Interleukin-12 Promotes Pathologic Liver Changes and Death in Mice Coinfected with *Schistosoma mansoni* and *Toxoplasma gondii*

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We previously demonstrated that mice concurrently infected with *Schistosoma mansoni* and *Toxoplasma gondii* undergo accelerated mortality which is preceded by severe liver damage. Abnormally high levels of serum tumor necrosis factor alpha (TNF- α) in the dually infected mice suggested a role for this and related proinflammatory mediators in the pathologic alterations. In order to evaluate the factors involved in increased inflammatory-mediator production and mortality, interleukin-12^{-/-} (IL-12^{-/-}) mice were coinfectd with *S. mansoni* and *T. gondii*, and survival and immune responses were monitored. These IL-12^{-/-} mice displayed decreased liver damage and prolonged time to death relative to wild-type animals also coinfectd with these parasites. Relative to the response of cells from the coinfectd wild-type animals, levels of TNF- α , gamma interferon, and NO produced by splenocytes from coinfectd IL-12^{-/-} mice were reduced, and levels of IL-5 and IL-10 were increased, with the net result that the immune response of the dually infected IL-12^{-/-} mice was similar to that of the wild-type mice infected with *S. mansoni* alone. While dually infected wild-type animals succumb in the absence of overt parasitemia, the delayed death in the absence of IL-12 is associated with relatively uncontrolled *T. gondii* replication. These data support the view that *S. mansoni*-infected mice are acutely sensitive to infection with *T. gondii* as a result of their increased hepatic sensitivity to high levels of proinflammatory cytokines; IL-12 and TNF- α are implicated in this process.

Type 1 inflammatory and type 2 anti-inflammatory cytokine responses form the basis in large part for understanding how the immune system responds to infection. It is now well established that these contrasting cytokine responses display cross-regulatory activity (1, 25, 30). For example, gamma interferon (IFN- γ) inhibits proliferation of Th2 cells, as well as increasing Th1 activity by promoting interleukin-12 (IL-12) production and maintaining IL-12R β 2 expression on Th cells (26, 38). Conversely, IL-4 displays anti-inflammatory activity by inhibiting macrophage activation and inhibits IFN- γ production by down-regulating IL-12R β 2 expression (18, 36). IL-4 also acts as an autocrine growth factor for Th2 T lymphocytes. For these reasons, the immune system tends to polarize towards either inflammatory or anti-inflammatory responses during infection. This is exemplified by immunity to *Toxoplasma gondii* and *Schistosoma mansoni*, respectively.

Schistosomiasis is a highly prevalent chronic parasitic infection that affects 200 million people in developing countries. While in its infectious stages, the parasite enters the host through the skin and eventually locates to the mesenteric veins, where worm pairs deposit hundreds to thousands of eggs per day. The eggs may cross into the lumen of the intestine to exit

the host, or they may be carried by the circulatory system via the portal vein into the liver. Immunologically, *Schistosoma mansoni* infection (Schistosomiasis) is notable for the strong Th2 response of humans and experimental animals and for the role of this response in host survival, as well as its role in mediating the granulomatous immunopathology that is a hallmark of the disease (3, 8, 27, 31, 37).

T. gondii is an opportunistic protozoan parasite with worldwide distribution. Infection with *T. gondii* is usually initiated when humans or other hosts eat undercooked meat containing cysts from an infected animal or ingest water or food contaminated with oocysts shed in the feces of infected cats. Control of infection is mediated by a strong inflammatory response, in which IL-12-dependent IFN- γ plays a central and crucial role (2, 10, 15, 34). Infection normally proceeds from an acute phase associated with rapid tachyzoite proliferation to a chronic stage characterized by the presence of quiescent cysts within the central nervous system and skeletal muscles. Nevertheless, mice orally infected with *T. gondii* develop an intestinal inflammatory response that, in certain strains typified by C57BL/6, can be severe and life-threatening. Intestinal disease in these animals is mediated in part by a strong Th1 response, with the associated production of high levels of IFN- γ , tumor necrosis factor alpha (TNF- α), and NO (21, 22). Thus, while these cytokines are crucial for the full expression of immune effector mechanisms that limit the growth and spread of *T. gondii*, the same cytokines can be detrimental when overpro-

