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Regulation of Liver Fibrosis during Murine Schistosomiasis Mansoni

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ABSTRACT

Background: The main pathology of *Schistosoma mansoni* (*S. mansoni*) infection is induced by a granulomatous tissue reaction against the parasite eggs. Unfortunately, no therapy has been proven to prevent the progression of hepatic fibrosis associated with granulomatous hypersensitivity to parasite eggs. Accumulating evidence has demonstrated a critical pathogenic role for both interleukin 17 (IL-17) and interleukin 13 (IL-13) in organ fibrosis.

Objective: The present study investigated the role of IL-17 and IL-13 in the pathogenesis of liver fibrosis during *S. mansoni* infection.

Material and Methods: Thirty female C57BL/6 mice were divided into three groups, normal, infected, and anti-mouse IL-17 treated groups. The infected and anti-mouse IL-17 groups were infected with 40±5 cercariae of *S. mansoni* per mouse. Neutralizing rat anti-mouse IL-17 mAb or an isotype-matched rat IgG mAb was first administered intraperitoneally 3 weeks after *S. mansoni* infection (62.5µg per mouse) then at the same dose every third day until 2 days before killing. Serum IL-17, IL-13, and proinflammatory cytokines levels were determined by ELISA. Liver granulomas were measured by an ocular micrometer.

Results: Serum level of IL-17 was significantly higher in infected mice compared with non-infected animals. Reducing IL-17 activity using anti-IL-17 monoclonal antibodies improves liver functions and reduces the size of the liver granulomas. Meanwhile, Th-2 profibrogenic cytokine IL-13 was also decreased in infected/anti-IL-17 mAB-treated mice. IL-17-induced proinflammatory mediators (IL-1β, IL–6 and TNF-α) that involved in liver fibrosis were markedly reduced in anti-IL-17 mAB-treated mice.

Conclusion: IL-17 and IL-13 contribute to granulomatous inflammatory and fibrosing reactions in murine schistosomiasis.

Keywords: *Schistosoma mansoni*, Liver fibrosis, Mice, IL-17, IL-13.

INTRODUCTION

Studies of schistosomiasis showed that 700 million at risk of infection with almost 200 million infected in Africa alone [(1)]**.** Infection begins with skin penetration by cercariae (infectious stages of schistosomes), and following maturation into sexual pairs, adult parasites of *S. mansoni* migrate to the mesenteric veins where they live up to 10 years or more, laying hundreds of eggs/ day. Some of the eggs were trapped in the liver microvasculature and induced a vigorous granulomatous response [(2)]. Subsequently, fibrosis, portal hypertension, and collateral vessels may develop which were the primary causes of morbidity in infected individuals and some cases would ultimately be lethal. So, much of the symptomatology of schistosomiasis is attributed to the egg-induced granulomatous inflammatory response and associated fibrosis [(3)].

Mechanisms underlying the development of pathology were not well-defined. Animal studies identified a moderate type 1 helper (Th1) response to parasite antigens; but, a robust Th2 response to egg-derived antigens dominates and propagates fibrogenesis within the liver [(4)].

The unique subset of new T-cell lineage characterized by IL-17 secretion was defined [(5)] and has demonstrated profibrogenic effects in different experimental models of hepatic, pulmonary, and myocardial fibrosis [(6, 7)]. The IL-17 family is composed of six members, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. Among them, IL-17A (generally called IL-17), the founding member was predominantly produced by Th 17 cells [(8)]. These cytokines exert their biological effects through binding to their cognate IL-17 receptors (IL-17R). The IL-17 receptor family consists of five members, IL-17RA–IL-17RE. IL-17A signals through binding to IL-17RA (also referred to as IL-17R) and IL-17RC that are expressed on various cell types [(9, 10)]. A crucial role for IL-17 in *S. japonicum* induced pathology, with severe morbidity correlating with high levels of IL-17 were identified in C57BL/6 mice. C57BL/6 mice display a vigorous IL-17 response following infection with *S. japonicum*, leading to severe liver pathology. Neutralization with anti-IL-17 mAb or using IL-17RA$^{-/-}$ mice markedly reduced granuloma size[(11)].

