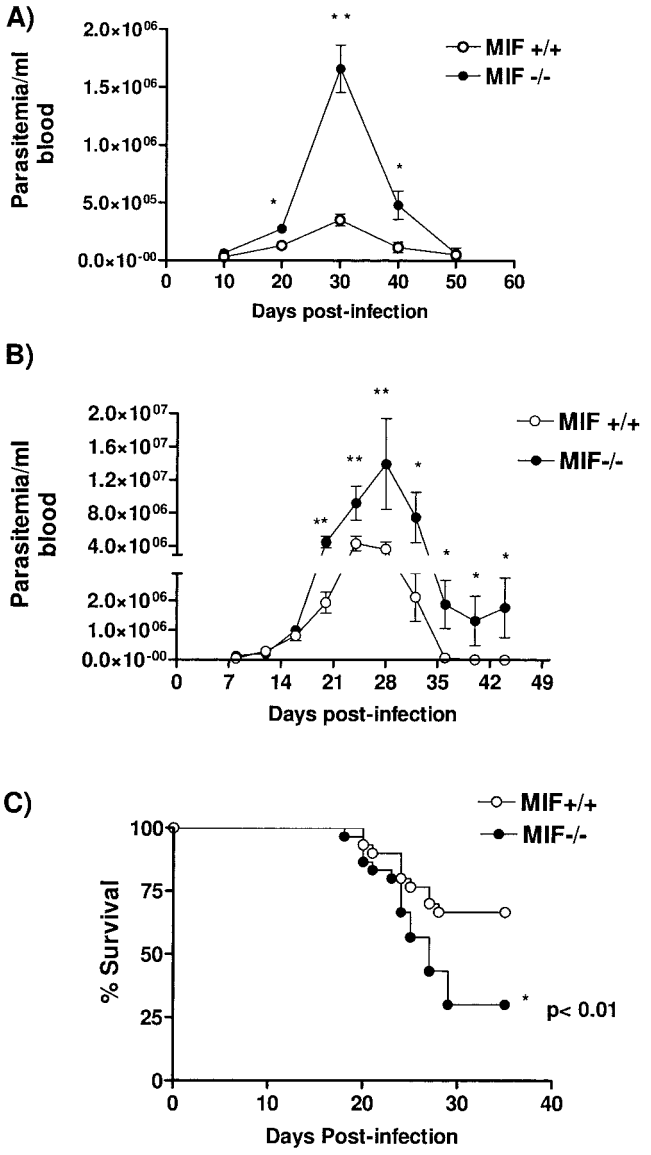


FIG. 1. Kinetics of parasitemia and mortality of MIF$^{-/-}$ mice infected with two different strains of *Trypanosoma cruzi*. (A) Course of *T. cruzi* infection in MIF$^{-/-}$ and MIF$^{+/+}$ mice infected subcutaneously with $1 \times 10^3$ metacyclic trypomastigotes of the nonpathogenic strain Ninoa, obtained from the natural vector (*Triatoma*). Parasitemia (B) and survival (C) of MIF$^{-/-}$ and MIF$^{+/+}$ mice infected i.p. with $5 \times 10^3$ blood trypomastigotes of the pathogenic Querétaro strain of *T. cruzi* are also shown. *, $P < 0.05$; **, $P < 0.001$ (indicates that differences are statistically significant between MIF$^{-/-}$ with MIF$^{+/+}$ infected mice). Data are expressed as means ± standard deviations (SD) and are representative of three independent experiments. Ten animals were used initially in each group.

cytokines are macrophages and dendritic cells. While it is known that macrophage TNF-α secretion is up-regulated by MIF (26), the role of MIF in regulating IL-1β and IL-18 remains elusive. To determine whether the production of proinflammatory cytokines is influenced by MIF during *T. cruzi* infection, we measured the levels of TNF-α, IL-1β, and IL-18 in the sera of MIF$^{-/-}$ and MIF$^{+/+}$ mice during the course of infection. TNF-α was detected in sera from both groups early

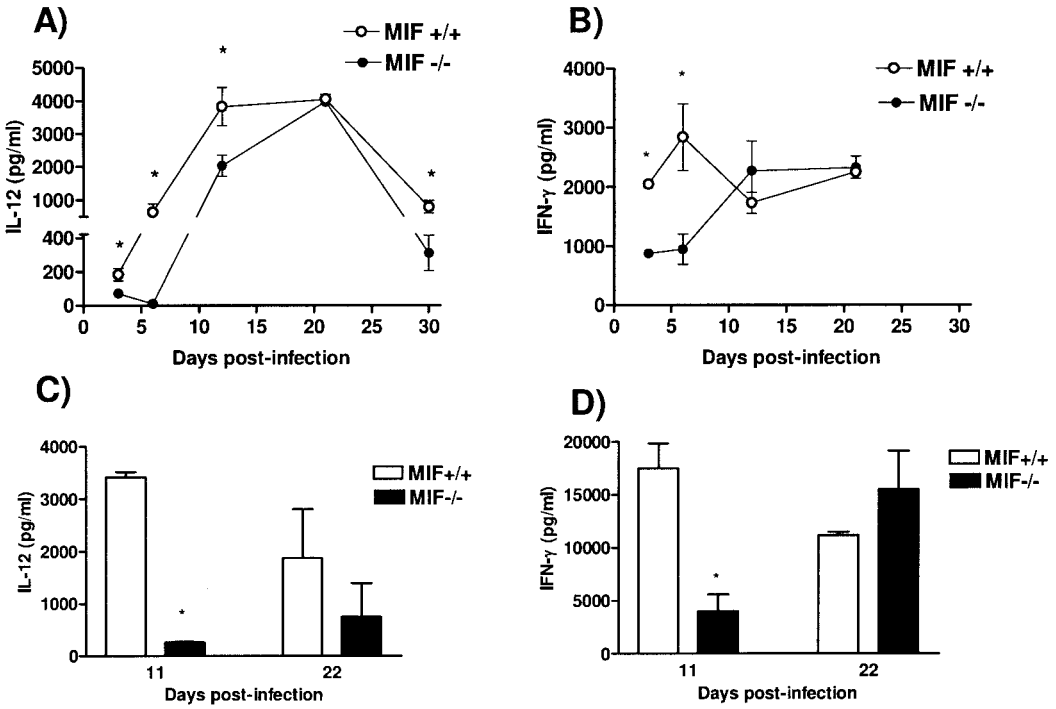


FIG. 2. Production of IL-12 and IFN-γ in $MIF^{-/-}$ and $MIF^{+/+}$ mice infected with *T. cruzi* (Querétaro). Mice were infected i.p. with $5 \times 10^3$ trypomastigotes. Blood was collected on days 3, 7, 11, 20, and 30 p.i., and IL-12 (A) and IFN-γ (B) levels were determined individually in sera by ELISA. Cytokine levels in the sera of noninfected mice were below the detection level. Additionally, splenocytes were obtained from mice on days 11 and 22 p.i. and stimulated with 25 μg/ml of *T. cruzi* soluble lysate. Following 72 h of in vitro stimulation, *T. cruzi* lysate-specific IL-12 (C) and IFN-γ (D) production from culture supernatants was evaluated by ELISA. Data are expressed as means ± SD ($n = 6$ animals per group). *, $P < 0.05$ for comparison of $MIF^{-/-}$ versus $MIF^{+/+}$ mice. Two more independent experiments were performed, which yielded similar results.

during the course of infection, but the levels were significantly higher in $MIF^{+/+}$ mice than in $MIF^{-/-}$ mice (Fig. 3A). However, as the infection progressed, both $MIF^{+/+}$ and $MIF^{-/-}$ mice displayed comparable levels of TNF-α in their sera. Similarly, TNF-α levels in spleen and lymph node cell culture supernatants were also elevated in WT mice during the early phase of infection (data not shown). Interestingly, the kinetics of IL-1β and IL-18 production also displayed similar patterns, and serum levels of both of these cytokines were significantly higher in $MIF^{+/+}$ mice than in $MIF^{-/-}$ mice throughout the course of the infection (Fig. 3B and C). These data suggest a pivotal role for MIF in regulating IL-1β and IL-18 production.

