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Role of IL-17A in enhancing liver fibrosis induced by TGF-β1 and IL-13 in *Schistosoma mansoni* infected mice

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Abstract

Schistosoma mansoni liver fibrosis is a complicated multicellular process involving numerous cytokines, chemokines, and growth factors. Transforming growth factor beta 1 (TGF-β1) and interleukin (IL)-13 have been identified as critical pro-fibrotic mediators in many studies. IL-17A was linked to enhanced TGF-β1 and IL-13-induced pathologies. This case-control study aimed to explore the effect of IL-17A on TGF-β1 and IL-13-induced liver fibrosis during experimentally schistosomiasis mansoni infection. A total of 40 laboratory-bred female C57BL/6 mice were divided into four equal groups (G), G1 non-infected, G2 infected wild type (WT), G3 infected/anti-IL-17 monoclonal antibodies (mAb) and G4 treated mice. Mice were infected percutaneously with 40±5 cercariae per mouse. Neutralizing IL-17 mAb was administered to G3 intraperitoneally 3 weeks after infection and then every third day until 2 days before sacrification; mice of G4 were treated with a single dose of praziquantel. Serum levels of TGF-β1, IL-13, IL-17A, and proinflammatory cytokines were measured by ELISA. Liver granulomas were identified by hematoxylin-eosin stain and measured by an ocular micrometer. There was a significantly increased serum concentration of TGF-β1, IL-13, and IL-17A in infected WT mice (P<0.01), but praziquantel treatment reduced cytokine levels (P<0.03). Neutralization of IL-17A activity remarkably reduced serum concentrations of TGF-β1 and IL-13 (P <0.03) resulting in improved liver functions and reduced granuloma size. Secretion of IL-1β, IL-6 and TNF-α were markedly enhanced by infection, however, mice that received anti-mouse IL-17 mAb displayed fewer inflammatory mediators (P<0.03). In conclusion, IL-17A might contribute to the progress of liver fibrosis by enhancing the profibrotic effect of TGF-β1 and IL-13 in mice infected with *S. mansoni*.

Keywords: *Schistosoma mansoni*, Liver fibrosis, TGF-β1, IL-13, IL-17.

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Introduction

Schistosomiasis mansoni, a disease caused by *Schisosoma mansoni* infection, was reported to be an important cause of severe morbidity and mortality in Egypt and worldwide with about 200 million infected individuals in Africa alone.[1]

The infection begins when free-swimming cercariae penetrate the skin and maturate into sexual pairs in the mesenteric veins where they lay hundreds of eggs/day. Some of the eggs are trapped in the liver microvasculature evoking strong granulomatous inflammation and subsequently progressive liver fibrosis and portal hypertension.[2]

Liver fibrosis is the excessive aggregation of extracellular matrix proteins (ECM) including collagen. Studies of the cellular and molecular mechanisms of liver fibrosis have identified activated hepatic stellate cells (HSCs), portal fibroblasts, and myofibroblasts of bone marrow origin as major collagen-producing cells in the injured liver.[3]

Transforming growth factor beta-1 (TGF-β1) plays a critical role in tissue fibrogenesis. Neutralization of its activity *in vivo* has proven to be an effective way of reducing the fibrotic response to injury in different organs. Also, transgenic mice in which TGF-β1 gene expression is enhanced develop marked spontaneous fibrosis or increased fibrotic response to injury. It regulates fibroplasia, not only by increasing extracellular matrix synthesis but also by coordinately regulating proteins that are required for connective tissue homeostasis.[4-7] In the liver, TGF-β1 is secreted by activated macrophages, regulatory T cells (T-regs), and activated HSCs in response to pro-inflammatory signals. It activates HSCs through the SMAD2/3 signaling pathway that leads to the transcription of pro-fibrotic molecules associated with the progression of liver fibrosis.[8,9]

In addition, many studies identified interleukin (IL)-13 as a potent mediator of fibrosis due to its marked fibrotic changes that were absent in lacking IL-13 even after several months of infection. It was suggested that IL-13 mediates its profibrotic effects by regulating the production and activation of TGF-β1.[10-13] However, some other studies demonstrated that IL-13-mediated fibrotic effects are not lonely dependent on the downstream signaling of TGF-β1, and suggested that IL-13 activates a mechanism of tissue fibrosis that is completely TGF-β1 independent as infected IL-13 knockout mice showed remarkable inhibition of fibrosis despite undiminished secretion of TGF-β1.[14]