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FIG. 1. Liver histopathology in WT and IL-12^{-/-} mice infected with *T. gondii* (Tg) or *S. mansoni* (Sm) plus *T. gondii*. (A and B) Liver samples from *T. gondii*-infected WT (A) and IL-12^{-/-} (B) mice; arrows point to inflammatory foci. (C through F) Liver samples from coinfected WT (C and E) and IL-12^{-/-} (D and F) mice. Original magnifications, $\times 100$ (A through D) and $\times 200$ (E through F). In WT dually infected mice (C and E), the hepatic parenchyma displays areas of severe coagulative necrosis (lower arrows) and cytoplasmic vacuolization (upper arrows), while in IL-12^{-/-} dually infected mice (D and F), the hepatic parenchyma and architecture are essentially preserved with minimal cytoplasmic vacuolization.

duced in the absence of appropriate regulatory mediators (16, 22, 29).

We recently became interested in determining how the immune system responds when the host is coinfects with these two contrasting parasites. Our approach was to infect mice percutaneously with *S. mansoni* and then 7 weeks later to orally administer *T. gondii* cysts (23). Deposition of eggs is the major type 2 cytokine stimulus during *S. mansoni* infection (17, 31) and begins at week 5 postinfection, resulting in a peak Th2 response by weeks 7 to 8. Hence, our protocol was designed to evaluate the ability of the host to respond to a strong type-1 cytokine-inducing pathogen under the influence of an ongoing type-2 immune response to an unrelated parasite.

Our initial prediction was that an *S. mansoni*-induced type-2 cytokine response would ameliorate type-1 cytokine inflammation induced during oral *T. gondii* infection (23). While this proved to be the case, the animals nevertheless displayed increased mortality and morbidity when infected with the two parasites. Further examination revealed that the double-infected mice developed severe liver damage marked by large

areas of tissue destruction and the presence of apoptotic hepatocytes. Associated with these pathologic changes, serum TNF- α levels in double-infected mice were highly elevated, leading us to hypothesize that this cytokine was involved in mediating damage to the liver. Notably, our results revealed alterations that could not be predicted based on previous studies on animals infected with either *T. gondii* or *S. mansoni* alone.

Our goal is to understand the immunological basis of the pathologic changes that develop in wild-type (WT) C57BL/6 mice coinfects with *T. gondii* and *S. mansoni*. Since our previous work implicated proinflammatory mediators in the development of the severe disease associated with dual infection, we used IL-12^{-/-} mice to determine whether *T. gondii*-induced IL-12 plays a role in liver damage and early mortality in dually infected animals. IL-12 is a key initiator cytokine in the *T. gondii*-induced inflammatory cascade (14, 15). Our results suggest that, compared to WT mice, IL-12^{-/-} mice display lower production of TNF- α , IFN- γ , and NO; decreased liver changes; and prolonged survival time during double infection. The

studies also suggest that *T. gondii* infection suppresses the *S. mansoni*-induced Th2 response in an IL-12-dependent manner.

MATERIALS AND METHODS

Mice. Female strain C57BL/6 (B6) mice were obtained from Taconic Farms (Germantown, N.Y.) and Swiss-Webster B6 IL-12 p35^{-/-} and B6 TNFRp55^{-/-} female mice were obtained from the Jackson Laboratory (Bar Harbor, ME). The animals were kept under specific-pathogen-free conditions in the animal facility at the College of Veterinary Medicine, Cornell University, Ithaca, N.Y., and used when they reached 8 to 10 weeks of age. For treatment with aminoguanidine, an inhibitor of NO synthase with a selective preference for inducible NO synthase, aminoguanidine hemisulfate (100 mM; Sigma, St. Louis, Mo.) was provided in the sole source of drinking water (7). Experimental groups consisted of five mice for survival studies and three mice for immunological assessments. Each experiment was performed at least twice.

Parasites and infections. Mice were percutaneously infected with 70 *S. mansoni* cercariae (NMRI strain) as previously described (8). The ME49 *T. gondii* strain was maintained by intraperitoneal inoculation of Swiss-Webster mice with brain homogenate from mice that had been infected with *T. gondii* 6 to 8 weeks earlier. For B6 infection, brain homogenate of *T. gondii*-infected mice was obtained and adjusted to 400 cysts/ml, and 250 μ l of this suspension was administered by gavage to ether-anesthetized mice to give a final dose of 100 cysts/mouse. For coinfection studies, the mice were infected with *T. gondii* 7 weeks after *S. mansoni* infection. On day 8 after *T. gondii* infection, mice were euthanized with CO₂, their spleens were removed for cell culture, and their livers were removed for reverse transcription (RT)-PCR and histopathology and immunohistochemistry analyses. In some experiments, blood was collected by cardiac puncture into EDTA-containing tubes and centrifuged (12,800 $\times$ g for 5 min), and the resulting plasma was stored at -70°C for cytokine measurements.