**Augmented Th2-type reactions are not responsible for impaired proinflammatory responses in $MIF^{-/-}$ mice.** Since Th2-type cytokines, such as IL-4 and IL-10, are involved in both susceptibility to *T. cruzi* infection and down-regulation of inflammatory cytokines (23, 30), we determined whether altered levels of these cytokines were responsible for the enhanced susceptibility of $MIF^{-/-}$ mice to acute *T. cruzi* infection. No significant differences were noted in the serum levels of IL-4 and IL-10 between *T. cruzi*-infected WT and $MIF^{-/-}$ mice at all time points examined throughout the course of the infection (Fig. 4A and B). The production of *T. cruzi* lysate-specific IL-4 by spleen cells from $MIF^{-/-}$ mice was lower early in the infection, whereas IL-10 production was similar between groups (Fig. 4C and D). Thus, it appears that elevated levels of Th2-type cytokines are unlikely to be responsible for the down-regulation of proinflammatory cytokine production observed in $MIF^{-/-}$ mice.

***T. cruzi*-infected $MIF^{-/-}$ mice develop severe heart and skeletal muscle pathologies.** On day 22 p.i., the hearts and skeletal muscles of $MIF^{+/+}$ mice showed low tissue parasitism with moderate inflammatory mononuclear cell infiltration but without major histopathological signs of lesions (Fig. 5A and C). In contrast, histopathology sections of infected $MIF^{-/-}$ mice showed an intense inflammatory reaction in the heart tissue (Fig. 5B and E), with a marked increase in amastigote nests (Fig. 5F) which was accompanied by severe heart injury due to large necrotic lesions. Damage was also more evident in the skeletal muscles of $MIF^{-/-}$ infected mice, which had large lesions and a larger number of amastigote nests than those of $MIF^{+/+}$ *T. cruzi*-infected mice (Fig. 5C and D). Nevertheless, the heart was the more severely affected tissue, with larger numbers of amastigote nests and greater inflammatory infiltrates than the skeletal muscles of $MIF^{-/-}$ mice (Fig. 5E and F). These results suggest that $MIF^{-/-}$ mice succumb to the classical pathophysiology of the infection, which is typical of a severe acute phase of experimental Chagas' disease.

**Analysis of IFN-γ, CCL5, IL-12 p35, IL12 p40, p19 (IL-23), and iNOS mRNA expression in infected hearts.** Since $MIF^{-/-}$ mice displayed severe cardiac pathology, we examined the cytokine and chemokine expression in this organ. On day 22 after *T. cruzi* infection, total mRNA was isolated from hearts, and RT-PCR analyses were performed. As shown in Fig. 6A and B,

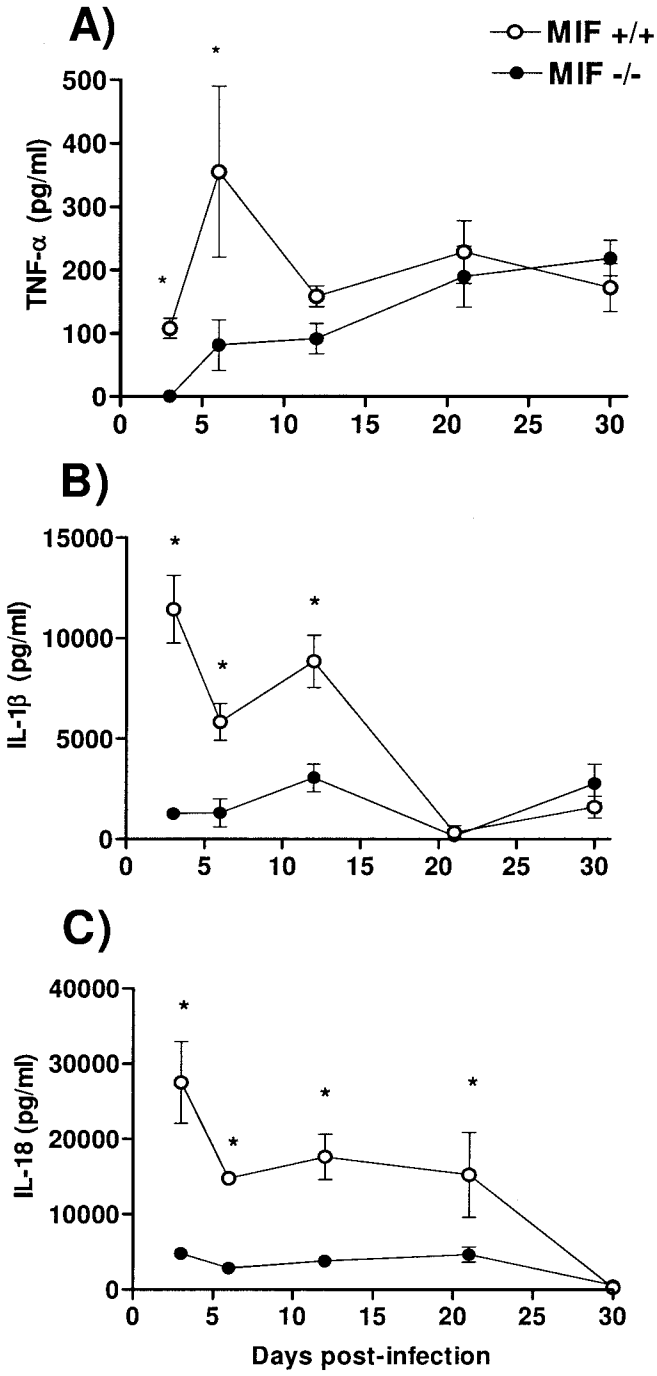


FIG. 3. Production of TNF-α, IL-1β, and IL-18 in $MIF^{-/-}$ and $MIF^{+/+}$ mice infected with *T. cruzi*. Mice were infected as stated in the legend to Fig. 2. Cytokines were determined in sera during the course of infection (8 to 10 mice per group). Levels of (A) TNF-α, (B) IL-1β, and (C) IL-18 were evaluated in individual serum samples by ELISA. Data are expressed as means ± SD. *, $P < 0.05$ for comparison of $MIF^{-/-}$ versus $MIF^{+/+}$ mice.