Th17 cells, a subpopulation of CD4 helper T cells, are important mediators in host defense against various pathogens. They are produced from naive CD4 T cells in response to the pro-inflammatory cytokines IL-1β, IL-6, and TGF-β1 and require IL-23 to become fully activated.[15] This unique subset of cells secretes pro-inflammatory cytokines like IL-17A, IL-17F, and TNF-α as well as the anti-inflammatory cytokine IL-22. Th17 cells were linked to liver fibrosis enhancement in different liver diseases including alcoholic hepatitis and hepatitis B&C viruses infections.[16,17] IL-17A is a pro-fibrogenic cytokine with no direct effect on HSCs. This cytokine enhances HSCs' response to TGF-β1 leading to increased secretions of collagen type I, α-SMA, and TIMP-I. It may also induce liver fibrosis progression indirectly via other means, including recruitment of macrophages through enhancement of chemokine secretion by HSCs. It then stimulates these newly recruited macrophages to secrete fibrogenic cytokines including TGF-β1.[18,19] Studies have also revealed that IL-17A regulates the development of IL-13-induced lung fibrosis and pathology by enhancing IL-13-induced STAT6 activation.[20]

In the present study, we developed an *in vivo* model of schistosomiasis mansoni liver fibrosis and explored the effect of IL-17A on TGF-β1 and IL-13-induced liver fibrosis during experimentally schistosomiasis mansoni infection.

Materials and Methods

The present study was conducted at the Theodor Bilharz Research Institute (TBRI) Giza, Egypt, during the period from June 2020 to January 2022.

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.[21] Specific pathogen-free 40 female 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 sacrificed at 8, 10, and 12 weeks after infection. Neutralizing rat anti-mouse IL-17 mAb (Biosource International, Camarillo, California, USA) was first administered intraperitoneally 3 weeks after infection (62.5µg per mouse) and then repeated every third day until 2 days before scarification.[22]

Treatment: Praziquantel (Distocide ®, EIPICO, Egypt) 600 mg tablets, were ground into powder and then dissolved in distilled water. The drug was given to mice orally by gastric gavage in a single dose of 500 mg/kg on the 42nd day post-infection (PI).[23]

Measurement of aspartate transaminase/ alanine aminotransferase, and gamma-glutamyl transferase serum levels: Blood samples from each mouse were collected via a small puncture of the caudal vein using a sterile needle. Sera were separated by centrifugation at 2817xg for 10 minutes. Levels of aspartate transaminase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) were detected using a commercial kit (SPINREACT, Ctra. Santa, Spain) according to the manufacturer's instructions.[24]

Cytokines assay: Serum concentrations of TGF-β1, IL-17A, IL-13, and proinflammatory cytokines (IL-1β, IL-6& TNF-α) were determined by commercial ELISA kits (Biosource International, Camarillo, California, USA), according to the manufacturer's instructions.

Histopathological examination: The liver from each mouse was removed, 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 5 µm and at a distance of 250 µm apart to avoid re-measurement. The sections were then examined microscopically under 100x and 400x magnification after staining with hematoxylin-eosin and Masson's trichrome stain which showed the amount and pattern of collagen formation in the granuloma.[25] The ocular micrometer was used to measure the diameter of liver granulomas under the microscope in 80-100 visual fields of liver sections (mounted on coded slides). Only cross-sections containing a visible central egg were counted and the granuloma size was expressed as the mean area in µm2± SD.[25]

Statistical analysis

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

Results

Changes in the serum levels of AST, ALT, and GGT during murine schistosomiasis mansoni

To determine the effect of *S. mansoni* infection on liver functions, uninfected control, *S. mansoni* infected, and praziquantel-treated C57BL/6 mice were subjected to the experiment. Serum levels of AST, ALT, and GGT were measured at 8, 10, and 12 weeks post infection (PI) and showed a significant increase in infected mice (*P*<0.03). Administration of praziquantel notably reduced levels of liver enzymes in infected animals, (*P* <0.03) (Figure. 1).