Also, IL-13 plays an important part in liver pathology during schistosomiasis [(12)]. IL-13 is bound by two distinct receptors; IL-13Ra1 (which forms a complex with the signaling IL-4Ra chain) and IL-13Ra2. IL-13Ra2 was first described as a soluble,

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high-affinity binding protein that inhibits IL-13 [13].A significant increase in IL-13Ra2 in the serum and liver of *S. mansoni* infected mice observed was parallel to the Th2 response against parasite eggs, suggesting that IL-13Ra2 was driven by Th2 responses. The upregulation of IL-13Ra2 is critical for controlling IL-13-mediated liver pathology as mice with a targeted deletion of IL-13Ra2 (IL-13Ra2$^{-/-}$) develop exacerbated fibrosis compared to infected controls [12, 14]. A soluble IL-13Ra2-Fc construct used to neutralize IL-13 was shown to reverse the excessive liver fibrosis observed in infected IL-13Ra2$^{-/-}$mice, showing that IL-13Ra2 acts as a potent decoy receptor for IL-13 in schistosomiasis [14].

The present study aimed to investigate the roles of profibrogenic cytokines IL-17 and IL-13 in the regulation of liver fibrosis induced by *Schistosoma mansoni* infection.

MATERIALS AND METHODS

Ethics statement:

The study was conducted at Theodor Bilharz Research Institute (SBSP/TBRI, Giza, Egypt) in strict accordance with the TBRI guidelines for ethical conduct in the use of animals in research.

Mice and infection: C57BL/6 mice were reported to be highly susceptible to *S. mansoni* infection and were used as a suitable animal model for evaluating host immune response to schistosome species [15]. Specific pathogen-free C57BL/6 mice, aged 8 weeks and weighing 18–20 g, were maintained in conditioned rooms at 21°C on sterile water and a balanced dry food containing 14% protein. The Mice were housed in groups of two to three mice each in wire-floored cages. Cercariae of *S. mansoni* were shed from infected *Biomphalaria alexandrina* snails kept in 200 mL of distilled water and exposed to artificial light. Mice were infected percutaneously with 40±5 cercariae and killed at 8 weeks after infection. Neutralizing rat anti-mouse IL-17 mAb or an isotype-matched rat IgGmAb was first administered intraperitoneally 3 weeks after *S. mansoni* infection (62.5µg per mouse) then at the same dose every third day until 2 days before killing [16].

Measurement of aspartate transaminase/alanine aminotransferase levels: Blood samples from each mouse were collected via a small puncture of the caudal vein by using a sterile needle. Sera were separated by centrifugation at 6000 rpm for 10 minutes. Levels of aspartate transaminase (AST) and alanine aminotransferase (ALT) were detected using a commercial kit (Boehringer reagent kit, Mannheim, Germany) according to the manufacturer's instructions [17].

Cytokines assay: Serum concentrations of IL-17, IL-13, and proinflammatory cytokines (IL-1β, IL−6 & TNF-α) were determined by ELISA (Biosource International, Camarillo, California, USA) as recommended by the supplier.

Histology: Livers were removed from the mice, washed three times with 0.01M PBS (pH 7.4), fixed in 10% formalin, embedded in paraffin, and microtome sections were cut at a thickness of 5µm and at a distance of 250µm apart to avoid re-measurement. The sections were then examined by light microscopy under 100x& 400x magnification after they were stained with hematoxylin-eosin and Masson's trichrome stain that showed the amount and pattern of collagen formation in the granuloma.

The ocular micrometer was used for measurement of the diameter of the liver egg granulomas [18].Granulomas were measured under a microscope in 80-100 visual fields of liver sections (mounted on coded slides) by an observer blind to treatment history. Only cross-sections containing a visible central egg were counted. Granuloma size is expressed as the mean area in $\mu m^2 \pm$ SD.