Histopathological analysis. Livers were removed from experimental animals and immediately fixed in 10% (wt/vol) buffered formaldehyde. Samples were then embedded in paraffin wax, cut into 6- μ m sections, and stained with hematoxylin and eosin prior to microscopic examination.

Cell culture. Spleens from three mice per group were pooled. Single cell suspensions were obtained by forcing tissues through sterile 70- μ m nylon mesh (Becton Dickinson) followed by extensive washing with Dulbecco's modified eagle medium (Sigma). Erythrocytes were removed by hypotonic lysis, and the remaining cells were resuspended in complete tissue culture medium (composed of Dulbecco's modified Eagle medium, 10% fetal calf serum, 25 mM HEPES, 5 $\times$ 10⁻⁵ M β -2-mercaptoethanol, 100 U of penicillin per ml, 100 μ g of streptomycin per ml, and 2 mM glutamine, all from Sigma). Cells were counted, adjusted to 10⁷ per ml, and cultured for 72 h at 37°C and 5% CO₂ in flat-bottom 24-well plates (Falcon) in complete tissue culture medium alone, with 20 μ g of soluble egg antigen (SEA) (5, 6) per ml, 20 μ g of soluble tachyzoite antigen (STAg) per ml (4), or with plate-bound MAb anti-CD3 (0.5 μ g per well). After 72 h, supernatants were recovered and assayed for cytokines and NO.

Cytokine ELISA. Culture supernatant and/or plasma cytokine levels of IFN- γ , TNF- α , IL-4, IL-5, and IL-10 were measured by two-site enzyme-linked immunosorbent assay (ELISA) using MAbs commercially available from Pharmingen or R&D. Standard curves were generated using recombinant cytokines, and absorbances were measured on a microplate reader (Bio-Rad).

Nitric oxide production. Levels of NO were measured using the Greiss reaction as described elsewhere (24).

Plasma transaminase assay. Presence of the liver-associated enzyme aspartate transaminase (AST) in plasma was measured as previously described (24). Briefly, 20 μ l of plasma was added to 100 μ l of 0.2 M DL-aspartate and 1.8 mM α -ketoglutaric acid in phosphate-buffered saline (pH 7.5). The solution was mixed and incubated at 37°C for 1 h, and then 100 μ l of 2,4-diphenylhydrazine was added, and the mixture was incubated for 20 min at room temperature. The reaction was stopped with 1 ml of 0.4 N NaOH, and sample absorbances were measured at 490 nm.

RT-PCR-mediated gene transcript amplification. *T. gondii* tachyzoite levels in the liver were evaluated by RT-PCR-mediated amplification of transcripts for the parasite surface protein SAG-2 (p22). RNA was isolated and reverse transcribed with 3'-specific primers, and SAG-2, as well as hypoxanthine phosphoribosyltransferase cDNA, was amplified exactly as described previously (23). The PCR products were resolved by electrophoresis in a 2% agarose gel, and visualization of the DNA bands was accomplished by staining with ethidium bromide and illumination under a UV light box. Photographs of gels were scanned and analyzed with the use of Adobe Photoshop software (Adobe Systems, Mountain View, Calif.). Integrated band size and pixel density were evaluated and ex-

FIG. 2. Plasma levels of the liver-associated enzyme AST. AST levels were measured in plasma taken from WT and IL-12^{-/-} mice at 8 days post-*T. gondii* infection. The results are expressed as means $\pm$ SD from three individual mice per group and are representative of two separate experiments (SD < 1.0 where error bar not visible). The difference in AST levels between WT and IL-12^{-/-} coinfecting mice was significant ($P < 0.005$). Levels of AST in control plasma (Control) from uninfected mice were below 10 U/ml.

pressed as a ratio of p22 band intensity divided by hypoxanthine phosphoribosyltransferase band intensity.