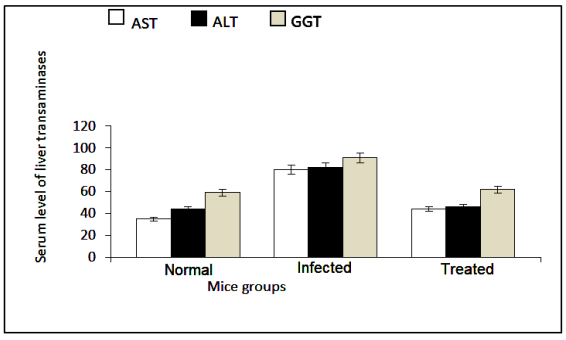
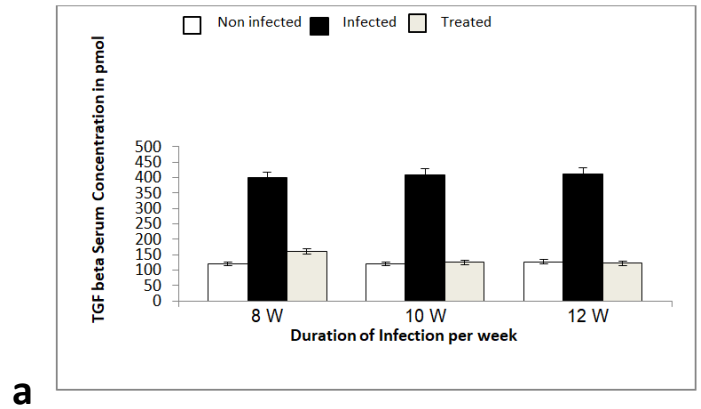


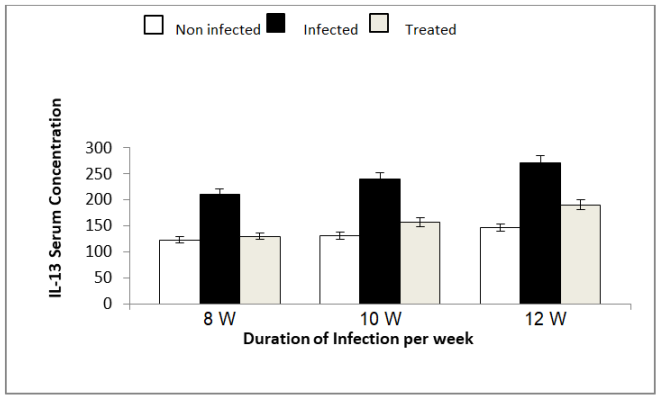
Figure 1. A histogram demonstrating liver enzymes among different mice groups. Liver enzymes were significantly higher in infected mice (*P*<0.03), praziquantel treatment notably reduced them <0.03).

Effect of S. mansoni infection on the serum levels of TGF-β1, IL-13 and IL-17 A

TGF-β1 secretion was substantially higher in infected mice at 8, 10, and 12 w PI than in non-infected controls (P<0.01). Administration of praziquantel (Figure 2a) reduced the growth factor levels in the infected group (P<0.03). Similarly, *S. mansoni* infection increased serum levels of IL-13 and IL-17 A in tested mice (P<0.01) (Figure 2b & 2c), but the level of both cytokines were significantly decreased in treated mice as compared to the infected non-treated group (P<0.03).



a



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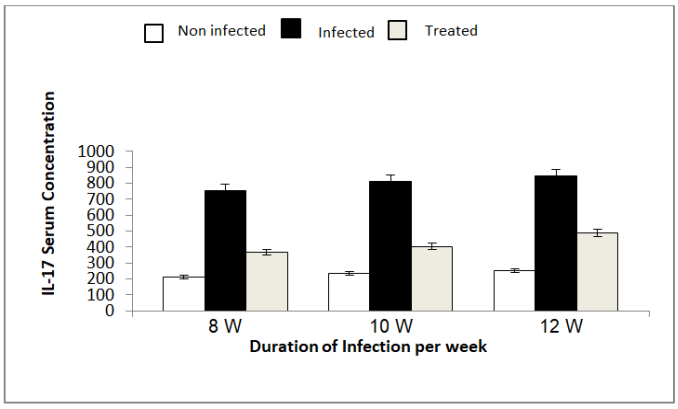

c

Figure 2. A histogram demonstrating the increased production of profibrogenic cytokines (a) TGF-β1, (b) IL-13, and (c) IL-17A in response to *S. mansoni* infection. The secretions of profibrogenic cytokines were significantly higher in infected mice at 8, 10, and 12 weeks PI when compared to non-infected mice (P<0.01). Praziquantel treatment significantly reduced serum concentration of TGF-β1, IL-13, and IL-17A in infected mice (P<0.03).

