Interleukin-17-Mediated Control of Parasitemia in Experimental Trypanosoma congolense Infection in Mice

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Received 18 February 2010/Returned for modification 22 March 2010/Accepted 8 September 2010

BALB/c mice are highly susceptible to experimental Trypanosoma congolense infections, whereas C57BL/6 mice are relatively resistant. Infected highly susceptible BALB/c mice die of systemic inflammatory response syndrome. Because interleukin-17 (IL-17) and Th17 cells regulate inflammatory responses, we investigated their role in the pathogenesis of experimental African trypanosomiasis in mice. We show that the production of IL-17 by spleen and liver cells and the serum IL-17 level increased after T. congolense infection in mice. Interestingly, infected highly susceptible BALB/c mice produced more IL-17 and had more Th17 cells than infected relatively resistant C57BL/6 mice. Paradoxically, neutralization of IL-17 with anti-IL-17 monoclonal antibody in vivo induced higher parasitemia in both the susceptible and the relatively resistant mice. Interestingly, anti-IL-17 antibody-treated mice had higher serum levels of alanine aminotransferase and aspartate aminotransferase, and the production of IL-10 and nitric oxide by liver cells was markedly decreased. Moreover, recombinant IL-17-treated mice exhibited significantly faster parasite control and lower peak parasitemia compared to control mice. Collectively, these results suggest that the IL-17/Th17 axis plays a protective role in murine experimental African trypanosomiasis.

African trypanosomes are extracellular protozoan parasites that cause fatal disease in humans and domestic livestock in sub-Saharan Africa. The disease is endemic in 36 countries, and millions of people are at risk of suffering from human African trypanosomiasis. Trypanosomiasis in animals is caused by Trypanosoma congolense, Trypanosoma brucei brucei, and Trypanosoma vivax, but T. congolense is the most important cause of disease for livestock (29). It is estimated that the disease costs $1.3 billion to livestock producers and consumers every year (17).

African trypanosomes have developed very sophisticated mechanisms to evade the host’s immune defenses (39, 40). The indigenous African and exotic European breeds of cattle are relatively resistant and susceptible, respectively, to African trypanosomiasis (28). In the laboratory, BALB/c mice are highly susceptible to experimental T. congolense infections, whereas C57BL/6 mice are relatively resistant, as measured by levels of parasitemia and survival time. When infected intraperitoneally (i.p.) with 10⁷ T. congolense, BALB/c mice have a mean survival time of 8.5 ± 0.5 days, whereas C57BL/6 mice survive for more than 100 days (40). Infected BALB/c mice die of systemic inflammatory response syndrome (SIRS), which is associated with high production of proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), IL-12, and gamma interferon (IFN-γ) (12, 37, 39, 40, 43, 45). Interestingly, blockade of IL-10 signaling either by anti-IL-10R monoclonal antibody (MAb) treatment (35, 36, 38) or knocking out the IL-10 gene (7) induces early death in the infected relatively resistant C57BL/6 mice. Thus, IL-10 is crucial for controlling proinflammatory cytokine storm that results lethal SIRS.

Recently, T helper 17 (Th17) cells have been identified as a new T-helper subset (3, 10, 16, 33). A hallmark of Th17 cells is the production of IL-17A (also called IL-17), a proinflammatory cytokine that favors Th17 differentiation and their effector cytokines have both pathological and protective effects. These observations indicate that Th17 cells and their effector cytokines have both pathological and protective roles during inflammation and infections, respectively. There is as yet no report on the role of IL-17 and Th17 cells in resistance to African trypanosomes. Because T. congolense-infected BALB/c mice have high serum levels of IL-6, and their macrophages elaborate high amounts of IL-6 after in vitro infection (12), a cytokine that favors Th17 differentiation and IL-17 production (3, 16), we hypothesized that IL-17 and/or Th17 cells play important roles in resistance to T. congolense infection in mice by contributing to excessive inflammatory response. However, the data presented here suggest that IL-17 may be playing some protective role, particularly in controlling early parasitemia in mice infected with T. congolense.