Statistical analysis: Data were collected, tabulated, and statistically analyzed by using statistical package for the social sciences program, version 18 (SPSS; SPSS Inc. Chicago, Illinois, USA). The obtained data were expressed as means and SD. Significance analysis was carried out using a two-tailed Student's *t*-test for unpaired means. Statistically significant was considered when *P*-value less than 0.05.

RESULTS

Elevated IL-17 level in infected mice sera: To investigate whether *S. mansoni* infection induces IL-17 production. We compared serum levels of IL-17 obtained from uninfected control, infected, and infected/anti-IL-17 mAB-treated mice. *S. mansoni* infection induces IL-17 production, the cytokine level was substantially higher in infected mice than in non-infected control. Administration of anti-IL-17 mAB reduced IL-17 serum levels in infected mice (Fig. 1).

Anti-IL-17 mAb reduced serum levels of aspartate transaminase/alanine aminotransferase in infected C57BL/6 mice: Having demonstrated that IL-17 was induced by *S. mansoni* infection, it was important to investigate the effect of anti-IL-17 mAb on liver functions of infected mice. Uninfected control, *S. mansoni* infected, and infected/anti-IL-17 mAB-treated mice were subjected to the experiment. Serum levels of aspartate transaminase (AST) and alanine aminotransferase (ALT) were measured at 8 weeks p.i. ALT/AST serum levels were significantly increased in mouse serum following infection with *S. mansoni*. Meanwhile, levels of ALT/AST were reduced in infected/anti-IL-17 mAB-treated mice when compared to infected non treated group (Fig. 2a&b).

Reduction of hepatic granulomatous inflammation by anti-IL-17 mAb in infected mice: Haematoxylin and eosin staining of liver sections revealed the normal cellular organization of uninfected hepatic lobules, with the typical actinomorphous distribution of hepatic cord centered around central veins. Infection by *S. mansoni* markedly altered the histological structure of the mouse liver. There were many more inflammatory cells in the infected liver lobules than in uninfected controls, while anti-IL-17 neutralizing mAb markedly reduced numbers of inflammatory cells in the infected liver (Fig. 3a). The infected livers developed large liver granulomas, whereas these granulomas were significantly smaller in infected/anti-IL-17 mAb-treated mice (Fig. 3b). Taken together, these results suggest that induction of IL-17 during *S. mansoni* infection may enhance hepatic granulomatous inflammation.

Elevated IL-13 serum level in infected mice: *S. mansoni* infection resulted in increased serum level of IL-13. The serum level of cytokine was significantly decreased in infected/anti-IL-17 mAB-treated mice as compared to infected non treated ones (Fig. 4).

Changes in the serum levels of proinflammatory cytokines: A variety of cytokines are involved in liver fibrosis**,** we, therefore, design the experiment to determine the role of IL-17 in the upregulation of certain inflammatory cytokines during infection. Levels of IL-1β, IL-6 & TNF-α in the sera of mice groups were determined by ELISA. Compared with infected non treated mice, serum levels of cytokines in the infected/anti-IL-17 mAB-treated group were greatly reduced (Fig. 5a, b &c).