infected hearts from $MIF^{-/-}$ mice displayed a significant increase in transcripts of IFN-γ, but similar levels for CCL5, compared to hearts from $MIF^{+/+}$ mice. Furthermore, transcripts of IFN-γ were detectable in $MIF^{-/-}$ mice by using amounts of cDNA as low as 7 ng (Fig. 6A), while using this amount of cDNA yielded barely detectable IFN-γ transcripts from $MIF^{+/+}$ mice. Interestingly, β-actin transcripts were more intensely detected in $MIF^{-/-}$ *T. cruzi*-infected mice. Using 1 μg of mRNA, similar β-actin intensities were detected for both groups (data not shown); however, when dilutions of heart mRNA were performed, it was evident that $MIF^{-/-}$ infected mice expressed β-actin more intensely than $MIF^{+/+}$ infected mice (Fig. 6A and B). This apparent lack of good quality control is explained by the known effects of intense *T. cruzi* infection on heart muscle cells, where the disruption of the actin cytoskeleton generates an increase in β-actin production (39). This observation is consistent with the higher parasite burdens and severe pathology in the infected hearts of $MIF^{-/-}$ mice. In contrast, when another housekeeping mRNA was tested (GAPDH), no differences in the transcripts were detected between $MIF^{-/-}$ and $MIF^{+/+}$ infected mice (Fig. 6A). Additionally, using real-time RT-PCR, we measured IL-12, IL-23, and iNOS mRNA levels that are likely involved in mediating the host defense against *T. cruzi*. As shown in Fig. 6C, *T. cruzi*-infected $MIF^{+/+}$ mice showed significant increases in the levels of IL-12 p35, IL-12 p40, IL-23 p19, and iNOS mRNAs in their hearts compared to similarly infected $MIF^{-/-}$ mice.

**IFN-γ-dependent, antigen-specific IgG2a antibody titers are decreased in $MIF^{-/-}$ mice.** IFN-γ and anti-*T. cruzi* specific antibodies have been shown to act synergistically in protecting mice against this parasite (31, 40). Given that IFN-γ is known to modulate IgG production, particularly that of IgG2a, and that IFN-γ levels were similar in late infections between $MIF^{-/-}$ and $MIF^{+/+}$ mice, we decided to evaluate the kinetics of parasite-specific IgG1 and IgG2a antibodies during *T. cruzi* infection. Throughout the course of infection, *T. cruzi*-infected $MIF^{-/-}$ mice displayed lower titers of *T. cruzi*-specific IgG2a than did $MIF^{+/+}$ mice, and the differences were statistically significant on days 21 and 28 p.i. (Fig. 7A), despite the fact that the levels of IFN-γ in both the sera and spleen cell supernatants were comparable between groups at these time points. In contrast, *T. cruzi*-specific IgG1 production was not severely affected by MIF deficiency (Fig. 7B).

## DISCUSSION

Our study shows that $MIF^{-/-}$ mice are highly susceptible to experimental Chagas' disease caused by *T. cruzi*. Infection of $MIF^{-/-}$ mice with $5 \times 10^3$ blood trypomastigotes of the pathogenic *T. cruzi* strain (Querétaro) resulted in prolonged and high parasitemia levels, severe cardiac immunopathology, and increased mortality. $MIF^{-/-}$ mice also developed relatively high blood parasitemia levels and cardiac inflammation when infected with $1 \times 10^3$ metacylic promastigotes of the nonpathogenic strain of *T. cruzi*, but they eventually resolved the infection as efficiently as $MIF^{+/+}$ mice. These findings indicate that MIF plays a role in the host defense against acute *T. cruzi* infection.

The proinflammatory cytokines IL-12, IFN-γ, and TNF-α play an important role in mediating resistance to *T. cruzi* infection (24, 44, 59), and MIF promotes IL-12 and TNF-α production in macrophages (11, 26). Furthermore, MIF also mediates host immunity against intracellular pathogens by ac-

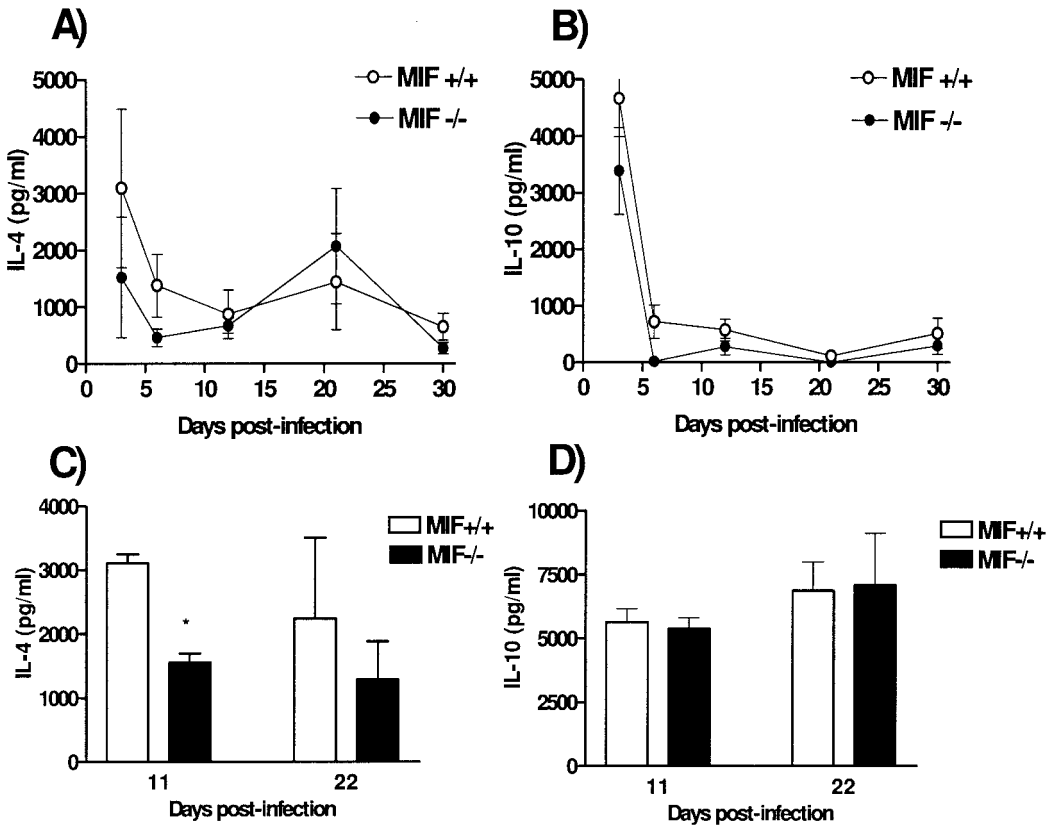


FIG. 4. Kinetics of in vivo and in vitro Th2-type cytokine production by $MIF^{-/-}$ and $MIF^{+/+}$ *T. cruzi*-infected mice. IL-4 (A) and IL-10 (B) levels were determined in the sera of both groups during the infection. *T. cruzi* lysate-specific IL-4 (C) and IL-10 (D) production by splenocytes following 72 h of in vitro stimulation with *T. cruzi* lysate (25 μg/ml) is also shown. Data are expressed as described in the legend to Fig. 2.

