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IL-10 and TGF- β Redundantly Protect against Severe Liver Injury and Mortality during Acute Schistosomiasis¹

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Abstract

The cytokines IL-10 and TGF- β regulate immunity and inflammation. IL-10 is known to suppress the extent of hepatic damage caused by parasite ova during natural infection with *Schistosoma mansoni*, but the role of TGF- β is less clear. Cytokine blockade studies in mice revealed that anti-IL-10R mAb treatment during acute infection modestly increased cytokine production and liver damage, whereas selective anti-TGF- β mAb treatment had marginal effects. In contrast, mice administered both mAbs developed severe hepatic inflammation, with enlarged, necrotic liver granulomas, cachexia, and >80% mortality by 8 wk postinfection, despite increased numbers of CD4⁺CD25⁺Foxp3⁺ T regulatory cells. Blocking both IL-10 and TGF- β at the onset of egg production also significantly increased IL-4, IL-6, TNF, IFN- γ , and IL-17 production and markedly increased hepatic, peritoneal, and splenic neutrophilia. In contrast, coadministration of anti-IL-10R and TGF- β mAbs had little effect upon parasite ova-induced intestinal pathology or development of alternatively activated macrophages, which are required to suppress intestinal pathology. This suggests that inflammation is controlled during acute *S. mansoni* infection by two distinct, organ-specific mechanisms: TGF- β and IL-10 redundantly suppress hepatic inflammation while intestinal inflammation is regulated by alternatively activated macrophages.

Although inflammation is fundamental to immune-mediated protection against most pathogens, counterregulatory mechanisms are required to limit collateral damage to host tissues (1,2). In the case of schistosomiasis, a tropical disease affecting over 250 million persons worldwide, excessive inflammation and cytokine production is associated with severe immunopathology and mortality (3–5). Ova produced by these parasites are highly immunogenic and cause T_H2 differentiation, granulomatous inflammation, and marked hepatic and intestinal fibrosis (6,7). In mice, T_H2-derived cytokines (IL-4, IL-5, and IL-13) can produce morbidity during the chronic phase of infection (16–20 wk postinoculation) but are less rapidly harmful than T_H1 (IFN- γ or T_H17 (IL-17)-derived cytokines, which can cause fatal disease during the acute phase (7–9 wk postinoculation) (8–11). Thus,

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mechanisms that restrict parasite egg-driven inflammation during schistosomiasis are critical for host survival.

We have previously demonstrated that alternatively activated macrophages, which are induced by IL-4 and IL-13, are required to regulate the intestinal inflammation that develops once *Schistosoma mansoni* worms begin to lay eggs (12). Chimeric mice that selectively fail to express IL-4R on bone marrow-derived cells and transgenic mice that selectively fail to express IL-4R on macrophages and neutrophils die of severe intestinal inflammation during the acute phase of infection (12,13). Surprisingly, although hepatic inflammation is pronounced in both mice and humans infected with *S. mansoni* (14,15), it was not increased to the same extent as intestinal inflammation in infected chimeric or transgenic mice (13). This led us to investigate whether an unrelated mechanism is primarily responsible for regulating hepatic inflammation.

Our studies evaluated two cytokines, IL-10 and TGF- β , that are both well known for their immunosuppressive properties; each can independently regulate the differentiation, proliferation, and activation of immune and nonimmune cells (2,16–18). Both cytokines are produced in increased quantity during *S. mansoni* infection of humans, nonhuman primates, and mice (19,20). IL-10 has been well studied in murine models of *S. mansoni* infection and has been shown to control excessive T_H1 or T_H2 responses, suppress macrophage and dendritic cell activation, and limit worm ova-induced hepatotoxicity during the acute phase of infection (9,21,22). In contrast, although macrophages and T cells produce TGF- β during parasitic worm infections and this cytokine is known to suppress Ag-specific immunity and promote the differentiation and suppressive function of CD4⁺CD25⁺Foxp3⁺ T regulatory cells (Tregs),³ TGF- β has not been shown to suppress *S. mansoni*-induced inflammation (23–25).

We now demonstrate that IL-10 and TGF- β redundantly promote host survival by suppressing proinflammatory cytokine production and liver injury during acute schistosomiasis. Although treatment of *S. mansoni*-infected mice with anti-TGF- β mAb induces only marginal changes, the combination of anti-TGF- β and anti-IL-10R mAbs profoundly increases cytokine production, hepatic inflammation, and mortality without having a major effect on alternatively activated macrophages or intestinal pathology.

Materials and Methods

Mice

Male BALB/c mice were purchased from Taconic. All mouse experiments were approved by the Institutional Animal Care and Use Committee at the Cincinnati Veterans Affairs Medical Center.

mAbs for neutralization and flow cytometry

Anti-IL-10R receptor (1B1.3), anti-TGF- β (1D11 16.8), and isotype control mAb (GL113) (IgG1) were produced in Pristane-primed (Acros Organic) athymic nude mice as ascites and purified by means of ammonium sulfate precipitation and diethyl-amino-ethyl-cellulose column chromatography. Endotoxin values of all mAb were determined to be <200 pg/ml (13). Spleen and PBMC were stained with fluorochrome-labeled rat mAbs to mouse CD11b (M1/70) (26), Gr-1 (RB6-8C5) (27), CD4 (RM4-5), CD25 (PC-61), and Foxp3 (FJK-16s).

³Abbreviations used in this paper: Treg, T regulatory cell; IVCCA, in vivo cytokine capture assay; AST, aspartate transaminase.