MATERIALS AND METHODS

Mice. Six- to eight-week-old female BALB/c, C57BL/6, and outbred Swiss white (CD1) mice were obtained from Charles River, St. Constante, Quebec, Canada. All mice were housed in the university animal facilities and were maintained according to the recommendations of the Canadian Council of Animal Care.
Parasites. Cryopreserved *T. congolense* variant antigenic type TC13 were passed in immunosuppressed CD1 mice as previously described (32). Parasites were isolated from the blood of CD1 mice 3 days after passage by DEAE-cellulose anion-exchange chromatography (19).

Infections, estimation of parasite burden, and cell preparations. For infection, mice were i.p. injected with $10^5$ TC13 in 100 μl of Tris-saline-glucose. To estimate daily parasitemia, a drop of blood was taken from the tail vein of each infected mouse and parasitemia was estimated by counting the number of parasites at a $\times 400$ magnification by microscopy. At different days postinfection, mice were sacrificed, and spleen and liver cells were prepared as previously described (1, 7), cultured for 48 h in complete medium (Dulbecco modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 U of penicillin/ml, and 100 μg of streptomycin/ml), and the supernatant fluids were used for cytokine determination by enzyme-linked immunosorbent assay (ELISA).

**In vivo IL-17 neutralization.** Lyophilized rat anti-mouse IL-17 MAb and control rat IgG (R&D Systems, Minneapolis, MN) were resuspended in phosphate-buffered saline (PBS). For BALB/c mice, anti-IL-17 antibody or rat IgG was injected i.p. into mice at days 1, 2, 4, and 6 (100 μg/mouse) postinfection. At day 7, mice were euthanized, and sera, spleens, and livers were collected for further analysis. For C57BL/6 mice, anti-IL-17 antibody was administered at days 1, 2, 4, 6, 8, and 10 (100 μg/mouse). Infected C57BL/6 mice were sacrificed at days 8 and 30 postinfection, and sera, spleens, and livers were collected for further analysis.

**Recombinant IL-17 treatment in vivo.** Lyophilized recombinant murine IL-17 (rIL-17; R&D Systems, Minneapolis, MN) was resuspended in sterile PBS at a final concentration of 100 μg/mouse. rIL-17 was injected i.p. into infected mice at the same time points as anti-IL-17 antibody.

**FIG. 1.** Increased numbers of IL-17-producing cells and IL-17 production in BALB/c mice infected with *T. congolense*. Infected BALB/c mice were sacrificed at different days postinfection (as indicated), and the levels of IL-17 in the culture supernatant fluids of spleen and liver cells (A and B) and in serum (C) were determined by ELISA. At the time of sacrifice, spleen and liver cells were directly stained ex vivo for intracellular IL-17, and the percentage of CD4$^+$ IL-17$^+$ (D and F) and CD4$^+$ IL-17$^+$ (E and G) cells was determined within CD3$^+$ cells by flow cytometry. The percentage of γδ TCR$^+$ cells in spleens and livers from infected mice (day 8 postinfection) was also determined within CD3$^+$ CD4$^+$ IL-17$^+$ cells (H). The results are presented as means ± standard errors (SE) and are representative of four (A to C), three (D to G), and two (H) independent experiments ($n = 3$ mice) with similar results. *, $P < 0.05$; ***, $P < 0.001$.
RESULTS

Kinetics of IL-17/Th17 in BALB/c mice infected with *T. congolense*. Because the IL-17/Th17 axis is associated with inflammation (3, 10, 16, 33) and BALB/c mice infected with *T. congolense* die of SIRS (39, 40), we hypothesized that the IL-17/Th17 axis contributes to excessive inflammation in infected mice. After *T. congolense* infection, the production of IL-17 by spleen cells from infected mice increased with time (Fig. 1A), and this was positively correlated with an increase in parasitemia (*r* = 0.74). Liver cells also produced a substantial amount of IL-17 after infection (Fig. 1B), and the serum IL-17 level also increased, peaking at day 8 postinfection (Fig. 1C).