tivating macrophages and enhancing their microbicidal activity by increasing NO production (26). In the present study, we found that *T. cruzi*-infected $MIF^{-/-}$ mice produced significantly less IL-12, IL-18, IL-1β, and TNF-α during the early phase of infection. Furthermore, we also found that hearts from *T. cruzi*-infected $MIF^{-/-}$ mice contained significantly less iNOS mRNA than hearts from $MIF^{+/+}$ mice. Taken together, these findings suggest that endogenous MIF is required for optimal production of proinflammatory cytokines and NO during the early phase of *T. cruzi* infection. Because $MIF^{-/-}$ mice produce significantly less NK-cell-activating IL-12 and IL-18, the lack of IFN-γ production early in infection in these mice can be explained by an inadequate activation of NK cells, which are by far the most important source of IFN-γ at early stages of *T. cruzi* infection (40). It is important that the numbers of NK cells and macrophages were not diminished in $MIF^{-/-}$ infected mice, as measured by flow cytometry (data not shown). However, the requirement of MIF for efficient production of IL-1β, IL-18, and IL-12 in innate immunity against *T. cruzi* infection appears to be a new finding.

The major pathway reported for MIF-induced proinflammatory cytokine production has been attributed to its ability to regulate lipopolysaccharide responses through the modulation of TLR4 expression on macrophages (53). Interestingly, TLR4-mediated responses to *T. cruzi* have been reported as one of the main pathways for inducing early cytokine production associated with innate immunity (48). Thus, low expression of TLR4 may also be involved in the low inflammatory response observed in $MIF^{-/-}$ *T. cruzi*-infected mice.

It is known that at early stages of acute *T. cruzi* infection, cell infiltrates are necessary to control parasite replication; however, excessive infiltration can be dangerous for the host. In the present study, the hearts from *T. cruzi*-infected $MIF^{-/-}$ mice expressed low levels of proinflammatory cytokines, such as IL-12 and IL-23, and the enzyme iNOS, which is responsible for the production of nitric oxide, an effector molecule involved in killing of *T. cruzi* (21). IL-23 has recently been associated with the promotion of autoimmune diseases (32), but its role in infectious diseases is still unknown. To our knowledge, this is the first time that a lack of IL-23 expression has been associated with MIF deficiency. Nonetheless, we also found that $MIF^{-/-}$ mice develop intense cardiac inflammation despite the lack of strong proinflammatory cytokine production. This controversial observation can be explained by the fact that the infected hearts of $MIF^{-/-}$ and $MIF^{+/+}$ mice displayed similar expression levels of CCL5, which facilitates the trafficking of leukocytes to the heart and favors the development of an inflammatory infiltrate. In fact, a recent study showed that mice lacking CCR5, a receptor for CCL5, develop high parasitemia levels and cardiac parasitism and show reduced survival following *T. cruzi* infection (19). In our study, we also found that infected hearts from $MIF^{-/-}$ mice contained sig-

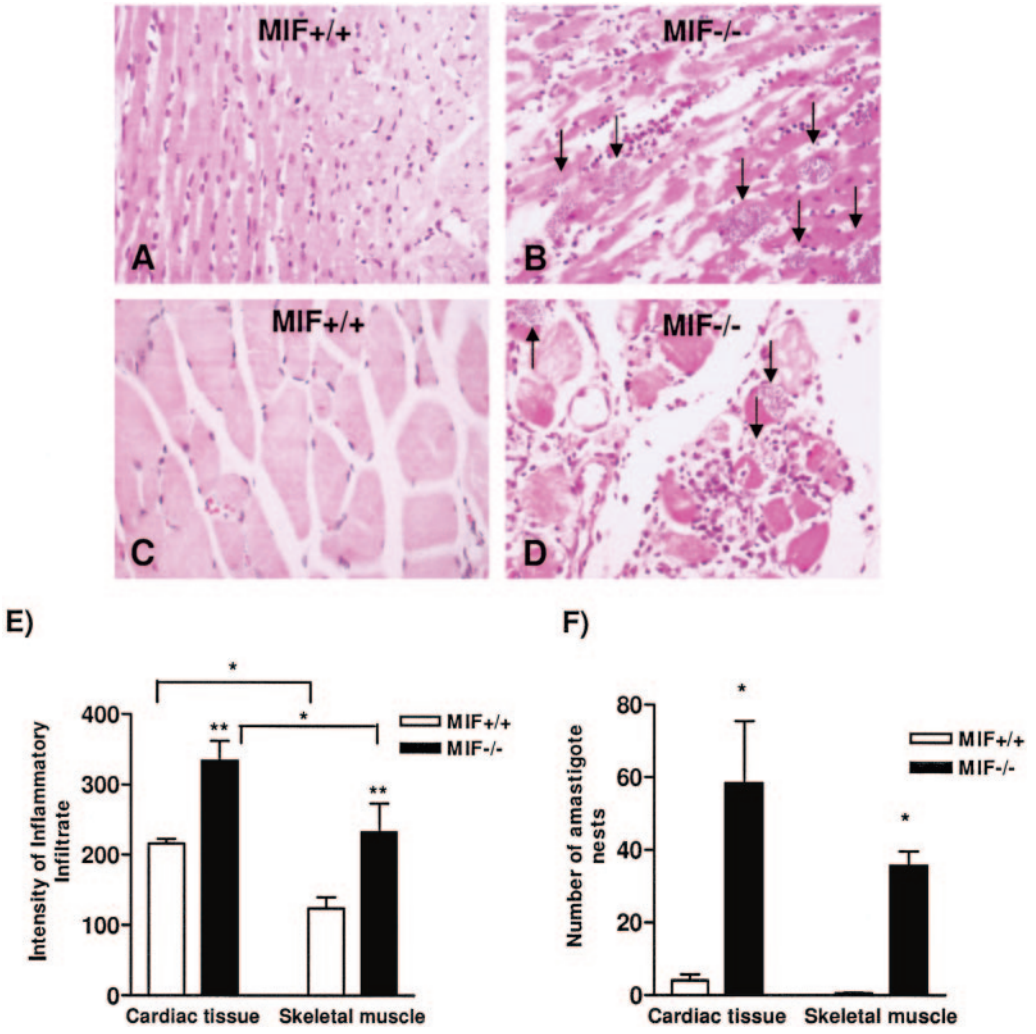


FIG. 5. Representative histology of *T. cruzi* parasitism and inflammation in cardiac and skeletal muscle tissues of $MIF^{+/+}$ and $MIF^{-/-}$ infected mice. Sections were stained with hematoxylin-eosin. (A and B) Heart histopathology of $MIF^{+/+}$ (A) and $MIF^{-/-}$ (B) mice infected with *T. cruzi*. (C and D) Histopathology of skeletal muscle in $MIF^{+/+}$ mice (C) and $MIF^{-/-}$ mice (D) infected with *T. cruzi*. (E) Number of inflammatory cells per field (25 histopathological fields per section) for cardiac and skeletal muscle sections in both $MIF^{+/+}$ and $MIF^{-/-}$ *T. cruzi*-infected mice. (F) Number of amastigote nests in 25 histopathological fields (*, $P < 0.02$). Data are expressed as means ± standard errors of the means ($n$ = 4 to 5 animals per group). Mice were killed at 22 days p.i. Arrows point to parasite nests. *, $P < 0.05$ for comparison of heart versus skeletal muscle in $MIF^{+/+}$ and $MIF^{-/-}$ mice; **, $P < 0.05$ for comparison of $MIF^{-/-}$ versus $MIF^{+/+}$ tissues.