Immunohistochemistry. Immunoperoxidase staining with polyclonal rabbit antibodies against *T. gondii* was used for detection of the parasites (35). The total number of parasitophorous vacuoles in 10 randomly selected fields per slide was counted.

Statistical analyses. Differences in cytokine production in plasma between IL-12^{-/-} and WT coinfecting mice as well as differences in parasite burdens and AST levels were determined by Student's *t* test. Differences in survival were determined using the nonparametric Wilcoxon test. Probability values of ≤ 0.05 were considered significant.

RESULTS

IL-12 plays a role in promoting liver damage in mice coinfecting with *S. mansoni* and *T. gondii*. As reported previously (23), in WT dually infected mice, the hepatic parenchyma had large areas of coalescing coagulative necrosis, marked hepatic cord dissociation, and moderate cytoplasmic vacuolation (Fig. 1C and E). Strikingly, in dually infected IL-12^{-/-} mice, the hepatic parenchyma and architecture were essentially preserved with minimal hepatocyte vacuolation (Fig. 1D and F). The hepatic lesions in WT and IL-12^{-/-} mice infected with *T. gondii* were characterized by similar small inflammatory foci scattered throughout the parenchyma (Fig. 1A and B). Consistent with our previous report (23), in WT mice, granulomas around schistosome eggs in dually infected animals appeared smaller (20% of reduction) relative to those in animals infected with *S. mansoni* alone; this size difference was less apparent in dually infected IL-12^{-/-} mice, whose granulomas were only 10% smaller than those in single-organism-infected animals (data not shown). Hepatic changes were not observed in control uninfected mice.

Plasma levels of the liver-associated enzyme AST were measured in WT and IL-12^{-/-} mice infected with *S. mansoni*, *T. gondii*, or *S. mansoni* plus *T. gondii* (Fig. 2). AST is normally contained within hepatocytes, but when the liver is damaged, AST is released, resulting in elevated levels of the enzyme in the blood. Consistent with the histopathological findings, levels of plasma AST were reduced in double-infected IL-12^{-/-} mice relative to coinfecting WT animals ($P < 0.005$).

FIG. 3. Proinflammatory mediator production is defective in IL-12^{-/-} mice. TNF- α (A through C), IFN- γ (D through F), and NO (G through I) were measured in spleen cell culture supernatants from *S. mansoni* (Sm), *T. gondii* (Tg), or *S. mansoni*-plus-*T. gondii*-infected mice euthanized 8 days after *T. gondii* infection. The cultures were stimulated with SEA, STAg, or anti-CD3 MAb, and after 3 days, supernatants were collected for cytokine and NO evaluation. Cytokines were measured by ELISA and NO by Greiss reaction. The results are representative of three separate experiments.

Production of inflammatory mediators is defective in IL-12^{-/-} animals. Since excessive inflammatory-mediator production is implicated in the severe liver disease observed in mice infected with *S. mansoni* plus *T. gondii* (23), we determined whether reduced liver disease in dually infected IL-12^{-/-} animals was correlated with a diminished inflammatory-mediator response. To examine splenocyte cytokine production, cells from single- and double-infected IL-12^{-/-} and WT mice were cultured in vitro with media, SEA, STAg, or anti-CD3 MAb. Cells from *T. gondii*-infected WT mice produced TNF- α (Fig. 3A), IFN- γ (Fig. 3D), and NO (Fig. 3G) without further restimulation in vitro. The addition of antigen had a minimal affect, but polyclonal stimulation with anti-CD3 significantly increased levels of TNF- α and IFN- γ . In comparison, under all in vitro culture conditions, production of all three mediators by spleen cells from *T. gondii*-infected IL-12^{-/-} mice was significantly lower than was the case for WT mice. These data demonstrate the pivotal role of IL-12 in promoting inflammatory responses during infection with *T. gondii*. As expected, cells from *S. mansoni*-infected mice made low or unmeasurable levels of TNF- α , IFN- γ , and NO following stimulation with parasite antigen (Fig. 3B, E, and H, respectively). Nevertheless, anti-CD3 stimulation led to the production of low levels of

all three mediators, at equivalent levels in WT and IL-12^{-/-} animals (Fig. 3B, E, and H). The latter observation is consistent with the view that IFN- γ production during *S. mansoni* infection is IL-12 independent (30a). In cells from coinfecting mice (Fig. 3C, F, and I), the pattern of cytokine production was generally similar to that seen for splenocytes from animals infected with *T. gondii* alone, although the levels of TNF- α and IFN- γ produced by cells from WT mice that were not restimulated in vitro were lower than was the case for cells from WT mice infected with *T. gondii* alone.