The majority of IL-17-producing cells in both the spleens and the livers of infected mice were predominantly CD3⁺ (data not shown). Interestingly, although the percentage of CD4⁺IL-17⁺ (conventional Th17) cells within the CD3⁺ population increased (Fig. 1D and F), the majority of IL-17⁺ cells did not coexpress CD4 molecules, suggesting that these CD4⁺ IL-17⁺ cells are nonclassical Th17 cells (Fig. 1E and G). To further investigate the phenotype of CD3⁺ CD4⁺ IL-17⁺-producing cells, we costained the cells for CD8 and γδ T-cell receptor (TCR) expression. We found that majority of the CD3⁺ CD4⁺ IL-17⁺ cells were γδ T cells (Fig. 1H), and only ca. 5% of these were CD8⁺ cells (data not shown).

We also determined the levels of other proinflammatory cytokines in the serum and culture supernatant fluids of spleen and liver cells. Similar to previous reports (36, 43, 48), the serum levels of IL-6 (Fig. 2A), TNF-α (Fig. 2B), and IFN-γ (Fig. 2C) gradually increased after infection. Interestingly, the liver (and not the spleen) was the earlier source of these proinflammatory cytokines after infection (Table 1). Similar to IL-17, liver cells expressed more IL-6 (Fig. 2D) and TNF (Fig. 2E) at day 5, but the highest IFN-γ production by liver cells was at day 8 postinfection (Fig. 2F). Collectively, these results show that the spleens and livers of the highly susceptible BALB/c mice produce IL-17 after *T. congolense* infection, and this is correlated with increased parasitemia and the production of proinflammatory cytokines.

### TABLE 1. Production of proinflammatory cytokines by spleen and liver cells from *T. congolense*-infected BALB/c mice at days 2, 5, and 8 postinfectiona

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Spleen cells</th>
<th>Liver cells</th>
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<tr>
<td></td>
<td>Day 2</td>
<td>Day 5</td>
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<tr>
<td>IL-17 (pg/ml)</td>
<td>ND</td>
<td>61 ± 10</td>
</tr>
<tr>
<td>IL-6 (ng/ml)</td>
<td>4 ± 2</td>
<td>44 ± 37</td>
</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>224 ± 116</td>
<td>4,011 ± 1,028</td>
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<tr>
<td>TNF (pg/ml)</td>
<td>666 ± 235</td>
<td>774 ± 310</td>
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* BALB/c mice were infected i.p. with *Trypanosoma congolense* clone TC13. At days 2, 5, and 8 postinfection, the mice were sacrificed, spleen and liver cells were cultured for 48 h, and the culture supernatant fluids were assayed for cytokines by ELISA. *P* < 0.05, versus spleen cells. ND, not detected.
Comparison of IL-17 level in BALB/c and C57BL/6 mice infected with *T. congolense*. The results above suggested that increased IL-17/Th17 response is associated with increased production of proinflammatory cytokines and may contribute to excessive inflammation and pathology in *T. congolense*-infected BALB/c mice. If this was true, IL-17 levels and numbers of Th17 cells should be different in infected highly susceptible BALB/c and relatively resistant C57BL/6 mice. As previously reported (43), infected BALB/c mice displayed higher parasitemia than infected C57BL/6 at days 7 and 8 postinfection (Fig. 3A). In addition, while the level of IL-17 increased in the serum of infected BALB/c mice, it remained relatively unchanged in infected C57BL/6 mice (Fig. 3B). Furthermore, spleen and liver cells of infected BALB/c mice produced higher amounts of IL-17 in cultures (Fig. 3C and D) and contain higher numbers of IL-17-producing cells than those from infected C57BL/6 (Fig. 3E to H). In agreement with previous reports (36, 43, 48), the serum levels of IL-6 (Fig. 4A) and TNF-α (Fig. 4B) were also higher in infected BALB/c mice than in samples from infected C57BL/6. In contrast, the disease-ameliorating cytokine, IL-10, was lower (although not significant) in the serum (Fig. 4D) and supernatant fluids of liver cells (Fig. 4H) from BALB/c than C57BL/6 mice. This finding is in contrast to a previous report that showed a higher level of IL-10 in serum of infected BALB/c mice (44) and could reflect differences in source of mice and housing facilities. Collectively, the data thus far show that IL-17 production is correlated with the production of proinflammatory cytokines and higher parasitemia and therefore suggest that this cytokine may contribute to susceptibility to experimental African trypanosomiasis.