Infection of mice with *S. mansoni*

Mice were anesthetized and percutaneously infected with 50–70 *S. mansoni* cercariae as previously described (12). Parasites were provided by the National Institute of Allergy and Infectious Diseases Schistosomiasis Resource Center at the Biomedical Research Institute, through National Institute of Allergy and Infectious Diseases Contract N0-AI-30026.

Flow cytometry

Splenocytes were isolated washed in FACS buffer (HBSS, 1% FBS, and 0.2% sodium azide) and incubated with anti-Fc γ RII/RIII mAb (2.4G2). Cells were then stained with the mAbs described and analyzed with a BD FACSCalibur and Cellquest software.

Evaluation of cytokine production and morbidity

IL-17A levels were measured by ELISA (eBioscience). IL-4, TNF, IL-6, IL-10, and IFN- γ were measured by in vivo cytokine capture assay (IVCCA) (28). A TGF- β 1 ELISA kit was obtained from R&D Systems. Serum AST concentration was measured by the Veterans Affairs Medical Center clinical pathology laboratory. Serum LPS concentration was measured as described (13).

S. mansoni granuloma measurement

Histologically processed sections of liver tissue (5 μ m) were stained with H&E or Masson's trichrome. Individual granuloma areas were measured on coded slides; only granulomas that possessed a central egg were evaluated. Quantitation of area was performed using a SPT Diagnostics imaging system and Simple PCI C-Imaging systems software. Data shown are mean \pm SE of 150 granulomas per group from two independent experiments.

Real-time PCR

RNA was obtained from hepatic tissue, DNase I-treated, and cDNA was generated using SuperScript II Reverse Transcriptase (Invitrogen). Real-time PCR was conducted on a Gene Amp 7500 instrument (PE Biosystems) with the Syber Green detection reagent. Cycle threshold values for Arginase I and Relm- α (FIZZ-1) were determined and fold induction was compared with vimentin using the $1/\Delta^{\text{cycle threshold}}$ method (12).

Vimentin For: 5'-TGA CCG GCT TGT ATG CTA TC-3'

Vimentin Rev: 5'-CAG TGT GAG CCA GGA TAT AG-3'

Arginase I For: 5'-CAG AAG AAT GGA AGA GTC AG-3'

Arginase I Rev: 5'-CAG ATA TGC AGG GAG TCA CC-3'

Relm- α /Fizz-1 For: 5'-AGA TGG GCC TCC TGC CCT GCT GGG-3'

Relm- α /Fizz-1 Rev: 5'-ACC TGG TGA CGG GCG ACG ACG GTT-3'

Determination of hydroxyproline content

Hepatic collagen content was measured as hydroxyproline concentration (12,29).

Statistical analysis

Statistical significance was assessed by either one-tailed Student's *t* test (two groups) or ANOVA for multiple groups and a post hoc Bonferroni's test to determine significance, all performed using Prism GraphPad software.

Results

IL-10 and TGF- β production are induced by worm eggs and promote host survival during acute *S. mansoni* infection

The inflammatory response to worm ova during murine schistosomiasis can be separated into acute (6–9 wk) and chronic (<13 wk) phases (15). Because chronic schistosomiasis is characterized by T cell anergy and reduced production of inflammatory cytokines (30,31), we evaluated whether IL-10 and TGF- β levels increase as the disease progresses toward chronicity. IL-10 production, as detected by IVCCA, changed from undetectable levels in serum at 5 wk postinoculation to 3 ± 1 ng/ml by 7.5 wk and 9 ± 2 ng/ml by 13 wk postinfection (Fig. 1A). TGF- β serum levels increased 3-fold between 5 and 7.5 wk postinoculation and plateaued afterward (Fig. 1B).

Ab blockade of IL-10 and/or TGF- β was performed to determine whether these cytokines are host-protective during the acute phase of infection. Treatment of *S. mansoni*-inoculated mice with anti-IL-10R mAb caused a significant increase in weight loss (Fig. 1C) and a trend toward increased mortality that was not statistically significant ($p < 0.068$) (Fig. 1D), while anti-TGF- β mAb, by itself, had little effect. Treatment of worm-inoculated mice with both anti-mouse IL-10R and TGF- β mAbs (α -IL-10R/ α -TGF- β) caused more than twice the weight loss induced by anti-IL-10 mAb alone and increased mortality to greater than 80% by 9 wk postinoculation. In contrast, treatment of uninoculated mice with α -IL-10R, α -TGF- β , or the combination of α -IL-10R/ α -TGF- β mAbs did not cause significant weight loss (data not shown).

Combined blockade of IL-10 and TGF- β exacerbates the acute inflammatory response

Because cytokine perturbations are associated with increased mortality during schistosomiasis (9), we used the IVCCA to evaluate whether neutralization of IL-10 and TGF- β modifies proinflammatory cytokine production (28). At 7.3 wk postinoculation, treatment of *S. mansoni*-inoculated mice with α -TGF- β mAb had little or no effect on IL-4, IL-6, IL-17, IFN- γ , or TNF production, whereas anti-IL-10R mAb significantly increased secretion of IL-4, IFN- γ , TNF, and IL-17 and combined α -IL-10R/ α -TGF- β treatment caused significantly greater increases in IFN- γ , TNF, and IL-17 secretion and a large, significant increase in IL-6 secretion (Fig. 2). As expected, α -TGF- β mAb treatment reduced TGF- β serum levels and anti-IL-10R mAb treatment increased serum levels of IL-10.