Effect of Anti-IL-17A neutralizing mAb on the serum levels of TGF-β1 and IL-13 during S. mansoni infection

Next, we investigated whether TGF-β1 and IL-13 secretions were affected by the neutralization of IL-17 activity. Neutralization of IL-17A activity significantly reduced levels of TGF-β1 (Figure 3a) and IL-13 (Figure 3b) in infected mice (*P*<0.03).

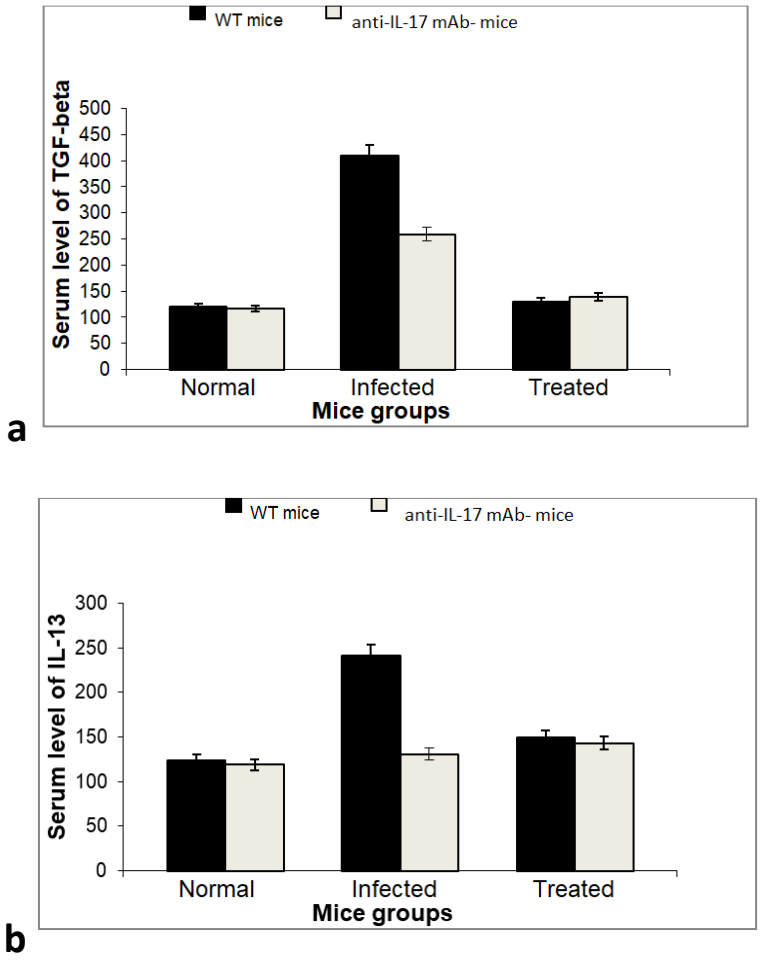

Figure 3. A histogram showing the effect of neutralization of IL-17A activity on (a) TGF-β and (b) IL-13. Serum concentrations of TGF-β and IL-13 were determined in infected WT and infected mice treated with rat anti-mouse -IL-17A mAb. Neutralization of IL-17 activity significantly decreased serum concentration of both cytokines in *S mansoni* infected mice (*P*<0.03). Mice treatment with praziquantel notably decreased serum concentration of TGF-β and IL-13 in infected mice with *S mansoni* (*P*<0.03).

Changes in the serum levels of proinflammatory cytokines in different mice groups

S. mansoni infection increased secretion of proinflammatory cytokines in mice sera. Compared to infected WT mice, the infected/anti-IL-17 mAb- group produced remarkably lower levels of IL-1β (Figure 4a), IL-6 (Figure 4b), and TNF-α (Figure 4c) during the infection (*P*<0.03).