**IL-17 is important for the control of parasitemia in *T. congolense*-infected mice.** To determine whether IL-17 plays a role in the pathogenesis of experimental *T. congolense* infection, we treated infected C57BL/6 mice with anti-IL-17 antibody to neutralize IL-17 activity and monitored the parasitemia levels. The dose and regimen of antibody treatment used here has been shown to effectively neutralize IL-17 activity in vivo in different experimental models (4, 8, 31). Surprisingly, anti-IL-17 antibody-treated mice had higher peak parasitemia throughout the infection period. At day 6 postinfection, neutralization of IL-17 caused statistically significant (*P* < 0.01) increase in parasitemia level (Fig. 5A). Furthermore, the subsequent peaks of undulating parasitemia in treated mice were higher than those of control mice. A similar protective effect was also observed in anti-IL-17 MAb-treated BALB/c mice (data not shown). To further confirm the protective role of IL-17 in experimental *T. congolense* infection, infected C57BL/6 mice were also treated with rIL-17. rIL-17-treated mice had significantly lower parasitemia at days 7, 8, and 9 postinfection and control their first wave of parasitemia faster than untreated controls (Fig. 5B). Taken together, these results indicate that IL-17 plays a role in the control of parasitemia in experimental murine *T. congolense* infection.

At day 8 postinfection, some antibody-treated C57BL/6 mice were sacrificed, and IL-17, IL-10, TNF, and IFN-γ levels were determined.
production by liver cells were determined. We found the production of IL-17, IL-10, and IFN-γ by liver cells significantly ($P < 0.05$) decreased in anti-IL-17 antibody-treated mice (Fig. 6A to C). In contrast, the production of TNF was not different between treated and control groups of mice (Fig. 6D). Consistent with the ELISA data, liver cells from anti-IL-17 antibody-treated mice contain significantly ($P < 0.05$) less CD4$^+$ IL-17$^+$ and CD4$^+$ IL-17$^-$ (Fig. 6E) cells than their isotype-treated controls. Similar to IL-17, liver cells from anti-IL-17 antibody-treated mice also contain less CD4$^+$ IL-10$^+$ cells and CD4$^+$ IL-10$^-$ cells (Fig. 6F) than their controls. Interestingly, anti-IL-17 antibody-treated mice had higher serum levels of ALT (Fig. 6G) and AST (Fig. 6H), suggestive of increased hepatic tissue damage after anti-IL-17 MAAb treatment. Moreover, the production of nitric oxide (NO), which has both cytostatic and cytolytic effects on trypanosomes (11, 21, 47), by spleen and liver cells from treated mice was significantly ($P < 0.05$) reduced (Fig. 6I). However, treatment with anti-IL-17 MAAb did not alter the production of parasitic-specific antibodies in infected mice (Fig. 6J). Collectively, these results suggest that impaired NO production and increased liver injury could be involved in the reduced ability of anti-IL-17 MAAb-treated mice to control parasitemia.