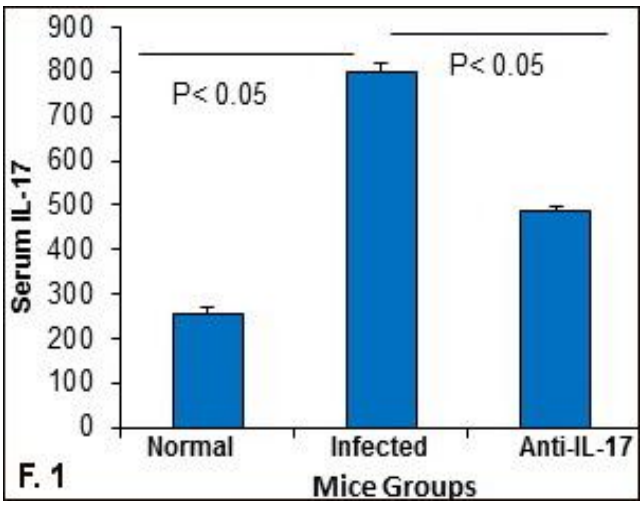


Fig.(1): *S. mansoni* induced the production of IL-17 in infected mice. Our mice were divided into three groups, normal, infected, and infected/ anti-mouse IL-17 groups. The infected groups were infected with 40±5 cercariae of *Schistosoma mansoni* per mouse. For the infected and infected/anti-IL-17group, 62.5µg control IgGmAb or anti-IL-17 mAb per mouse were administered intraperitoneally every 3 days, respectively. Our data indicated that *S. mansoni* infection increased the serum level of IL-17 in infected mice. The serum levels of cytokine were significantly less in the infected/anti-IL-17 mAb group than in mice exposed to parasite only.

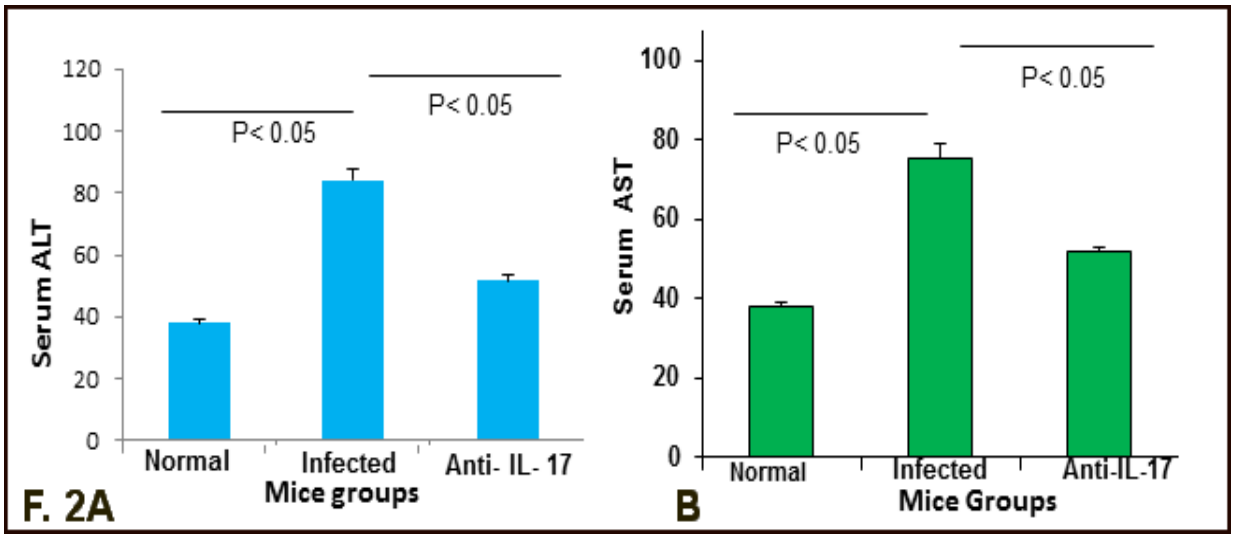
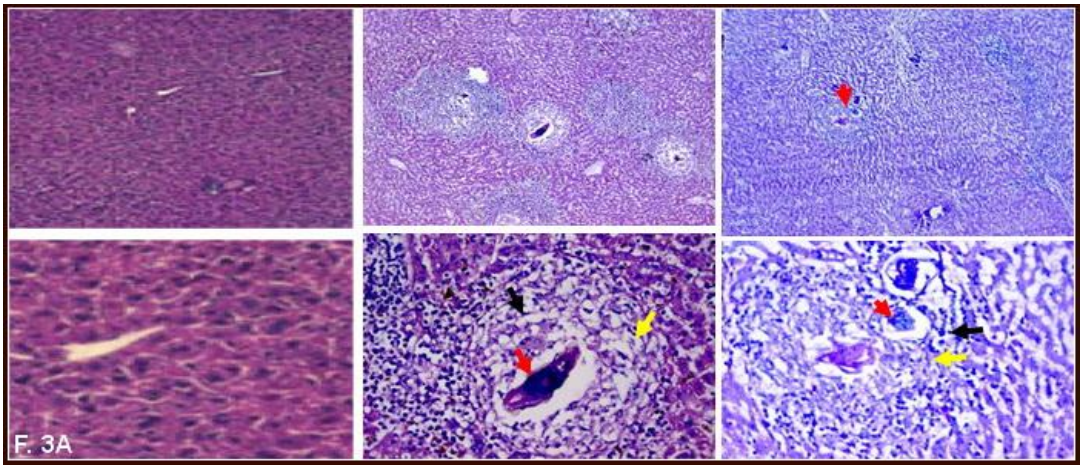