TGF- β neutralization increases the effects of IL-10 blockade on hepatic granuloma size, liver injury, and neutrophil accumulation

In most inbred mouse strains, *S. mansoni* liver granulomas are well-circumscribed, fibrotic lesions that contain lymphocytes, eosinophils, and macrophages (15). Granuloma size was increased by IL-10 blockade and even more by combined blockade of IL-10 and TGF- β (Fig. 3A). Hepatocyte injury, as indicated by serum levels of aspartate transaminase (AST), was also increased in *S. mansoni*-inoculated mice to some extent by blockade of IL-10 and to a considerably greater extent by combined IL-10/TGF- β blockade (Fig. 3B). In contrast, liver fibrosis, while induced by *S. mansoni* infection, was not significantly affected by IL-10/TGF- β blockade (Fig. 3C).

These observations were consistent with analysis of hepatic granuloma morphology in *S. mansoni*-inoculated mice (Fig. 4), which demonstrated severe necrosis of hepatocytes within granulomas only in mice that received both mAbs (Fig. 4D) and no increase in hepatic fibrosis beyond that induced by *S. mansoni* itself (Fig. 4, E–H). Although IL-10/TGF- β blockade did not increase hepatic inflammation or neutrophil number in naive mice (data not shown), tissue sections showed a remarkable increase in neutrophil infiltration at the margin

of granulomas in α -IL-10R/ α -TGF- β mAb-treated, *S. mansoni*-infected mice (Fig. 4D, inset). Consistent with this, numbers of neutrophils (CD11b⁺/Gr-1^{bright} cells) were dramatically elevated in both the peritoneal cavity (Fig. 5A) and spleen (Fig. 5B) of these infected mice and smaller increases in peritoneal neutrophil number were observed in uninfected, α -IL-10R/ α -TGF- β mAb-treated mice.

Anti-IL-10R/anti-TGF- β mAb treatment does not exacerbate gut immunopathology or block alternative macrophage activation

The absence of IL-4/IL-13-responsive macrophages during *S. mansoni* infection causes weight loss and death by increasing endotoxemia and gut pathology (12,13). Consequently, we evaluated whether α -IL-10R/ α -TGF- β treatment had similar effects. Contrary to our expectation, we observed no exacerbated gut injury or dysregulation of intestinal granuloma architecture in *S. mansoni*-inoculated, α -IL-10R/ α -TGF- β mAb-treated mice (Fig. 6, A–D) or increase in serum levels of endotoxin (Fig. 6E). Consistent with this, alternative macrophage activation was unaffected by IL-10/TGF- β blockade, as shown by a lack of effect on intestinal mRNA transcripts for the alternatively activated macrophage proteins arginase I (Fig. 6F) and RELM α (FIZZ-1) (Fig. 6G), although anti-TGF- β mAb seemed to suppress the small increases in Arginase I and RELM- α expression that were induced by anti-IL-10R mAb (32).

Treg generation is unaffected by IL-10/TGF- β blockade

Both IL-10 and TGF- β have been reported to promote the generation of Tregs (33,34). Consequently, it seemed possible that blockade of both cytokines might increase immunopathology indirectly by suppressing Treg generation. Indeed, CD4⁺CD25⁺Foxp3⁺ cells have been linked with down-modulation of both T_H1 and T_H2 cell expansion during acute murine schistosomiasis (35,36). Surprisingly, however, combined IL-10/TGF- β blockade markedly increased numbers of both regulatory (CD4⁺CD25⁺Foxp3⁺) (Fig. 7A) and effector (CD4⁺CD25⁺Foxp3⁻) (Fig. 7B) T cells in *S. mansoni*-inoculated mouse spleen.

Discussion

This work demonstrates that TGF- β redundantly controls hepatic immunopathology and promotes survival of hosts infected with the parasitic worm *S. mansoni*. Although several reports have demonstrated that IL-10 limits inflammation in murine schistosomiasis (22,30,37), the potential role in this disease of TGF- β , a second cytokine that can limit inflammation, has not previously been explored. Our studies confirm that anti-IL-10R mAb modestly increases weight loss and appears to increase mortality in *S. mansoni*-inoculated mice during the acute phase of infection, but show that anti-TGF- β mAb, by itself, has little effect. Administration of anti-IL-10R mAb, but not anti-TGF- β mAb also significantly increased IL-4, IFN- γ , TNF, and IL-17 production 7.3 wk postinfection and increased liver granuloma size and exacerbated hepatocellular injury (increased serum AST). Although anti-TGF- β mAb, by itself, had little effect on *S. mansoni*-induced inflammation, it greatly exacerbated inflammation and mortality in anti-IL-10R mAb-treated, *S. mansoni*-inoculated mice. Mice administered both anti-IL-10 receptor and anti-TGF- β mAbs from 5 to 6.5 wk postinoculation developed increased T_H1, T_H2, and T_H17 cytokine production, neutrophilia, and marked necrosis within hepatic granulomas by 7.5 wk postinoculation. These mice lost 15–20% of their body weight and >80% died by 8 wk. This demonstrates a large degree of redundancy between IL-10 and TGF- β in limiting hepatic inflammation during *S. mansoni* infection. This redundancy cannot be explained by a compensatory increase in TGF- β production in the absence of IL-10 because *S. mansoni*-infected mice treated with anti-IL-10R mAb had slightly lower levels of TGF- β and vice versa.