**Expression of IL-17RA in BALB/c and C57BL/6 mice.** Anti-IL-17 MAAb treatment resulted in higher parasitemia, indicating
that IL-17 might be playing a protective role in *T. congolense* infection in mice. To understand why infected BALB/c mice die despite having higher IL-17 levels in serum, we compared the kinetic of IL-17RA expression by hepatic cells in infected BALB/c and C57BL/6 mice. At all times tested, the percentage of IL-17RA⁺ cells was not significantly different in liver from infected BALB/c and C57BL/6 mice (Fig. 7A). However, the mean fluorescence intensity (MFI) of IL-17RA⁺ cells was significantly higher in C57BL/6 mice than in BALB/c mice at day 2 postinfection (Fig. 7B). However, beyond this time, there was no significant difference in the expression of this receptor in these two strains of mice.

**DISCUSSION**

The primary objective of the present study was to determine whether experimental *T. congolense* infection in mice induces the production of IL-17 and/or Th17 cells and whether these play any significant role in the pathogenesis of the diseases. After infection with *T. congolense*, the highly susceptible BALB/c mice produce high amounts of proinflammatory cytokines, including TNF, IL-6, and IL-12 (36, 43, 48). The production of these cytokines, particularly IL-6 is usually associated with Th17 development and production of high levels of IL-17, which is generally regarded as a master regulator of inflammation (15, 20, 24, 46). In the present study, we found that *T. congolense* infection led to a higher serum level of IL-17 and more IL-17 production by spleen and liver cells in the highly susceptible BALB/c than those in the relatively resistant C57BL/6 mice. These results prompted us to think that IL-17 might be associated with excessive inflammation and play critical role in development of SIRS and death in infected highly susceptible mice.
Surprisingly, neutralization of IL-17 activity in both the susceptible and the relatively resistant mice resulted in higher parasitemia, indicating that IL-17 might be playing a protective role in *T. congoense* infection in mice. This observation is consistent with previous reports showing that IL-17 plays a protective role in several infectious diseases (2, 9, 14, 34). Indeed, we found that treatment of infected mice with rIL-17 dramatically reduced peak parasitemia and led to faster parasite control, confirming a critical role of IL-17 in parasite infection. If IL-17 plays a protective role in trypanosome infection, why did infected BALB/c mice die despite having higher IL-17 levels in their serum, and their liver and spleen cells contain more IL-17+ cells than the relatively resistant C57BL/6 mice? The difference in parasitemia between anti-IL-17 MAb-treated and control mice was unrelated to differences in anti-*T. congoense*-specific antibody response because we found no differences in the magnitude and quality of anti-*T. congoense* antibody levels in the sera of anti-IL-17 MAb-treated and control mice (see Fig. 6J). We speculate that, among many reasons, differences in IL-17 receptor (IL-17R) expression leading to differences in responsiveness to IL-17 in BALB/c and C57BL/6 might play an important role in this process. IL-17 (particularly IL-17A and IL-17F) requires homo/heterodimeric complexes of IL-17 receptor A (IL-17RA) and IL-17RC for mediating productive physiologic signaling (6, 41). It is possible that IL-17R expression by IL-17-responsive cells is different in BALB/c and C57BL/6 mice after *T. congoense* infection. In line with this, we have found that by day 2 postinfection, the intensity of IL-17RA expression on liver cells from infected C57BL/6 mice was significantly higher than those from infected BALB/c mice. We speculate that this difference in early IL-17RA expression might significantly impact on early responsiveness to IL-17 and hence the outcome of parasite control in these mice.