Fig.(2):A-Serum ALT and B- serum AST of uninfected control, *S. mansoni* infected, and infected/anti-IL-17 mAB-treated mice were measured at week 8 p.i. Results are shown as means ± SD from 10 mice in each group (*P< 0.05). Significantly decreased levels of ALT/AST in anti-IL-17 treated mice were observed when compared to the infected group.



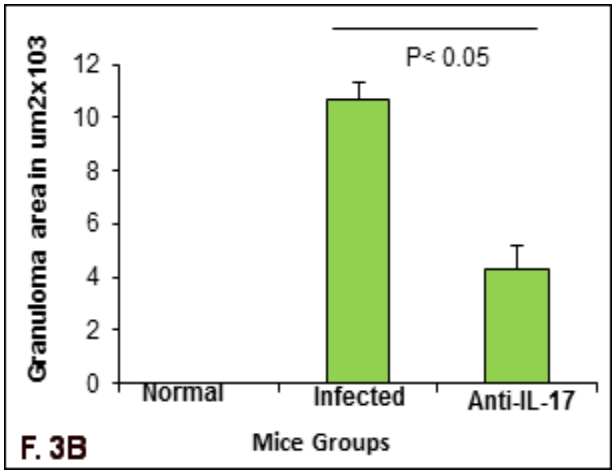


Fig.(3): A-Anti IL-17 mAb treated mice developed decreased granuloma size compared to non-treated infected mice. Sections of normal liver (left panels), infected mice (middle panels), and anti-mouse IL-17 mAb treated mice (right panels) examined by H & E staining (original magnification x100 for upper panels and x 400 for lower panels). Infected non treated mice showing larger size fibrocellular granuloma formed of central ova with living miracidia (red arrow), surrounded mainly by lymphocytes, esinophils (black arrow) and collagen fibers (yellow arrow). B-Granuloma size measured by oculometer, only granulomas with a visible central egg analyzed for accuracy. Between 80 and 100 granulomas for each group were measured under a microscope (*P<005, error bars indicate SD).

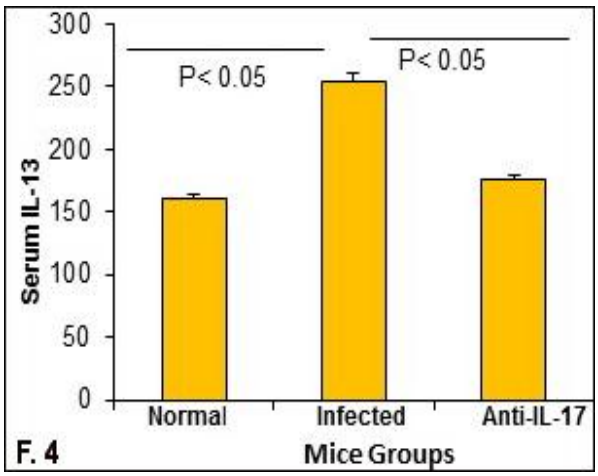


Fig. (4): Anti-IL-17 mAB reduced serum level of IL-13 in infected mice. *S. mansoni* infection increased IL-13 serum level. Anti-IL-17 mAB-treatment reduced cytokine production in infected mice as compared to infected non -treated mice.