T. gondii infection in WT mice led to increased plasma levels of TNF- α (Fig. 4A) and IFN- γ (Fig. 4B). In dually infected mice, TNF- α levels increased even further while IFN- γ levels tended to be lower than those in mice infected with *T. gondii* alone. In comparison, levels of these cytokines were very low in the plasma of mice infected with *S. mansoni* alone. Elevations in levels of TNF- α and IFN- γ were IL-12 dependent, since *T. gondii*- and *S. mansoni*-plus-*T. gondii*-infected IL-12^{-/-} animals had plasma levels of these cytokines that were lower than in WT animals. The difference in TNF- α production between double-infected WT and IL-12^{-/-} mice was statistically significant ($P < 0.05$). However, the difference in IFN- γ production

FIG. 4. Elevated plasma levels of TNF- α and IFN- γ are dependent upon IL-12. Mice infected with *S. mansoni* (Sm), *T. gondii* (Tg), or *S. mansoni* plus *T. gondii* were bled at 8 days post-*T. gondii* infection, and ELISA was used to determine cytokine levels. The results are expressed as means $\pm$ SD of three individual mice per group, and the experiment was repeated three times with similar results. The difference in TNF- α production between dually infected WT and IL-12^{-/-} mice was significant ($P < 0.05$).

between these two groups was not significant statistically ($P = 0.09$).

To address the roles of TNF- α and NO in liver damage and death during coinfection, we used TNFRp55^{-/-} mice and WT mice treated with aminoguanidine. Five mice were infected per group with *S. mansoni* plus *T. gondii*, and survival outcome was assessed. Coinfected TNFRp55^{-/-} mice and aminoguanidine-treated mice survived a mean of 2 days longer than WT coinfecting controls (11.6 ± 1.5 versus 9.8 ± 0.4 days [$P = 0.0476$] and 10.25 ± 0.96 versus 8.25 ± 0.5 days [$P = 0.0433$]), confirming a participation for TNF- α and NO in the severe disease seen in coinfecting mice.

IL-12^{-/-} mice display increased resistance to coinfection. Correlating with their less severe liver damage, dually infected IL-12^{-/-} mice survived significantly longer than did dually infected WT controls (mean $\pm$ standard deviation [SD] time to death: 13.7 ± 1.5 versus 9.5 ± 0.7 days; $P < 0.005$ [Fig. 5]). Strikingly, the dually infected IL-12^{-/-} animals also exhibited

FIG. 5. IL-12^{-/-} mice display increased resistance to coinfection with *S. mansoni* and *T. gondii* (Tg). As in other experiments, mice were infected percutaneously with 70 *S. mansoni* cercariae and then 7 weeks later were administered an oral dose of 100 *T. gondii* cysts (ME49 strain). (A) Survival of IL-12^{-/-} mice infected with *T. gondii* alone (open squares) or with *S. mansoni* plus *T. gondii* (closed circles). (B) Survival of WT (open circles) compared with IL-12^{-/-} (closed triangles) mice coinfecting with *S. mansoni* plus *T. gondii*. Five mice were used in each group, and the experiments were repeated three times. IL-12^{-/-} dually infected mice survived significantly longer than both the IL-12^{-/-} mice infected with *T. gondii* alone ($P < 0.005$) and the WT coinfecting mice ($P < 0.005$).

prolonged time to death relative to control IL-12-deficient mice infected with *T. gondii* alone (mean $\pm$ SD time to death: 13.7 ± 1.5 versus 10.5 ± 0.7 days; $P < 0.005$ [Fig. 5]). As expected from previous reports (11), IL-12^{-/-} mice infected with *T. gondii* alone died sooner than *T. gondii*-infected WT mice (Fig. 5).