Although anti-IL-17 antibody treatment in vivo induced higher parasitemia in BALB/c and C57BL/6 mice, the production of other proinflammatory cytokines, including TNF, by spleen (data not shown) and liver (Fig. 6D and F) cells from control and anti-IL-17 antibody-treated mice was not significantly different. However, the percentage of IL-10+ cells and the production of IL-10 in cultures by liver cells were significantly lower in anti-IL-17 MAb-treated mice at day 8 postinfection (Fig. 6B). Previous reports have shown that IL-10-deficient mice on the relatively resistant C57BL/6 background die within 10 days of *T. congoense* infection, a finding akin to the highly susceptible mice (7), and that blockade of IL-10 signaling by treatment with anti-IL-10R MAb also shortened the survival period of the relatively resistant mice (35, 36, 38, 40). In addition, IL-10 has been proposed to play an important role in dampening inflammation in the liver, thereby protecting this important organ for parasite clearance from inflammation-induced tissue damage (7). Thus, the decreased IL-10 production in the liver of anti-IL-17 MAb-treated mice might favor excessive inflammation and tissue damage leading to impaired parasite clearance. However, when we measured this cytokine by day 30 postinfection, there was not much difference in its production by cells from control and anti-IL-17-antibody-treated mice (data not shown). It is conceivable that the protective role of IL-17 in *T. congoense* infection might be effective only during the early days of infection, and its effect becomes insignificant once the chronic disease state is attained.

The production of nitric oxide (NO) via the inducible NO synthase (iNOS) is required for effective control of parasitemia in *T. congoense*-infected mice (22, 23). Nitric oxide has both cytolytic and cytostatic effects on African trypanosomes, and inhibition of NO production via the iNOS pathway exacerbates parasite growth in vitro and in vivo (11, 21, 47). Recently, it has been reported that IL-17 enhances the expression of iNOS gene and NO production via signaling through the p38 mitogen-activated protein kinase pathway (18, 25–27). Interestingly, we found that treatment of infected mice with anti-IL-17 MAb leads to significant reduction in IL-17 and NO production by both spleen and liver cells (see Fig. 6A and I). It is conceivable that during *T. congoense* infection in mice, IL-17 might also induce NO production by liver cells, and this plays a protective role in *T. congoense* infection by limiting parasite growth in vivo. Although anti-IL-17 antibody treatment in vivo induced higher parasitemia in BALB/c and C57BL/6 mice, the production of other proinflammatory cytokines, including TNF, by spleen (data not shown) and liver (Fig. 6D and F) cells from control and anti-IL-17 antibody-treated mice was not significantly different. However, the percentage of IL-10+ cells and the production of IL-10 in cultures by liver cells were significantly lower in anti-IL-17 MAb-treated mice at day 8 postinfection (Fig. 6B).
growth. Moreover, since IFN-γ induces NO production (11), and there was lower IFN-γ production by liver cells from anti-IL-17-treated mice (see Fig. 6C), it is possible that the suppression of NO production in anti-IL-17-treated mice might be related to the observed decrease in IFN-γ production by cells from antibody-treated mice. Furthermore, we found that anti-IL-17 MAb treatment resulted in greater hepatic injury, as evidenced by increased levels of AST and ALT in the sera of treated mice. Previous reports suggest that increased liver injury is associated with an inability to control parasitemia and susceptibility to experimental African trypanosomiasis (7). Thus, it is conceivable that the suppression of NO production and increased hepatic damage act together to negatively impact on parasite control in anti-IL-17 MAb-treated mice.

In summary, we have shown that experimental infection of mice with *T. congolense* induces IL-17 production and Th17 cells in the spleens and livers of infected mice, and IL-17 plays a protective role (control of parasitism) in both highly susceptible and relatively resistant mice. Although neutralization of IL-17 activity did not affect the survival time of infected mice, it led to decreased IL-10 and nitric oxide production by liver cells early during infection. We hypothesize that IL-17-related increase in IL-10 and NO production by liver cells of mice that might be able to inhibit excessive inflammation and promote parasite killing, respectively, leading to enhanced resistance to *T. congolense*.

**ACKNOWLEDGMENTS**

We thank members of the Parasite Vaccine Development Laboratory for their technical assistance, insightful comments, and constructive criticism.

This study was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC). Z.M. is supported by the Manitoba Institute of Child Health (MICH) postdoctoral fellowship.

We have no financial conflict of interest.

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