nificantly more IFN-γ than those from wild-type mice, which is likely to contribute to cardiac immunopathology. Nonetheless, $MIF^{-/-}$ mice were unable to clear the parasites from the hearts despite intense cardiac inflammation, suggesting that the cardiac damage may be due to the large numbers of amastigote nests instead of immunity-mediated damage caused by inflammation. Although the mechanism by which MIF contributes to protective host immunity against *T. cruzi* is not entirely clear, perhaps the activation of infiltrating cells is adversely affected in the absence of MIF signaling, resulting in poor effector function and reduced killing of parasites.

A previous study demonstrated that MIF plays a critical role in the activation of T cells (2). However, adaptive immunity appears not to be affected by MIF deficiency, since *T. cruzi* antigen-stimulated spleen cells from both $MIF^{+/+}$ and $MIF^{-/-}$ mice proliferated similarly and produced comparable levels of Th1- and Th2-type cytokines 3 weeks after infection. Thus, impaired proinflammatory cytokine production cannot be attributed to excess production of IL-4 or IL-10, both of which are known to have anti-inflammatory effects and to participate in susceptibility to *T. cruzi* infection. These data are consistent with previous reports using $MIF^{-/-}$ mice for other parasitic models (51, 55). In fact, those studies demonstrated that the absence of MIF prevents the development of protective immunity against another intracellular parasite, *Leishmania major*, as well as the extracellular parasite *Taenia crassiceps*, by inhibiting macrophage microbicidal activity rather than preventing the development of Th1-like responses. Moreover, for both parasitic infections, higher levels of antigen-specific IFN-γ were detected in $MIF^{-/-}$ mice which failed to mediate host resistance. Thus, the finding that *T. cruzi* lysate-specific IFN-γ production was not impaired in $MIF^{-/-}$ mice late in infection

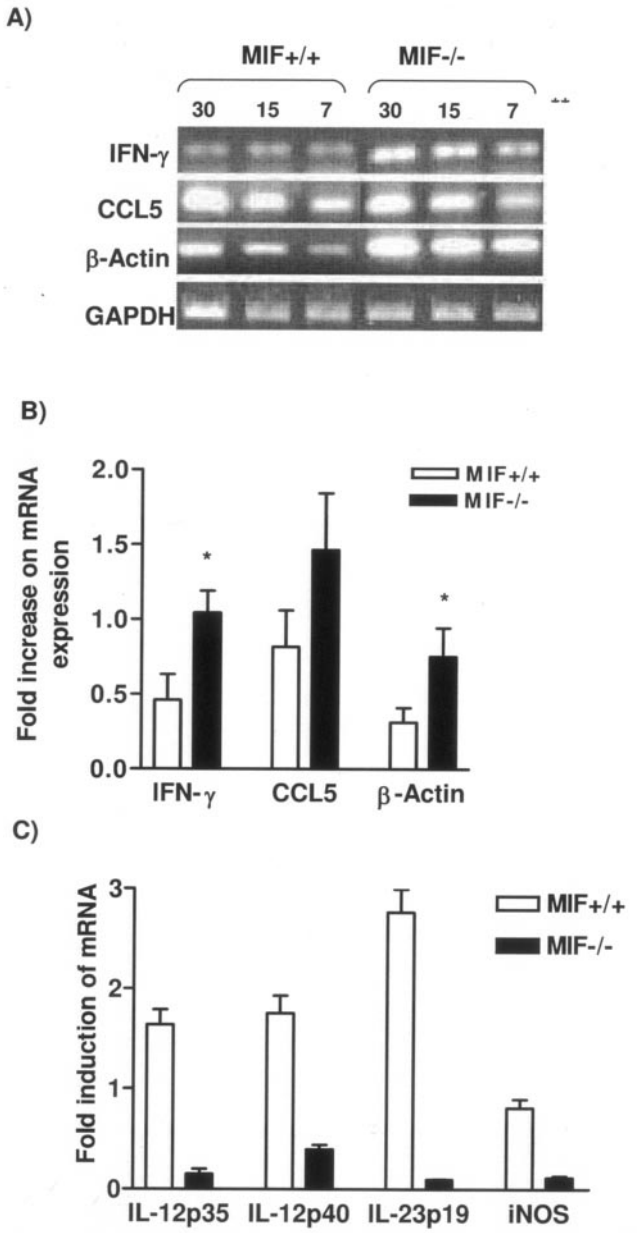


FIG. 6. RT-PCR and real-time RT-PCR analyses of cytokine mRNA expression in cardiac tissues isolated from $MIF^{+/+}$ and $MIF^{-/-}$ mice on day 22 after *T. cruzi* infection. (A) Semiquantitative RT-PCR analysis was performed using three different animals per group. (B) Densitometry analysis (using 7 ng of cDNA) showed a significant increase in the expression of IFN-γ and β-actin mRNAs in $MIF^{-/-}$ infected mice compared to $MIF^{+/+}$ infected mice. (C) Real-time RT-PCRs for IL-12 p35, IL-12 p40, and p19 (IL-23) were performed on the same tissues. *, $P < 0.05$ for comparison of $MIF^{-/-}$ versus $MIF^{+/+}$ mice regarding their respective cytokines. **, amount (ng) of cDNA used for PCR analysis. Experiments were repeated two times with similar results.

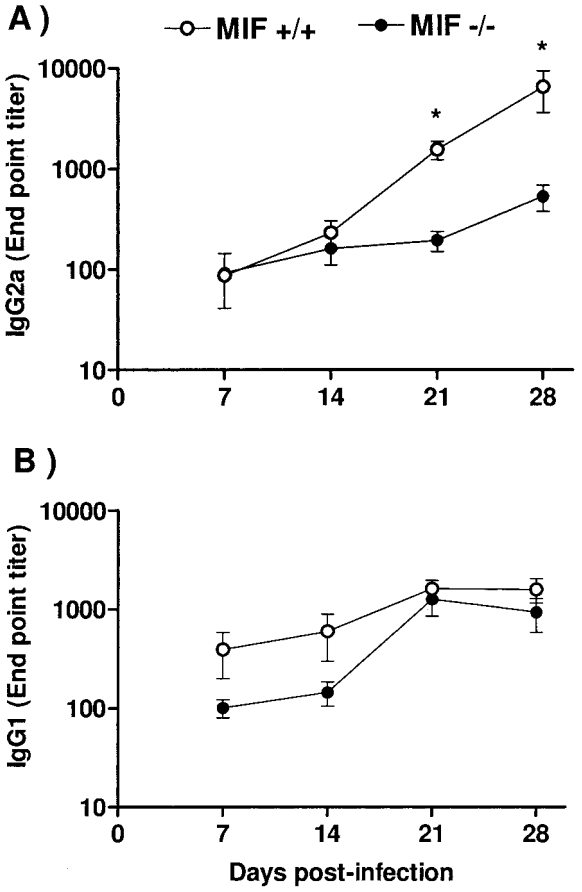


FIG. 7. Kinetics of antibody responses during *T. cruzi* infection in $MIF^{-/-}$ and $MIF^{+/+}$ mice. (A) Anti-*T. cruzi* lysate IgG2a; (B) anti-*T. cruzi* lysate IgG1. The graphs show means ± standard errors of the means ($n$ = at least 8 animals per group) and are representative of three independent experiments. *, $P < 0.05$ for comparison of $MIF^{-/-}$ versus $MIF^{+/+}$ mice at the same time point.