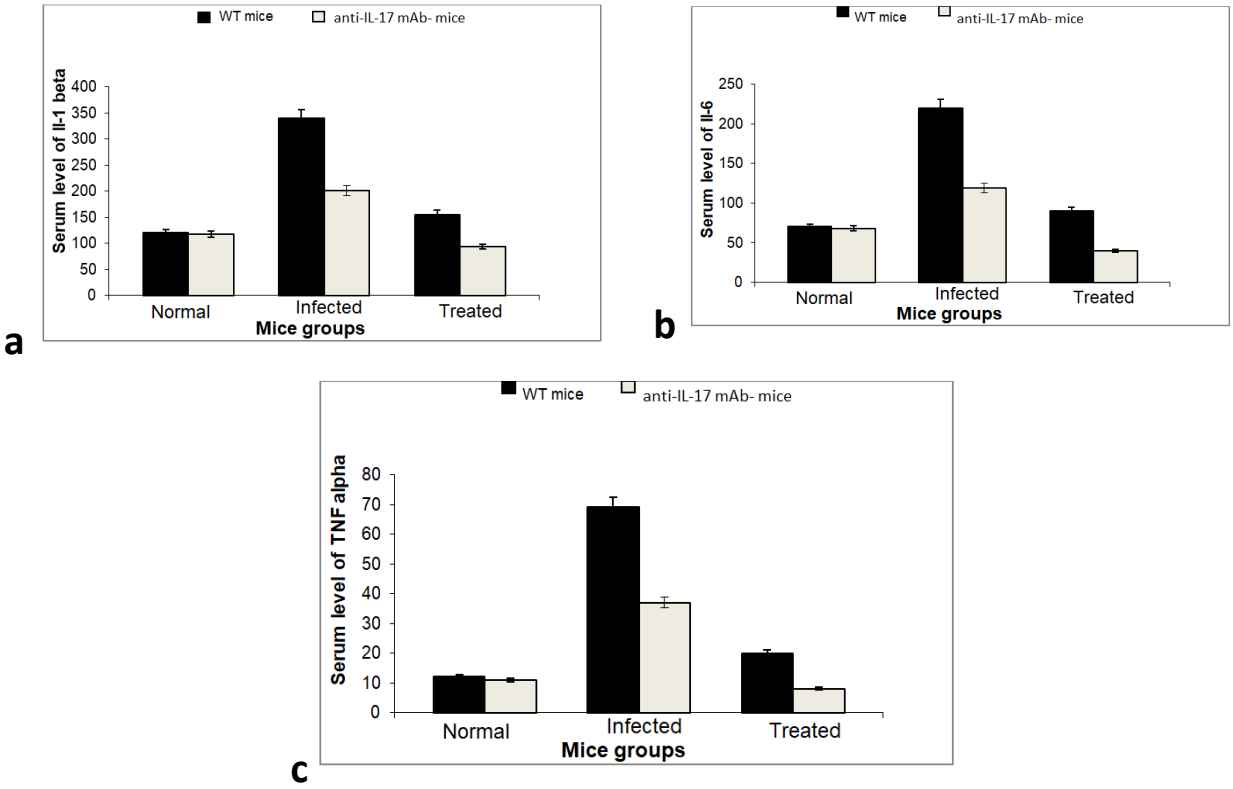

Figure 4. A histogram showing the role of IL-17A neutralization on the production of proinflammatory cytokines in *S mansoni*-infected mice. (a) IL-1β (b) IL-6, and (c) TNF--β Compared with infected WT mice, serum levels of cytokines in the infected/anti-IL-17 mAb-treated group were greatly reduced (*P* <0.03). Praziquantel treatment markedly decreased the levels of cytokines in infected animals (*P* <0.03).

Effect of IL-17 A neutralization on liver granulomatous inflammation induced by S. mansoni infection

Histological examination of liver sections revealed a normal cellular organization of the uninfected hepatic lobules. Infection with *S. mansoni* markedly altered the histological structure of the liver. There was more peri-ovular granulomatous inflammation in the liver of infected WT mice than in the infected/anti-IL-17 mAb treated group (*P*<0.02). Granuloma size increased over time, however, mice that received anti- IL-17 mAb showed significantly smaller granulomas during the infection (*P*<0.05) (Figure 5 A, B).

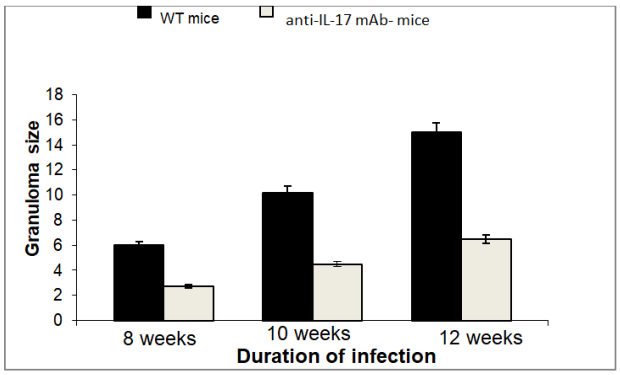

Figure 5A. A histogram showing the effect of IL-17A neutralization on the diameters of *S mansoni*-induced liver granulomas. Granulomas were measured by the ocular micrometer and expressed as the mean area in $\mu m^2 \pm$ SD. *S mansoni*-infected WT mice showed larger granulomas than infected/anti-IL-17 mAB animals (*P*<0.02). Granuloma size increased significantly over time in both mice groups (*P*<0.05).