The role of reduced inflammatory mediator levels and of *T. gondii* burden in the prolonged survival of coinfecting IL-12^{-/-} mice. Because proinflammatory cytokines are crucial for the control of *T. gondii* infection, it might be predicted that the levels of IFN- γ and TNF- α would be related to parasite burden and to the outcome of infection in mice and moreover that parasitemia would be directly correlated with disease severity. However, compared to coinfecting IL-12^{-/-} mice, coinfecting WT animals produced higher levels of IFN- γ , TNF- α , and NO (Fig. 3 and 4), and yet they died sooner (Fig. 5). Therefore, we examined *T. gondii* levels in *T. gondii*- and *S. mansoni*-plus-*T. gondii*-infected WT and IL-12^{-/-} mice (Fig. 6). In WT mice infected with *T. gondii* alone, SAG-2 transcripts indicative of active *T. gondii* infection were present, and in *T. gondii*- as well as *S. mansoni*-plus-*T. gondii*-infected IL-12^{-/-} mice, levels of SAG-2 appeared approximately twofold higher (as determined by scanning densitometric analysis) (Fig. 6A). This result is consistent with the reported inability of mice to control *T. gondii* infection in the absence of IL-12 (11, 14, 15). It is interesting that SAG-2 expression in WT coinfecting mice appeared lower relative to that in mice infected with *T. gondii* alone (Fig. 6A).

T. gondii-specific immunohistochemical staining was performed on liver sections from infected and control groups. As shown in Fig. 6, in *T. gondii*-infected WT mice (panel B), *T. gondii* levels were low relative to those in *T. gondii*-infected IL-12^{-/-} mice (panel D) (0.3 ± 0.6 versus 68 ± 23 parasite foci per field for WT and IL-12^{-/-}, respectively; $P = 0.01$). In WT coinfecting animals, we counted 2.3 ± 1.5 parasite foci per field (Fig. 6C), compared with 23 ± 6 (Fig. 6E) in IL-12^{-/-} mice carrying a dual infection ($P < 0.005$). Two conclusions can be drawn from these data. First, as anticipated, *T. gondii* parasitemia is controlled by IL-12. Second, the immunohistochemical stain suggests that *S. mansoni* may confer some resistance to *T. gondii* (panels D and E).

***T. gondii* induces an IL-12-dependent suppression of *S. mansoni*-induced type 2 cytokine responses.** Severe disease in dually infected mice could be due not only to exacerbated inflammatory cytokine production but also to suppression of the anti-inflammatory *S. mansoni*-specific Th2 response that is important for survival with *S. mansoni* alone (8). To examine this, we assessed production of the signature type 2 cytokines IL-5 and IL-10 in single and double infections in WT and IL-12^{-/-} mice. Because these cytokines could not be detected in the plasma (data not shown), we examined production in antigen- and anti-CD3-stimulated splenocyte cultures. In no case could we detect IL-5 by using cells from mice infected with *T. gondii* alone, although cells from these animals did make IL-10 but only in response to anti-CD3 (data not shown). However, cells from WT mice infected with *S. mansoni* produced high levels of IL-5 and IL-10 in response to SEA and anti-CD3 but not to STAg. Splenocytes from *S. mansoni*-infected IL-12^{-/-} animals made similar levels of IL-5 to those produced by WT mice, but it is interesting that they failed to make IL-10 in response to

FIG. 6. Liver *T. gondii* (Tg) burden in WT and IL-12^{-/-} mice infected with *T. gondii* and *S. mansoni* (Sm) plus *T. gondii*. (A) High levels of SAG-2 transcripts (measured by RT-PCR amplification) were evident in the livers of IL-12^{-/-} mice infected with *T. gondii* or with *S. mansoni* plus *T. gondii*. Immunohistochemical staining of liver sections for *T. gondii* showed low levels of *T. gondii* in single-infected WT (B) and double-infected (C) animals, although in multiple sections there was a trend toward fewer *T. gondii* foci in the coinfecting group (see text). Large numbers of *T. gondii*-positive foci were evident in singly (D) and dually (E) infected IL-12^{-/-} livers, although in the coinfecting group fewer *T. gondii*-positive foci were evident (cf. D versus E). Control sections from *S. mansoni*-infected mice were negative for *T. gondii* staining. Original magnification, $\times 200$. This experiment was repeated twice with similar results.

antigen, although anti-CD3-driven responses appeared intact (Fig. 7A and C). Superinfection of *S. mansoni*-infected WT mice with *T. gondii* led to a suppression of IL-5 and IL-10 production (cf. Fig. 7A and C versus B and D); this suppression is mediated at least in part by endogenous IL-12 production since dually infected IL-12^{-/-} mice had no defect in their ability to make IL-5 (Fig. 7B) in response to antigen or anti-CD3 or to make IL-10 after stimulation with anti-CD3 (Fig. 7D). Similar results were found when IL-4 was measured (data not shown).