was not unexpected. It is significant, however, that despite the similar levels of circulating IFN-γ and the increase in mRNA expression of IFN-γ in cardiac tissue, $MIF^{-/-}$ mice displayed a deficit in *T. cruzi* lysate-specific IgG2a production. Interestingly, a recent report using an asthma model with $MIF^{-/-}$ mice showed a similar finding, in which specific anti-OVA IgG2a antibodies were significantly reduced compared with those in $MIF^{+/+}$ mice (42). In line with this, the absence of MIF could affect the ability of macrophages and B cells to respond to IFN-γ, and hence IFN-γ produced by $MIF^{-/-}$ mice may fail to induce the efficient macrophage activation and NO production required to eliminate *T. cruzi*. The reason for this lack of response remains unknown but can partially explain the lack of parasite clearance by $MIF^{-/-}$ mice.

The pathogenicity of *T. cruzi* strains has been associated with the expression of some virulence factors, such as *trans*-sialidases (50) and the molecule gp83, that parasites use to invade cells (62). Weakly invasive strains, such as Ninoa (20), either express low levels of these markers or do not express them at all. Thus, these strains are handicapped in the ability to rapidly invade target cells, and the host may have a longer opportunity to eliminate these parasites—through both innate and adaptive mechanisms, such as complement activation and specific antibodies—before they cause tissue damage. Hence, it is likely that a less intense immune response in $MIF^{-/-}$ mice is sufficient to control the nonpathogenic strains of *T. cruzi*.

In conclusion, our findings show that endogenous MIF is involved in host resistance against acute *T. cruzi* infection; the higher susceptibility of $MIF^{-/-}$ mice may involve mechanisms that impair innate cytokine production, suggesting that MIF participates in the activation of innate immunity to induce an early inflammatory cytokine response against *T. cruzi* infection.

## ACKNOWLEDGMENTS

This work was supported by DGAPA-PAPIIT-UNAM grant IN212804 to M.R.S. and grant IN226402 to B.E. and by an NIH grant to A.R.S.

We thank Ignacio Martínez, Benito S. Sánchez, and Rodrigo Velázquez for their technical assistance.