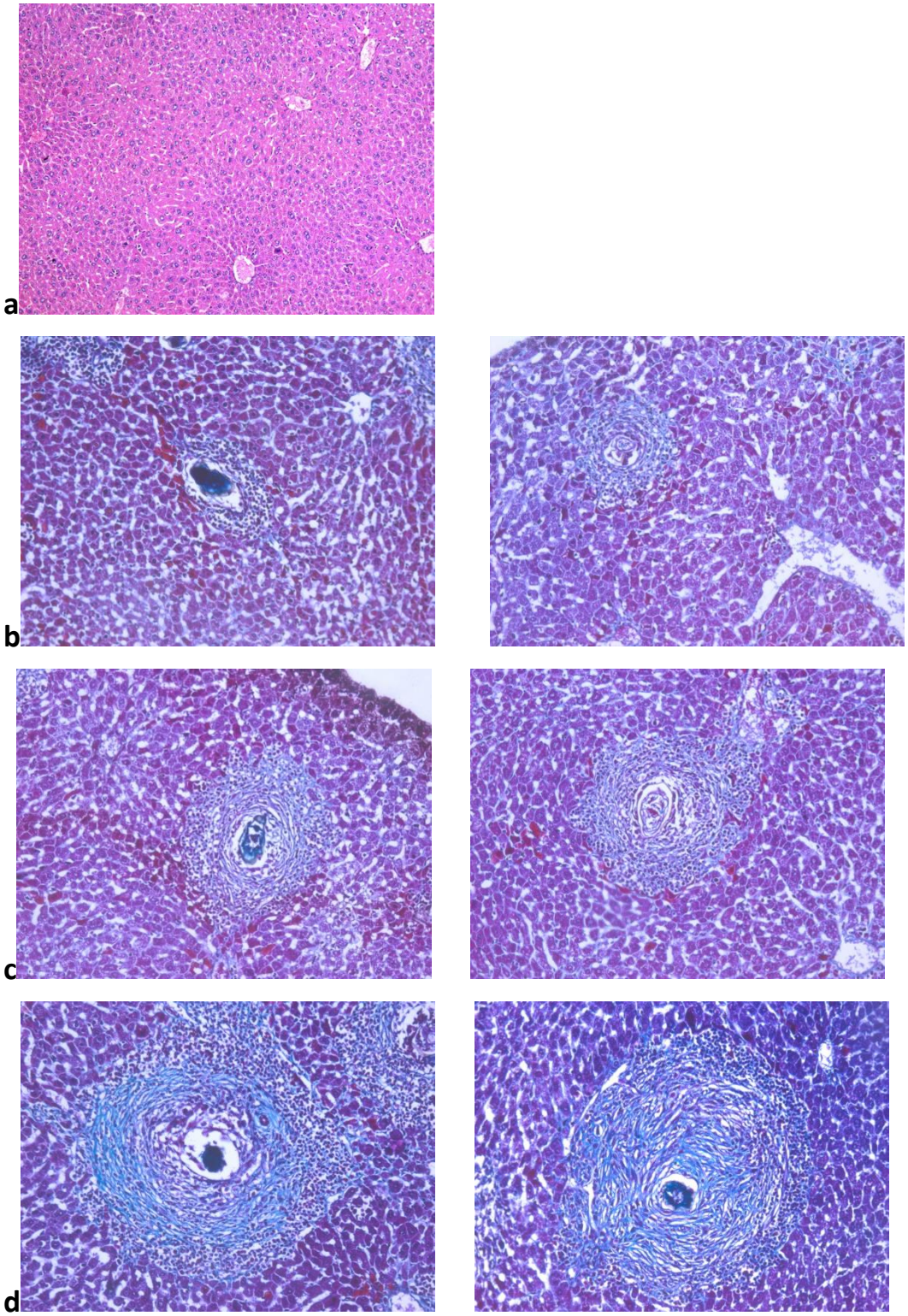

Figure 5B. Hematoxylin-eosin and Masson's trichrome-stained liver sections (magnification x400) demonstrating variable sizes of granulomas in *S. mansoni* infected mice. (a) Normal liver, (b) *S mansoni* infected liver with small-sized granuloma (c) moderate-sized granuloma, (d) large-sized granuloma.