DISCUSSION

C57BL/6 mice infected with *S. mansoni* are acutely susceptible to peroral infection with *T. gondii* (23). Disease due to dual infection is characterized by precipitous weight loss followed by death, with severe liver damage implicated as the cause (23). We have hypothesized that, during schistosomiasis, the liver is acutely sensitive to the high levels of inflammatory mediators that are produced in response to *T. gondii* infection. Support for this idea is provided by the prior observations that

(i) *S. mansoni*-infected mice, like D-gal-primed animals, succumb to liver failure when challenged with low doses of endotoxin (13, 20, 28) and (ii) *T. gondii* extract behaves like endotoxin inasmuch as it causes lethal liver failure in D-gal-sensitized mice (24). In this report we have used IL-12^{-/-} mice to directly examine the role of proinflammatory mediator production in the severe disease that accompanies dual infection. Compared to coinfecting WT mice, coinfecting IL-12-deficient animals had reduced levels of IFN- γ , TNF- α , and NO, elevated Th2 responses, and diminished liver disease. Consistent with the view that, during concurrent infection, IL-12 promotes the production of life-threatening levels of inflammatory mediators, dually infected IL-12^{-/-} mice survived significantly longer than dually infected WT animals. In contrast, IL-12^{-/-} mice infected with *T. gondii* alone died before *T. gondii*-infected WT mice (data not shown), confirming the underlying importance of IL-12 in survival against this intracellular infection. As was expected from a host compromised in its ability to mount a Th1-like response, dually infected IL-12^{-/-} mice had more *T. gondii* within their livers than did dually infected WT mice.

FIG. 7. *T. gondii* (Tg) infection suppresses IL-5 and IL-10 production in *S. mansoni* (Sm)-infected mice in an IL-12-dependent manner. IL-5 and IL-10 in supernatants of spleen cells stimulated with SEA, STAg, or anti-CD3 for 3 days were measured by ELISA. (A and C) Mice infected with *S. mansoni*; (B and D) mice infected with *S. mansoni* plus *T. gondii*. IL-5 levels are shown in A and B, and IL-10 levels are shown in C and D. The experiment was performed twice with similar results.

However, at least by immunohistochemical staining, the coinfecting IL-12-deficient mice appeared to have a lower hepatic *T. gondii* burden than did single-parasite-infected IL-12^{-/-} mice, suggesting that an IL-12-independent *S. mansoni*-induced mechanism of temporarily controlling *T. gondii* growth may exist. Thus prolonged survival in coinfecting IL-12^{-/-} mice may be due to diminished inflammatory mediator production combined with a reduced parasite burden.

Comparing WT and IL-12^{-/-} mice infected with *S. mansoni* alone, the absence of IL-12 has little or no effect on major measures of morbidity such as weight loss or increased mortality (data not shown), although plasma AST levels were lower in the IL-12^{-/-} animals, raising the possibility of an underlying role for IL-12 in tissue damage during schistosomiasis. Consistent with previous findings (30a), measured immune responses revealed no differences between schistosome-infected IL-12^{-/-} mice and schistosome-infected WT mice. The situation was quite different during *T. gondii* infection, however, when, as previously described, IL-12^{-/-} mice rapidly succumbed to infection. This increased susceptibility is accompanied by significantly increased parasitemia and decreased production of inflammatory mediators in vivo and in vitro. The most straightforward interpretation of these data is that, in WT mice, IL-12 plays the pivotal role in promoting Th1 response development and that without amplification of the production of inflammatory mediators such as IFN- γ , TNF- α , and NO, parasite replication proceeds in an uncontrolled fashion, and the host dies of direct cellular damage occurring as a result of this process. Death in the WT mice that succumb after exposure to *T. gondii* appears to have a different cause, since in these animals liver parasite burdens were very low. Previous reports (22) have indicated that the CD4 cell- and IFN- γ -dependent intestinal

immunopathology contributes to morbidity and death in these mice.