## REFERENCES

1. **Aliberti, J. C., M. A. Cardoso, G. A. Martins, R. T. Gazzinelli, L. Q. Vieira, and J. S. Silva.** 1996. Interleukin-12 mediates resistance to *Trypanosoma cruzi* in mice and is produced by murine macrophages in response to live trypomastigotes. Infect. Immun. **64:**1961–1967.
2. **Bacher, M., C. N. Metz, T. Calandra, K. Mayer, J. Chesney, M. Lohoff, D. Gemsa, T. Donnelly, and R. Bucala.** 1996. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. Proc. Natl. Acad. Sci. USA **93:**7849–7854.
3. **Becker, C., S. Wirtz, M. Blessing, J. Pirhonen, D. Strand, O. Bechthold, J. Frick, P. R. Galle, I. Autenrieth, and M. F. Neurath.** 2003. Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. J. Clin. Investig. **112:**693–706.
4. **Bernhagen, J., T. Calandra, R. A. Mitchell, S. B. Martin, K. J. Tracey, W. Voelter, K. R. Manogue, A. Cerami, and R. Bucala.** 1993. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. Nature **365:**756–759.
5. **Bloom, B. R., and B. Bennett.** 1966. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. Science **153:**80–82.
6. **Bosseno, M. F., C. Barnabe, E. Magallon Gastelum, F. Lozano Kasten, J. Ramsey, B. Espinoza, and S. F. Breniere.** 2002. Predominance of *Trypanosoma cruzi* lineage I in Mexico. J. Clin. Microbiol. **40:**627–632.
7. **Bozza, M., A. R. Satoskar, G. Lin, B. Lu, A. A. Humbles, C. Gerard, and J. R. David.** 1999. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. J. Exp. Med. **189:**341–346.
8. **Calandra, T., J. Bernhagen, R. A. Mitchell, and R. Bucala.** 1994. The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. J. Exp. Med. **179:**1895–1902.
9. **Calandra, T., B. Echtenacher, D. L. Roy, J. Pugin, C. N. Metz, L. Hultner, D. Heumann, D. Mannel, R. Bucala, and M. P. Glauser.** 2000. Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat. Med. **6:**164–170.
10. **Calandra, T., C. Froidevaux, C. Martin, and T. Roger.** 2003. Macrophage migration inhibitory factor and host innate immune defenses against bacterial sepsis. J. Infect. Dis. **187**(Suppl. 2)**:**S385–S390.
11. **Calandra, T., and T. Roger.** 2003. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat. Rev. Immunol. **3:**791–800.
12. **Campos, M. A., and R. T. Gazzinelli.** 2004. *Trypanosoma cruzi* and its components as exogenous mediators of inflammation recognized through Toll-like receptors. Mediators Inflamm. **13:**139–143.
13. **David, J. R.** 1966. Delayed hypersensitivity in vitro: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. Proc. Natl. Acad. Sci. USA **56:**72–77.
14. **de Jong, Y. P., A. C. Abadia-Molina, A. R. Satoskar, K. Clarke, S. T. Rietdijk, W. A. Faubion, E. Mizoguchi, C. N. Metz, M. Alsahli, T. ten Hove, A. C. Keates, J. B. Lubetsky, R. J. Farrell, P. Michetti, S. J. van Deventer, E. Lolis, J. R. David, A. K. Bhan, and C. Terhorst.** 2001. Development of chronic colitis is dependent on the cytokine MIF. Nat. Immunol. **2:**1061–1066.
15. **Faunce, D. E., and J. Stein-Streilein.** 2002. NKT cell-derived RANTES recruits APCs and CD8+ T cells to the spleen during the generation of regulatory T cells in tolerance. J. Immunol. **169:**31–38.
16. **Froidevaux, C., T. Roger, C. Martin, M. P. Glauser, and T. Calandra.** 2001. Macrophage migration inhibitory factor and innate immune responses to bacterial infections. Crit. Care Med. **29:**S13–S15.
17. **Gazzinelli, R. T., A. Talvani, M. M. Camargo, H. C. Santiago, M. A. Oliveira, L. Q. Vieira, G. A. Martins, J. C. Aliberti, and J. S. Silva.** 1998. Induction of cell-mediated immunity during early stages of infection with intracellular protozoa. Braz. J. Med. Biol. Res. **31:**89–104.
18. **Gomez-Garcia, L., R. Alejandre-Aguilar, A. Aranda-Fraustro, R. Lopez, and V. M. Monteon.** 2005. Description of inflammation and cytokine profile at the inoculation site and in heart tissue of mice re-infected with *Trypanosoma cruzi* vector derived-metacyclic trypomastigotes. Parasitology **130:**511–522.
19. **Hardison, J. L., R. A. Wrightsman, P. M. Carpenter, W. A. Kuziel, T. E. Lane, and J. E. Manning.** 2006. The CC chemokine receptor 5 is important in control of parasite replication and acute cardiac inflammation following infection with *Trypanosoma cruzi*. Infect. Immun. **74:**135–143.
20. **Hernandez, R., J. Herrera, M. F. Bosseno, S. F. Breniere, and B. Espinoza.** 2001. *Trypanosoma cruzi*: data supporting clonality in Mexican stocks. J. Parasitol. **87:**1178–1181.
21. **Holscher, C., G. Kohler, U. Muller, H. Mossmann, G. A. Schaub, and F. Brombacher.** 1998. Defective nitric oxide effector functions lead to extreme susceptibility of *Trypanosoma cruzi*-infected mice deficient in gamma interferon receptor or inducible nitric oxide synthase. Infect. Immun. **66:**1208–1215.
22. **Holscher, C., M. Mohrs, W. J. Dai, G. Kohler, B. Ryffel, G. A. Schaub, H. Mossmann, and F. Brombacher.** 2000. Tumor necrosis factor alpha-mediated toxic shock in *Trypanosoma cruzi*-infected interleukin 10-deficient mice. Infect. Immun. **68:**4075–4083.
23. **Hunter, C. A., L. A. Ellis-Neyes, T. Slifer, S. Kanaly, G. Grunig, M. Fort, D. Rennick, and F. G. Araujo.** 1997. IL-10 is required to prevent immune hyperactivity during infection with *Trypanosoma cruzi*. J. Immunol. **158:**3311–3316.
24. **Hunter, C. A., T. Slifer, and F. Araujo.** 1996. Interleukin-12-mediated resistance to *Trypanosoma cruzi* is dependent on tumor necrosis factor alpha and gamma interferon. Infect. Immun. **64:**2381–2386.
25. **Hunter, C. A., A. Villarino, D. Artis, and P. Scott.** 2004. The role of IL-27 in the development of T-cell responses during parasitic infections. Immunol. Rev. **202:**106–114.
26. **Juttner, S., J. Bernhagen, C. N. Metz, M. Rollinghoff, R. Bucala, and A. Gessner.** 1998. Migration inhibitory factor induces killing of *Leishmania major* by macrophages: dependence on reactive nitrogen intermediates and endogenous TNF-alpha. J. Immunol. **161:**2383–2390.
27. **Kleinert, H., C. Euchenhofer, I. Ihrig-Biedert, and U. Forstermann.** 1996. In murine 3T3 fibroblasts, different second messenger pathways resulting in the induction of NO synthase II (iNOS) converge in the activation of transcription factor NF-kappaB. J. Biol. Chem. **271:**6039–6044.
28. **Koebernick, H., L. Grode, J. R. David, W. Rohde, M. S. Rolph, H. W. Mittrucker, and S. H. Kaufmann.** 2002. Macrophage migration inhibitory factor (MIF) plays a pivotal role in immunity against *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **99:**13681–13686.
29. **Kong, Y. Z., X. Yu, J. J. Tang, X. Ouyang, X. R. Huang, G. Fingerle-Rowson, M. Bacher, L. A. Scher, R. Bucala, and H. Y. Lan.** 2005. Macrophage migration inhibitory factor induces MMP-9 expression: implications for destabilization of human atherosclerotic plaques. Atherosclerosis **178:**207–215.
30. **Kumar, S., and R. L. Tarleton.** 2001. Antigen-specific Th1 but not Th2 cells provide protection from lethal *Trypanosoma cruzi* infection in mice. J. Immunol. **166:**4596–4603.
31. **Kumar, S., and R. L. Tarleton.** 1998. The relative contribution of antibody production and CD8+ T cell function to immune control of *Trypanosoma cruzi*. Parasite Immunol. **20:**207–216.
32. **Langrish, C. L., Y. Chen, W. M. Blumenschein, J. Mattson, B. Basham, J. D. Sedgwick, T. McClanahan, R. A. Kastelein, and D. J. Cua.** 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J. Exp. Med. **201:**233–240.
33. **Laucella, S. A., M. Postan, D. Martin, B. Hubby Fralish, M. C. Albareda, M. G. Alvarez, B. Lococo, G. Barbieri, R. J. Viotti, and R. L. Tarleton.** 2004. Frequency of interferon-gamma-producing T cells specific for *Trypanosoma cruzi* inversely correlates with disease severity in chronic human Chagas disease. J. Infect. Dis. **189:**909–918.
34. **Livak, K. J., and T. D. Schmittgen.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods **25:**402–408.
35. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
36. **Lue, H., R. Kleemann, T. Calandra, T. Roger, and J. Bernhagen.** 2002. Macrophage migration inhibitory factor (MIF): mechanisms of action and role in disease. Microbes Infect. **4:**449–460.
37. **Martiney, J. A., B. Sherry, C. N. Metz, M. Espinoza, A. S. Ferrer, T. Calandra, H. E. Broxmeyer, and R. Bucala.** 2000. Macrophage migration inhibitory factor release by macrophages after ingestion of *Plasmodium chabaudi*-infected erythrocytes: possible role in the pathogenesis of malarial anemia. Infect. Immun. **68:**2259–2267.
38. **Matsumura, T., H. Hayashi, T. Takii, C. F. Thorn, A. S. Whitehead, J. Inoue, and K. Onozaki.** 2004. TGF-beta down-regulates IL-1alpha-induced TLR2 expression in murine hepatocytes. J. Leukoc. Biol. **75:**1056–1061.
39. **Meirelles, M. N., M. C. Pereira, R. H. Singer, M. N. Soeiro, L. R. Garzoni, D. T. Silva, H. S. Barbosa, T. C. Araujo-Jorge, M. O. Masuda, M. A. Capella, A. G. Lopes, and A. B. Vermelho.** 1999. *Trypanosoma cruzi*-cardiomyocytes: new contributions regarding a better understanding of this interaction. Mem. Inst. Oswaldo Cruz **94**(Suppl. 1)**:**149–152.
40. **Michailowsky, V., N. M. Silva, C. D. Rocha, L. Q. Vieira, J. Lannes-Vieira, and R. T. Gazzinelli.** 2001. Pivotal role of interleukin-12 and interferon-gamma axis in controlling tissue parasitism and inflammation in the heart and central nervous system during *Trypanosoma cruzi* infection. Am. J. Pathol. **159:**1723–1733.
41. **Mikulowska, A., C. N. Metz, R. Bucala, and R. Holmdahl.** 1997. Macrophage