There is precedent for the redundant effects of IL-10 and TGF- β in limiting inflammation (38–42). Treatment of IL-10KO mice with anti-TGF- β mAb during *Trichinella spiralis* infection greatly exacerbates inflammatory cell recruitment to the site of parasite invasion (43). IL-10 redundancy with other cytokines is also established: *S. mansoni* causes much greater inflammation and mortality in the absence of IL-4 and IL-10 or IL-12 and IL-10 than when only one of these cytokines is absent (9). Even in naive mice, combined neutralization of IL-10 and TGF- β substantially increased numbers of peritoneal (but not liver) neutrophils. This may have predisposed these animals to develop lethal, neutrophilic hepatic inflammation following *S. mansoni* infection.

Although combined anti-IL-10/anti-TGF- β mAb treatment greatly exacerbates *S. mansoni*-induced inflammation in the liver, it has little effect on the intestine, the other organ that is a major site of ova-induced damage during infection with this parasite (12). In contrast, intestinal inflammation is markedly increased in the absence of IL-4R α , a necessary component of the receptors for IL-4 and IL-13 (44). IL-4R α -mediated limitation of intestinal inflammation depends to a great extent on alternative activation of macrophages by these cytokines (12). Thus, *S. mansoni*-induced inflammation is limited in the two major target organs by independent mechanisms: macrophage activation by IL-4 and/or IL-13 limits intestinal inflammation, whereas effects of IL-10 and TGF- β prevent hepatic necrosis.

The mechanism by which IL-10 and TGF- β limit hepatic inflammation is not clear and may be multifactorial. However, the remarkable increase in IL-17 production, previously observed links between increased IL-17 production and lethality during murine schistosomiasis, and the considerable, presumably IL-17-related hepatic infiltration of neutrophils in anti-IL-10R/TGF- β mAb-treated, *S. mansoni*-inoculated mice suggest that IL-17 may contribute importantly to hepatocellular damage when neither IL-10 nor TGF- β is available to limit inflammation (10,45). The increased production of IL-17 in the absence of IL-10 and TGF- β , even in uninfected mice, is remarkable because TGF- β is thought to be required to stimulate naive T cells to differentiate into IL-17-secreting T_H17 cells and to promote T_H17 cell survival in the mouse (46,47). Our data suggest that increased TGF- β production over baseline is not essential to induce and maintain T_H17 cells and even inhibits this population, at least in the combined absence of IL-10 and the presence of a strong inflammatory stimulus. In-deed, IL-6 secretion promotes T_H17 differentiation (48), and production of this cytokine was significantly increased following neutralization of IL-10 and TGF- β in naive and *S. mansoni*-infected mice. Alternatively, our anti-TGF- β mAb may efficiently suppress paracrine effects of this cytokine but not autocrine effects. Consistent with this hypothesis, the TGF- β that promotes the differentiation of naive CD4⁺ T cells into T_H17 cells is produced by the CD4⁺ T cells themselves (16).

The same considerations hold for the surprising stimulatory effect of anti-TGF- β mAb on the expansion of Tregs in *S. mansoni*-infected mice. TGF- β is thought to be required for the differentiation of Tregs as well as T_H17 cells (the combination of TGF- β and IL-6 promotes T_H17 differentiation, whereas TGF- β in the absence of IL-6 induces Tregs) (46,48). Expansion of the Treg population might, in theory, keep inflammation from becoming even worse in *S. mansoni*-inoculated mice that have been treated with anti-IL-10 and anti-TGF- β mAbs; however, we and others (49) have found that Treg depletion with anti-CD25 mAb has little effect on survival or immunopathology in *S. mansoni*-inoculated mice, regardless of whether they have been treated with anti-IL-10R/anti-TGF- β mAb (data not shown).

In sum, our observations demonstrate a partial redundancy between two anti-inflammatory cytokines during *S. mansoni* infection, suggest that cytokine control of T_H17 and Treg differentiation may be more complex than has been previously thought, and show that separate anti-inflammatory mechanisms control inflammation in different organs. These

observations have considerable practical importance, inasmuch as both anti-IL-10 and anti-TGF- β mAbs are being evaluated for the treatment of human disease (50,51).

Acknowledgments

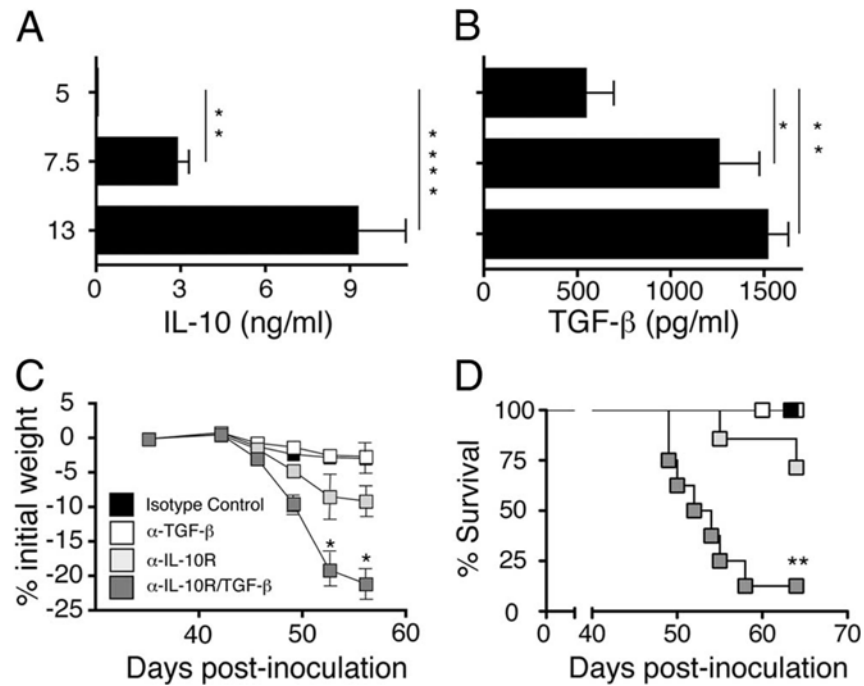
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**FIGURE 1.**