Discussion

In the present study we explored the effect of IL-17A on TGF-β1 and IL-13-induced liver fibrosis during experimentally schistosomiasis mansoni infection. The production of profibrotic cytokines TGF-β1, IL-13, and IL-17A was measured in control and *S. mansoni*-infected mice by ELISA. The data showed that increased production of these cytokines was observed in the sera of infected mice than in the uninfected animals (P<0.01). Treatment of infected animals with praziquantel remarkably decreased TGF-β1, IL-13, and IL-17A in mice sera and improved liver functions (P<0.03). Previous studies have demonstrated that treatment with praziquantel was effective in eradicating adult worms of *S. mansoni*, which may reverse or reduce fibrosis in pulmonary[28] and liver tissues.[29] This finding may indicate the critical role of TGF-β1, IL-13, and IL-17A in hepatic granulomatous inflammation associated with schistosomal infection.

Our findings showed that unlike infected WT mice, infected/anti-IL-17 mAb treated mice group secreted fewer levels of TGF-β1, IL-13, and IL-17A. Importantly, the granulomatous reaction was less evident and restricted to a few small-sized granulomas (P<0.02). These results may reflect the role of IL-17A in enhancing the production of TGF-β1 and IL-13 and potentiating their profibrotic effect during infection. This was in agreement with other studies, demonstrated that IL-17A induces the recruitment and stimulation of macrophages to secrete fibrogenic cytokines including TGF-β1.[18,19, 27] Furthermore, IL-17A increased HSCs response to TGF-β1 by upregulating the expression of TGF-β1 receptor on the cell surface, leading to enhanced liver fibrosis.[18,30] Manipulating TGF-β1 by inhibiting its expression, activation, or signaling successfully reduce tissue fibrosis, and vice versa transgenic mice in which TGF-β1 gene expression is enhanced develop marked spontaneous fibrosis or increased fibrotic response to injury.[31,32]

Our results demonstrated that neutralization of IL-17 activity, by injecting rat anti-mouse IL-17 mAb, notably reduced IL-13 secretion in infected mice. This is an important observation as IL-13 is not only a potent inducer and activator of TGF-β1[13] but also activates a mechanism of tissue fibrosis that is completely TGF-β1 independent.[14] Previous studies have also reported that convergence of IL-13 and IL-17A signaling in pulmonary structural cells may contribute to the pathogenesis of severe forms of allergic asthma.[20]

As inflammation enhances the progression of liver fibrosis and corticosteroids have been recommended for the treatment of liver fibrosis in patients with autoimmune and acute alcoholic hepatitis.[33] The role of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α, were examined in our mice groups. The present study demonstrated that IL-1β, IL-6, and TNF-α, were at high levels in infected WT mice, but were less in the infected/anti-IL-17 mAb treated group (P<0.03). These results were in line with previous studies that demonstrated the role of IL-17 A in the upregulation of inflammatory response and subsequent progression of fibrogenesis.[34]

In conclusion, our data indicated that IL-17A might contribute to the progress of liver fibrosis by enhancing the profibrotic effect of TGF-β1 and IL-13 in mice infected with *S. mansoni*. It is important to clarify the immune mechanisms involved in the pathogenesis of liver fibrosis caused by chronic infection with *S mansoni* to discover more effective therapeutic targets.

Author Contributions

MHA, TKZ, GAA, IRSh, and ASB planned and participated in the study design, performed the laboratory work, and data analysis, and review the manuscript. TKZ, GAA, and ASB were involved in the writing of the manuscript, data interpretation, revising the paper, and approving the final version. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The protocol of the study was reviewed and approved by the Research Ethical Committee at the Faculty of Medicine, Al-Azhar University, Cairo, Egypt (Approval date: March 2020).