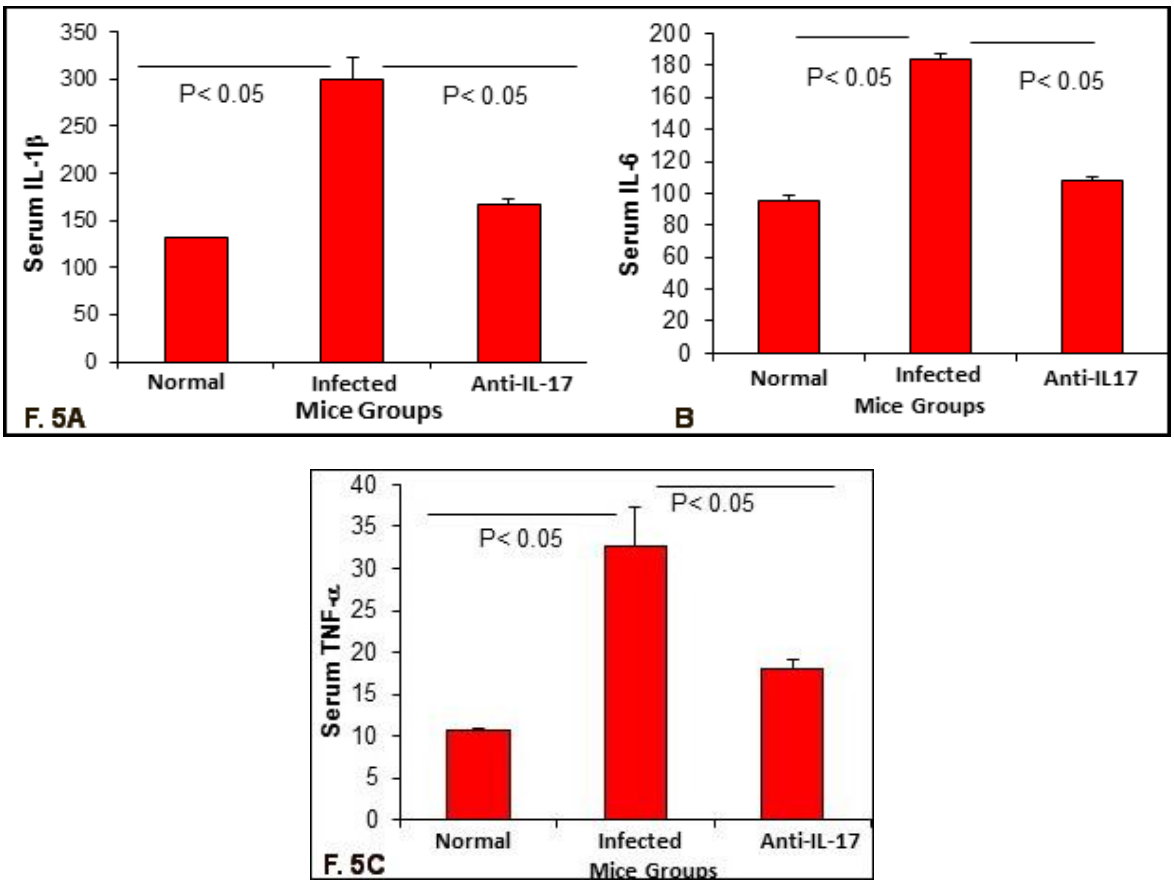


Fig.(5): A, B & C: Effect of anti-IL-17 mAb on proinflammatory cytokines. To assess the role of IL-17 in the upregulation of certain inflammatory cytokines during infection. Levels of IL-1β, IL-6, and TNF-α in the sera of our mice groups were determined by ELISA. Compared with those in infected non- treated, serum levels of these cytokines in infected/anti-IL-17 mAB-treated mice were greatly reduced.