IL-10 and TGF- β redundantly protect against lethal schistosomiasis. Serum levels of IL-10 (A) and TGF- β (B) determined by IVCCA and ELISA, respectively, at the indicated time points in mice infected with 60–70 *S. mansoni* cercariae. Means \pm SE of 8–10 mice/group. Experiment performed twice. Weight change (C) and survival kinetics (D) of BALB/c mice infected with 60–70 *S. mansoni* cercariae were injected i.p on days 38, 42, and 46 with 1 mg of anti-TGF- β mAb (clone 1D11), 1 mg of anti-IL-10 receptor mAb (1B1.3), 1 mg each of both mAbs, or 2 mg of an isotype control mAb (clone J1.2). Data shown as means \pm SE of eight mice/group. Experiment performed four times (*, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.0001$ compared with isotype control treated group). Significance determined by ANOVA.

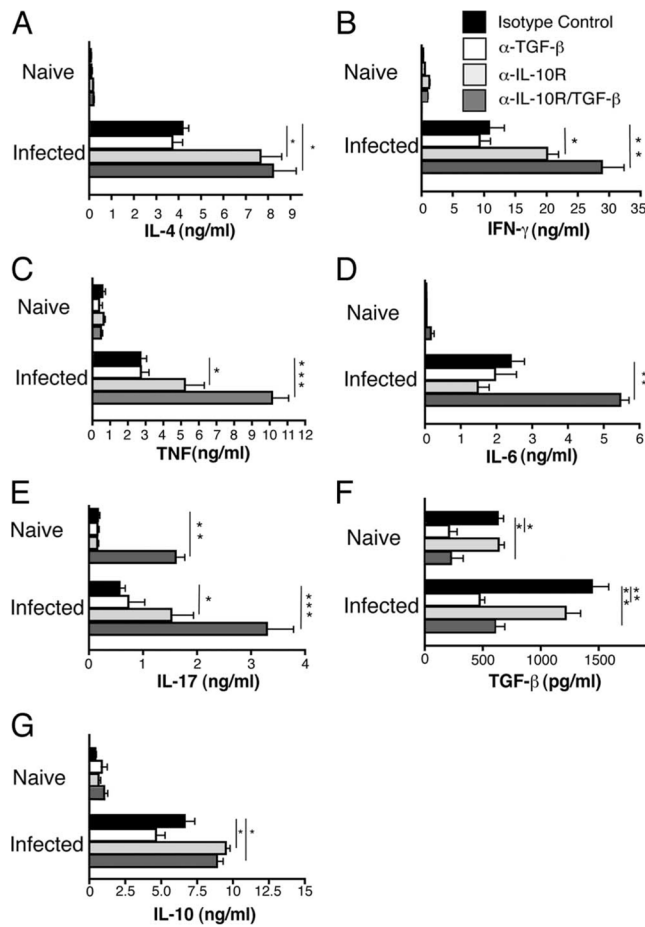
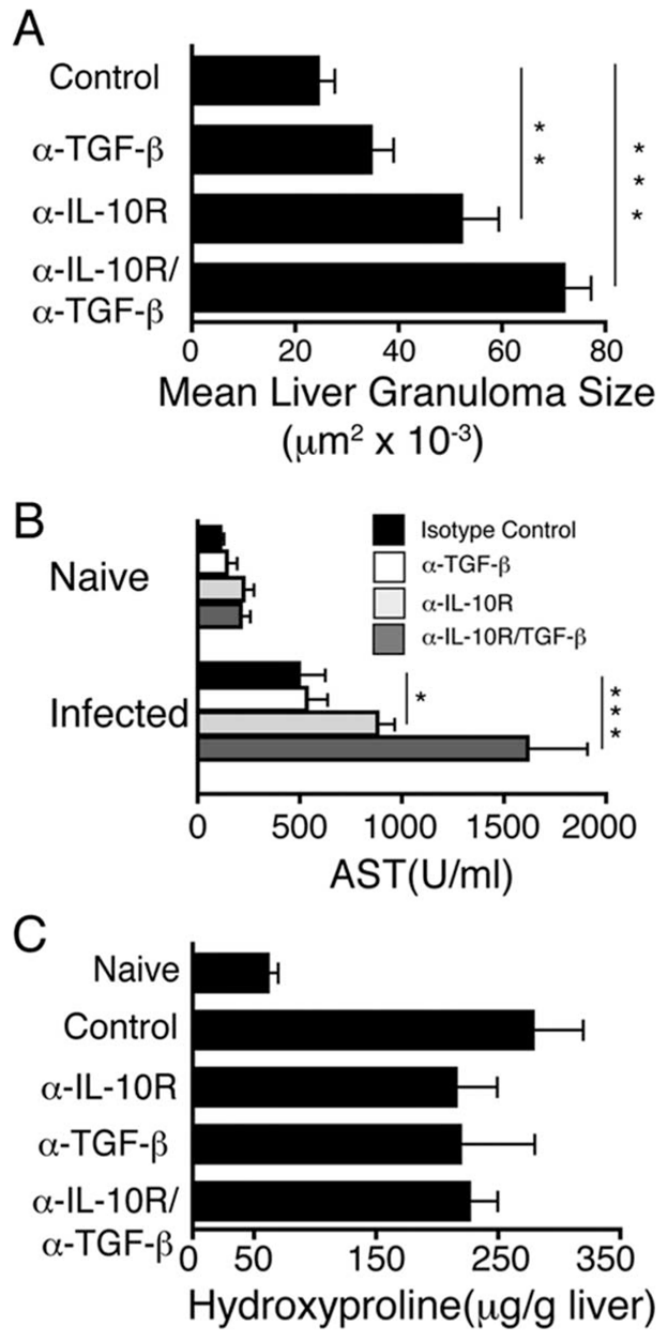