migration inhibitory factor is involved in the pathogenesis of collagen type II-induced arthritis in mice. J. Immunol. **158:**5514–5517.
42. **Mizue, Y., S. Ghani, L. Leng, C. McDonald, P. Kong, J. Baugh, S. J. Lane, J. Craft, J. Nishihira, S. C. Donnelly, Z. Zhu, and R. Bucala.** 2005. Role for macrophage migration inhibitory factor in asthma. Proc. Natl. Acad. Sci. USA **102:**14410–14415.
43. **Monteon, V. M., J. Furuzawa-Carballeda, R. Alejandre-Aguilar, A. Aranda-Fraustro, J. L. Rosales-Encina, and P. A. Reyes.** 1996. American trypanosomosis: in situ and generalized features of parasitism and inflammation kinetics in a murine model. Exp. Parasitol. **83:**267–274.
44. **Muller, U., G. Kohler, H. Mossmann, G. A. Schaub, G. Alber, J. P. Di Santo, F. Brombacher, and C. Holscher.** 2001. IL-12-independent IFN-gamma production by T cells in experimental Chagas' disease is mediated by IL-18. J. Immunol. **167:**3346–3353.
45. **Murray, L. J., R. Lee, and C. Martens.** 1990. In vivo cytokine gene expression in T cell subsets of the autoimmune MRL/Mp-lpr/lpr mouse. Eur. J. Immunol. **20:**163–170.
46. **Oddo, M., T. Calandra, R. Bucala, and P. R. Meylan.** 2005. Macrophage migration inhibitory factor reduces the growth of virulent *Mycobacterium tuberculosis* in human macrophages. Infect. Immun. **73:**3783–3786.
47. **Ohkawara, T., H. Takeda, J. Nishihira, K. Miyashita, M. Nihiwaki, Y. Ishiguro, K. Takeda, S. Akira, T. Iwanaga, T. Sugiyama, and M. Asaka.** 2005. Macrophage migration inhibitory factor contributes to the development of acute dextran sulphate sodium-induced colitis in Toll-like receptor 4 knockout mice. Clin. Exp. Immunol. **141:**412–421.
48. **Oliveira, A. C., J. R. Peixoto, L. B. de Arruda, M. A. Campos, R. T. Gazzinelli, D. T. Golenbock, S. Akira, J. O. Previato, L. Mendonca-Previato, A. Nobrega, and M. Bellio.** 2004. Expression of functional TLR4 confers proinflammatory responsiveness to *Trypanosoma cruzi* glycoinositolphospholipids and higher resistance to infection with *T. cruzi*. J. Immunol. **173:**5688–5696.
49. **Ponchel, F., C. Toomes, K. Bransfield, F. T. Leong, S. H. Douglas, S. L. Field, S. M. Bell, V. Combaret, A. Puisieux, A. J. Mighell, P. A. Robinson, C. F. Inglehearn, J. D. Isaacs, and A. F. Markham.** 2003. Real-time PCR based on SYBR-green I fluorescence: an alternative to the TaqMan assay for a relative quantification of gene rearrangements, gene amplifications and micro gene deletions. BMC Biotechnol. **3:**18.
50. **Risso, M. G., G. B. Garbarino, E. Mocetti, O. Campetella, S. M. Gonzalez Cappa, C. A. Buscaglia, and M. S. Leguizamon.** 2004. Differential expression of a virulence factor, the trans-sialidase, by the main *Trypanosoma cruzi* phylogenetic lineages. J. Infect. Dis. **189:**2250–2259.
51. **Rodriguez-Sosa, M., L. E. Rosas, J. R. David, R. Bojalil, A. R. Satoskar, and L. I. Terrazas.** 2003. Macrophage migration inhibitory factor plays a critical role in mediating protection against the helminth parasite *Taenia crassiceps*. Infect. Immun. **71:**1247–1254.
52. **Roger, T., J. David, M. P. Glauser, and T. Calandra.** 2001. MIF regulates innate immune responses through modulation of Toll-like receptor 4. Nature **414:**920–924.
53. **Roger, T., C. Froidevaux, C. Martin, and T. Calandra.** 2003. Macrophage migration inhibitory factor (MIF) regulates host responses to endotoxin through modulation of Toll-like receptor 4 (TLR4). J. Endotoxin Res. **9:**119–123.
54. **Roger, T., M. P. Glauser, and T. Calandra.** 2001. Macrophage migration inhibitory factor (MIF) modulates innate immune responses induced by endotoxin and gram-negative bacteria. J. Endotoxin Res. **7:**456–460.
55. **Satoskar, A. R., M. Bozza, M. Rodriguez-Sosa, G. Lin, and J. R. David.** 2001. Migration inhibitory factor gene-deficient mice are susceptible to cutaneous *Leishmania major* infection. Infect. Immun. **69:**906–911.
56. **Stavitsky, A. B., C. Metz, S. Liu, J. Xianli, and R. Bucala.** 2003. Blockade of macrophage migration inhibitory factor (MIF) in *Schistosoma japonicum*-infected mice results in an increased adult worm burden and reduced fecundity. Parasite Immunol. **25:**369–374.
57. **Talvani, A., C. S. Ribeiro, J. C. Aliberti, V. Michailowsky, P. V. Santos, S. M. Murta, A. J. Romanha, I. C. Almeida, J. Farber, J. Lannes-Vieira, J. S. Silva, and R. T. Gazzinelli.** 2000. Kinetics of cytokine gene expression in experimental chagasic cardiomyopathy: tissue parasitism and endogenous IFN-gamma as important determinants of chemokine mRNA expression during infection with *Trypanosoma cruzi*. Microbes Infect. **2:**851–866.
58. **Tarleton, R. L.** 1995. The role of T cells in *Trypanosoma cruzi* infections. Parasitol. Today **11:**7–9.
59. **Tarleton, R. L., M. J. Grusby, and L. Zhang.** 2000. Increased susceptibility of Stat4-deficient and enhanced resistance in Stat6-deficient mice to infection with *Trypanosoma cruzi*. J. Immunol. **165:**1520–1525.
60. **Teixeira, M. M., R. T. Gazzinelli, and J. S. Silva.** 2002. Chemokines, inflammation and *Trypanosoma cruzi* infection. Trends Parasitol. **18:**262–265.
61. **Ulett, G. C., N. Ketheesan, and R. G. Hirst.** 2000. Cytokine gene expression in innately susceptible BALB/c mice and relatively resistant C57BL/6 mice during infection with virulent *Burkholderia pseudomallei*. Infect. Immun. **68:**2034–2042.
62. **Villalta, F., Y. Zhang, K. E. Bibb, J. M. Burns, Jr., and M. F. Lima.** 1998. Signal transduction in human macrophages by gp83 ligand of *Trypanosoma cruzi*: trypomastigote gp83 ligand up-regulates trypanosome entry through the MAP kinase pathway. Biochem. Biophys. Res. Commun. **249:**247–252.
63. **Yoshida, A., H. Maruyama, T. Kumagai, T. Amano, F. Kobayashi, M. Zhang, K. Himeno, and N. Ohta.** 2000. *Schistosoma mansoni* infection cancels the susceptibility to *Plasmodium chabaudi* through induction of type 1 immune responses in A/J mice. Int. Immunol. **12:**1117–1125.

*Editor:* J. F. Urban, Jr.