The differences between single-infected and dually infected WT mice were as previously described (23), in that infection with *S. mansoni* rendered WT mice acutely sensitive to *T. gondii* infection. On the basis of our data, we argued previously (23) that this increase in disease severity due to dual infection was the result of *T. gondii*-induced TNF- α -mediated severe damage to a liver already inflamed as a result of the granulomatous response to schistosome eggs trapped in the sinusoids. New data presented here indicate that *T. gondii* infection also leads to suppression of the *S. mansoni*-induced Th2 response. This latest observation raises the possibility that the exacerbated disease in dually infected mice may be in part the result of a diminished Th2 response to *S. mansoni*, a condition that mimics that seen in infected IL-4^{-/-} mice, in which *S. mansoni* infection alone is lethal (8). Consistent with this hypothesis, morbidity was reduced and mortality delayed when proinflammatory-mediator production was suppressed as a result of IL-12 gene deletion and, to a lesser extent, when inflammatory signaling by TNF- α was decreased through TNFRp55 deletion and when NO production was inhibited. The absence of IL-12 resulted in greatly diminished IFN- γ and TNF- α production in dually infected mice. Additionally, the suppression of Th2 responses observed in dually infected WT mice was partially reversed in the absence of IL-12, raising the possibility that, through the production of regulatory mediators such as IL-10, the schistosome-induced Th2 response could be playing a role in IL-12^{-/-} mice in further controlling inflammation associated with the *T. gondii* infection.

Increased survival time in coinfecting IL-12^{-/-} mice was accompanied by less severe liver damage as assessed microscopically and by plasma levels of AST. Lesions in the livers of coinfecting WT mice were largely as described previously (23): extensive granulomatous inflammation surrounding schistosome eggs, accompanied by excessive damage in the liver parenchyma, where hepatocytes were vacuolated, and in which large areas of coagulation necrosis were apparent. Additionally, the granulomas around schistosome eggs were smaller in double-infected mice than those in mice infected with *S. mansoni* alone, as previously demonstrated (23). None of these effects were evident in the dually infected IL-12^{-/-} mice, which had liver changes that were histologically similar to those seen in *S. mansoni*-infected WT mice. These data argue strongly that the enhanced, novel liver damage observed in dual-infected WT mice is entirely the result of an IL-12-driven immunopathologic process. Consistent with this theme, a role for IL-12 in promoting hepatic immunopathology in *Leishmania donovani* infection has been recently reported (32).

The suppression of Th2 responses that accompanies *T. gondii* infection in *S. mansoni*-infected mice is a previously unrecognized aspect of dual infection. Previously we examined IgE levels in coinfecting mice, but we found little evidence for a decrease in levels of this IL-4-dependent isotype (23). However, given the short duration of *T. gondii* infection (approximately 8 days) and the half-life of the antibody, this is perhaps not surprising. In the present study we focused on Th2 cytokine production by T cells from dually infected mice and noted that IL-5 and IL-10 levels were depressed compared to those produced by cells from mice infected with *S. mansoni* alone; this

suppression is mediated by a process that is IL-12 dependent, since it is not apparent in dually infected IL-12^{-/-} animals. The mechanism by which *T. gondii*-induced IL-12 down-regulates the *S. mansoni*-induced Th2 response is unclear.

The inference of these studies is that, during schistosomiasis, the liver is acutely sensitive to inflammatory mediators associated with a Th1 response. Studies using IL-4^{-/-} (8), IL-4/IL-10^{-/-} (19), and IL-4/IL-13^{-/-} (12) mice, which mount Th1 rather than Th2 responses during *S. mansoni* infection and develop lethal wasting disease, strongly support the view that type-1 responses can be deleterious during schistosomiasis. We have argued that the primary function of the Th2 response that normally develops during *S. mansoni* infection is to limit Th1-like inflammatory responses while simultaneously sequestering parasite eggs away from the surrounding liver tissue (7). Data presented show that mice infected with *S. mansoni* are susceptible to IL-12-mediated inflammatory responses even when they are already mounting a strong Th2 response. These data suggest that the use of IL-12 to promote antifibrotic Th1 responses and thereby minimize liver damage in schistosomiasis may not be risk free (9, 33, 39).

Given the prevalence of *T. gondii* infection in areas with endemic schistosomiasis, it seems likely that individuals are simultaneously afflicted with both diseases, although to the best of our knowledge this has not been directly investigated. Our current studies are directed towards determining the frequency of seropositivity for *T. gondii* in areas where it is endemic versus areas where it is nonendemic with a view to examining the relevance of the experimental data presented here to the clinical situation in areas of endemicity.

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