FIGURE 2.

Blocking IL-10 and TGF- β causes increased levels of cytokines associated with T_H1, T_H2, and T_H17 polarization. Production of (A) IL-4, (B) IFN- γ , (C) TNF, (D) IL-6 (G), IL-10 (measured by IVCCA), (E) IL-17, and (F) TGF- β 1 (measured by ELISA) in sera from naive or *S. mansoni*-inoculated BALB/c mice on day 52 postinfection. Animals were injected i.p. on days 38, 42, and 46 with 1 mg of anti-TGF- β (clone 1D11), 1 mg of anti-IL-10 receptor (1B1.3), 1 mg of both anti-TGF- β /anti-IL-10R, or 2 mg of isotype control (clone J1.2). Means \pm SE of six to eight mice/group. Experiment performed three times (*, $p < 0.05$; **, $p < 0.01$, and ***, $p < 0.0001$ compared with isotype control treated group).

**FIGURE 3.**

Blocking both IL-10 and TGF- β exacerbates hepatocellular damage and granulomatous pathology, but does not affect fibrosis. *A*, Liver granuloma cross-sectional area was determined 52 days postinoculation. 300 granulomas were measured per group. *B*, Serum levels of AST in naive or *S. mansoni* infected mice 52 days postinfection. *C*, Liver hydroxyproline levels 52 days postinfection. Means \pm SE of six to eight mice/group. Experiments performed three times with similar results (*, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$ compared with isotype control-treated group).

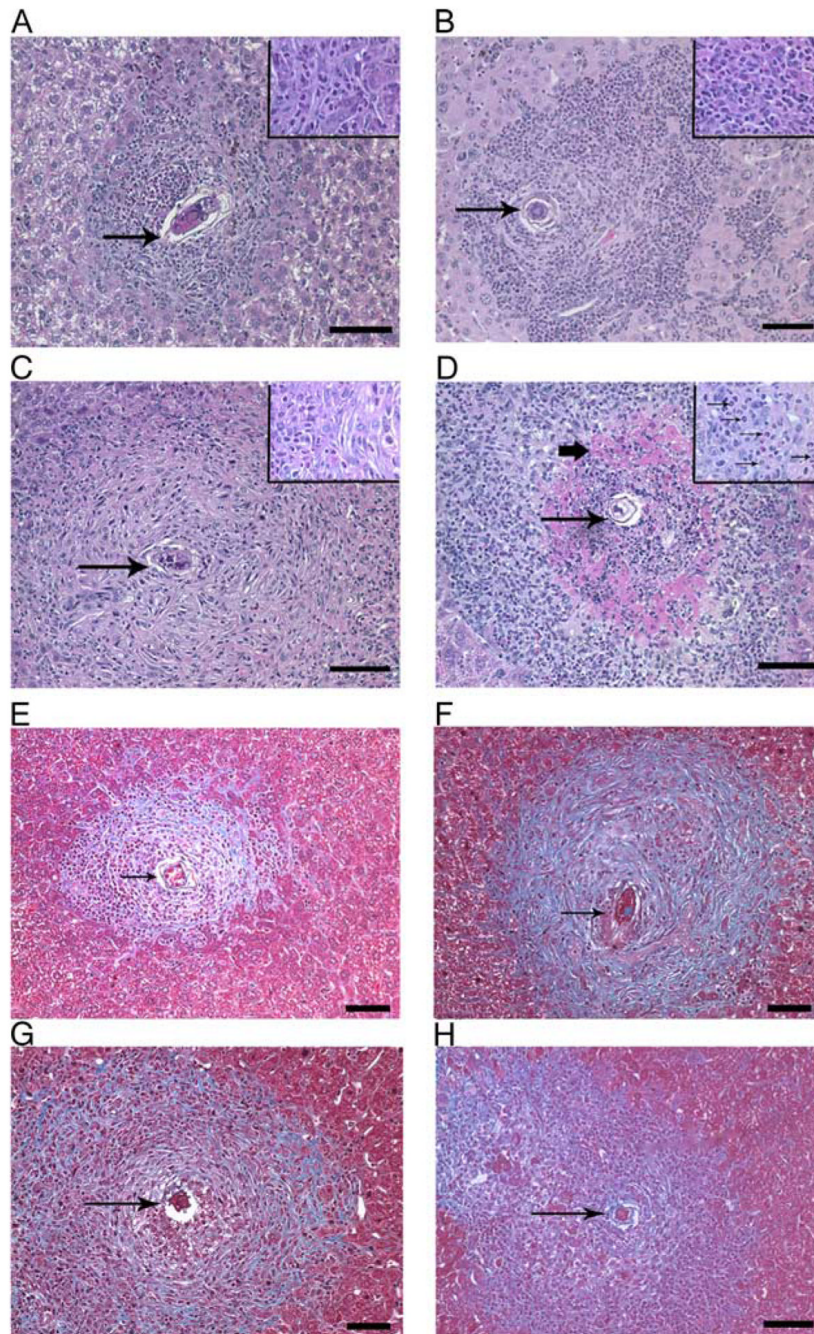
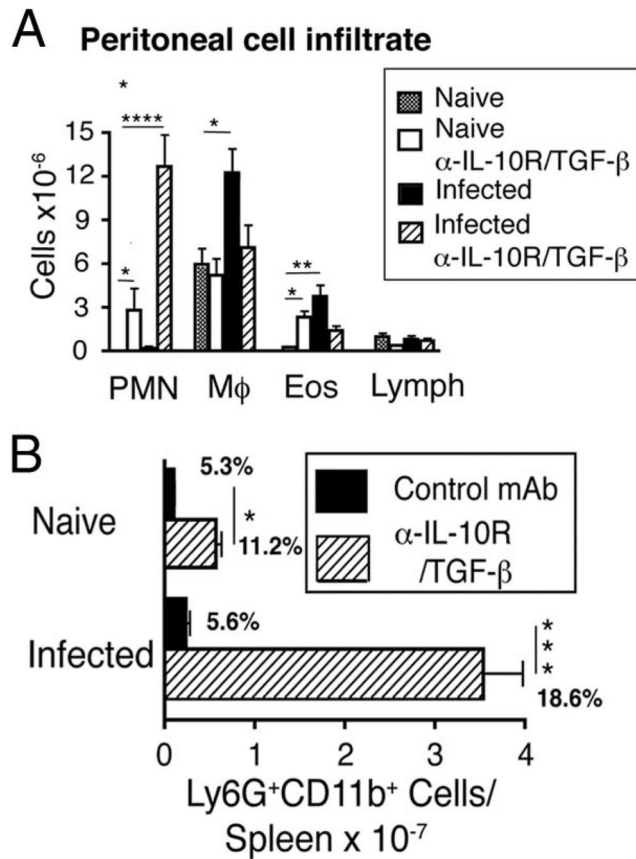