DISCUSSION

Schistosomiasis *mansoni* is often asymptomatic, however severe morbidity occurs in a subset of patients. The main pathological lesion of hepatic schistosomiasis is the granuloma formed around *Schistosoma* eggs in acute stages of infection and subsequent liver fibrosis at chronic and advanced stages[3, 4]. Previous studies using cytokine-deficient mice indicated opposing effects of Th 1 and Th 2 responses in fibrosis. The Th 2 response, characterized by a high production of IL-4 and IL-13, contributes to fibrogenesis. In contrast, the Th 1 response, by producing IFN-γ, inhibits liver fibrosis [19, 20, 21]. However, accumulating evidence has also demonstrated the severity of liver pathogenesis may be correlated with IL-17 activity, possibly released by inflammatory cells such as neutrophils and eosinophils in the granulomas [22, 23, 24].

The present study measured IL-17 production in healthy and infected mice by ELISA. The data showed that much greater IL-17 production was observed in the sera of *S. mansoni* infected mice than in uninfected animals (P<0.05). To identify the role of IL-17 in host granulomatous inflammatory and fibrosing reactions against *S. mansoni* infection, we administered an anti-IL-17 mAb to infected mice. Sera obtained from normal, infected and anti-IL-17 mAb treated mice were examined for ALT/AST levels; all collected samples were obtained from 8-week-infected mice. The results showed that anti-IL-17 mAb improves liver functions, as demonstrated by a reduction in ALT/AST in anti-IL-17 mAb treated mice when compared to infected non treated group (P<0.05). The granulomatous inflammation and granuloma size in infected mice was significantly reduced by anti-IL-17 mAb (P<0.05 compared with infected non treated mice) as measured by granuloma cross-sectional area. The results agreed with data demonstrating a positive relationship between IL-17 activity and the severity of liver pathogenesis and reports implicating IL-17 in the granulomatous inflammatory reaction during *S. japonicum* infection [13, 24].

Previous animal studies showed that IL-13 was responsible for the majority of immunopathology and fibrosis during infection [12, 15, 18]. In our Il-13 experiment, a comparison of infected & infected/anti-IL-17 mAb-treated mice showed that IL-17 induced IL-13 production, as IL-13 was significantly reduced in mice treated with anti-IL-17 mAb. This showed that anti-IL-17 mAb attenuates fibrosis by reducing both IL-17 and IL-13 synthesis.

Interleukin -17 signaling induced expression of inflammatory cytokines [8, 11] that were reported to involve in liver fibrosis [25, 26]. In the present study, proinflammatory cytokines levels were compared in sera obtained from mice groups. The results showed that pro-inflammatory mediators (IL-1β, IL-6 and TNF-α) were significantly higher in infected mice than in the uninfected control group. This effect was partially blocked by anti-IL-17 mAb, which strongly implicating IL-17 in inflammatory cytokines upregulation.

The regulation of schistosomiasis induced hepatic fibrosis may be complex with multiple mediators regulating the progression of the disease. More studies are required to elucidate the full spectrum of the mechanism of IL-17 and IL-13 in the increasing liver fibrosis during infection are ongoing and will be published in due time.

CONCLUSION

The outcome results suggested that IL-17 and IL-13 contribute to granulomatous inflammatory and fibrosing reactions in murine schistosomiasis and anti-IL-17mAb may be a potential therapy in the treatment of fibrosis formed around *Schistosoma* eggs. Thus, IL-17 may be considered a promising target for antifibrotic therapy.

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