FIGURE 4.

Blocking both IL-10 and TGF- β causes hepatic necrosis. Liver sections from *S. mansoni*-inoculated mice on day 52 postinfection following treatment with isotype control (A and E), anti-TGF- β (B and F), anti-IL-10R (C and G), or anti-TGF- β /anti-IL-10R (D and H) Ab. Thin arrows point to parasite ova. Bold arrow points to area of necrosis. Representative photos are shown. A–D, H&E-stained sections. $\times 100$ magnification (*large panel*) $600\times$ (*inset*) Scale bar $100\ \mu\text{m}$. E–H, Masson's trichrome-stained sections at a $\times 200$ magnification. Staining for collagen (blue) and hepatocytes (reddish/purple). Scale bar $50\ \mu\text{m}$.

**FIGURE 5.**

Blocking IL-10 and TGF- β during acute schistosomiasis causes profound neutrophilia in peritoneal cavity and spleen. *A*, Inflammatory cells from peritoneal lavage from naive or *S. mansoni*-inoculated mice (day 52) treated with 1 mg each of anti-IL-10R/TGF- β mAbs or left untreated. *B*, Spleen cells from naive or *S. mansoni*-inoculated mice (day 52) were stained for Ly6G/C (GR-1⁺) and Mac-1 (CD11b⁺) following injection with 1 mg of anti-IL-10R/TGF- β or control mAbs. Means \pm SE of total numbers per spleen from four mice/group. Percent gated population shown on graph. Experiments performed twice with similar results (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; and ****, $p < 0.0001$ compared with isotype control treated group).

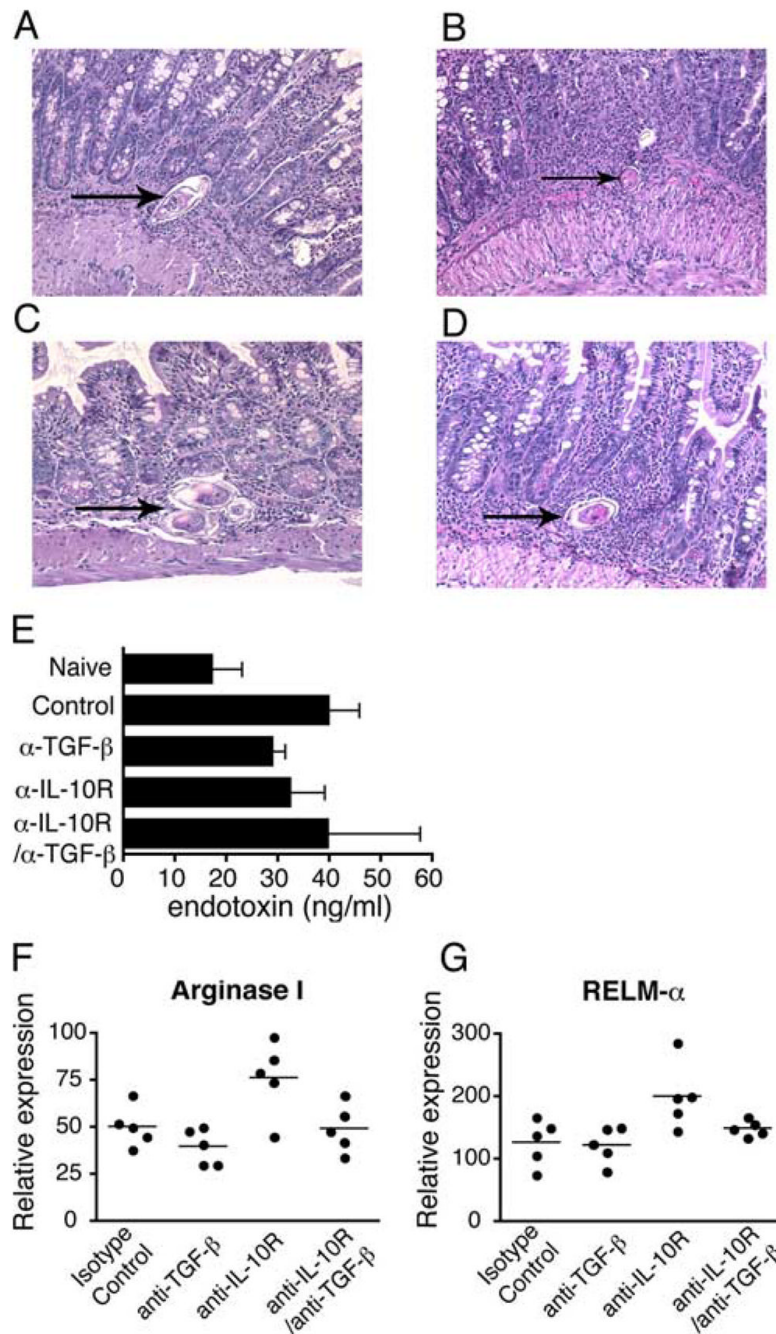
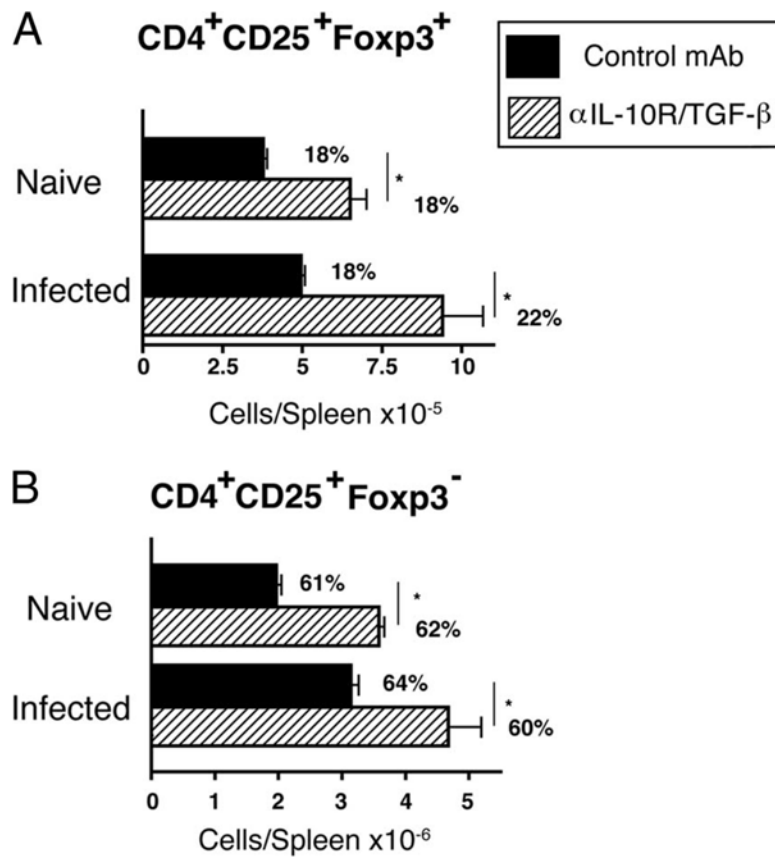


FIGURE 6. Blocking IL-10 and TGF- β does not affect intestinal pathology, alternative macrophage activation, or mucosal integrity. H&E staining of ileal tissue sections from *S. mansoni* infected mice on d 52 postinfection following treatment with isotype control (A), anti-TGF- β (B), anti-IL-10R (C), or anti-TGF- β /anti-IL-10R (D) mAb. Magnification 200 \times . Arrows point to parasite ova. Measurement of serum endotoxin levels on day 52 postinfection (E). Real-time PCR quantification of gut mRNA transcripts for (F) Arginase I and (G) RELM- α . Means \pm SE of five mice/group. Experiment performed twice.

**FIGURE 7.**

Blocking IL-10 and TGF- β during acute schistosomiasis infection increases Treg number. Spleen cells from naive or *S. mansoni*-inoculated mice (day 52) that received 2 mg of GL113 or were treated with 1 mg each of anti-IL-10R/TGF- β mAbs were stained with fluorochrome-labeled mAbs for CD4 (RM4-5), CD25 (PC-61), and Foxp3 (FJK-16s) and analyzed by flow cytometry for number of $CD4^+CD25^+Foxp3^+$ cells. Percent of gated $CD4^+$ cell population shown on graph. Means \pm SE of four mice/group. Experiments were performed twice (A and B) with similar results (*, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$ compared with isotype control-treated group).