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References

1. King, C.H., Dickman, K., Tisch, D.J.(2005). Reassessment of the cost of chronic helminthic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet*, 365, 1561–9.
2. Anthony, B., Allenm, J.T., Li, Y.S., *et al.* (2010). Hepatic stellate cells and parasite-induced liver fibrosis. *Parasit Vectors*, 3: 60. DOI: 10.1186/ 1756-3305-3-60.
3. Abath, F.G., Morais, C.N., Montenegro, C.E., *et al.* (2006). Immunopathogenic mechanisms in schistosomiasis: what can be learned from human studies? *Trends Parasitol.*, 22:85–91. Doi 10.1016/j.pt.2005.12.004
4. Dessein, A., Kouriba, B., Eboumbou, C., *et al.* (2004). Interleukin-13 in the skin and interferon-gamma in the liver are key players in immune protection in human schistosomiasis. *Immunol Rev.*, 201:180–190. DOI: 10.1111/j.0105-2896.2004.00195.x
5. Fairfax, K., Nascimento, M., Huang, SC-C., *et al.* (2012). Th2 responses in schistosomiasis. *Semin Immunopathol.*, 34: 863–71. doi: 10.1007/ s00281-012-0354-4
6. Wynn, T.A., Thompsonm R.W., Cheever, A.W., et al. (2004). Immunopathogenesis of schistosomiasis. *Immunol Rev.*, 201: 156–67. doi: 10.1111/j.0105-2896.2004.00176.x
7. McGonigle, S., Beall, M.J., Pearce, E.J. (2002). Eukaryotic initiation factor 2 alpha subunit associates with TGF beta receptors and 14-3-3 epsilon and acts as a modulator of the TGF beta response. *Biochemistry*, 41:579–87. doi: 10.1021/bi011407z
8. Puche, J.E., Saiman, Y., Friedman, S.L. (2013). Hepatic stellate cells and liver fibrosis. *Compr Physiol.*, 3:1473–92. doi: 10.1002/cphy.c120035
9. Minutti, C.M., Knipper, J.A., Allen, J.E., *et al.* (2017). Tissue-specific contribution of macrophages to wound healing. Semin Cell Dev Biol., 61, 3–11.
10. Chiaramonte, M. G., Donaldson, D. D., Cheever, A. W., *et al.* (1999). An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J. Clin. Invest.,* 104:777.
11. Fallon, P. G., Richardson, E. J., McKenzie, G. J., *et al.* (2000). Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J. Immunol.,* 164: 2585.
12. Chiaramonte, M. G., Donaldson, D. D., Cheever, A. W., *et al.* (2001). Studies of murine schistosomiasis reveal interleukin-13 blockade as a treatment for established and progressive liver fibrosis. *Hepatology,* 34:273.
13. Lee, C. G., Homer, R. J., Zhu, Z., *et al.* (2001). Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor (1). *J. Exp. Med.,* 194: 809.
14. Kaviratne, M., Hesse, M., Leusink, M., *et al.* (2004). IL-13 activates a mechanism of tissue fibrosis that is completely TGF beta independent. *J Immunol.*, 173:4020–4029.
15. Bettelli, E., Korn, T., Oukka, M., *et al.* (2008). Induction and effector functions of T(H)17 cells. *Nature*, 453:1051–1057.
16. Quertinmont, E., Vercruysse, V., Le Moine, O., *et al.* (2009). The interleukin-17 pathway is involved in human alcoholic liver disease. *Hepatology*, 49:646–657.
17. Zhang, J.Y., Zhang, Z., Lin, F., *et al.* (2010). Interleukin-17-producing CD4(+) T cells increase with the severity of liver damage in patients with chronic hepatitis B. *Hepatology*, 51:81–91.
18. Meng, F., Wang, K., Aoyama, T., *et al.* (2012). Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. *Gastroenterology*, 143:765–776.
19. Yan, S., Wang, L., Liu, N., *et al.* (2012). Critical role of interleukin-17/interleukin-17 receptor axis in mediating Con A-induced hepatitis. *Immunology and cell biology*, 90:421–428.
20. Hall, S.L., Baker, T., Lajoie, S., *et al.* (2017). IL-17A enhances IL-13 activity by enhancing IL-13-induced signal transducer and activator of

transcription 6 activation. *J Allergy Clin Immunol.*, 139,(2):462-471.

21. Wilson, M., Kane, M., Pesce, J., *et al.* (2007). Immunopathology of schistosomiasis. *Immunol Cell Biol.*, 85:148-54.
22. Chen, D., Luo, X., Xie, H. *et al.*(2013). Characteristics of IL-17 induction by *Schistosoma japonicum* infection in C57BL/6 mouse liver. *Immunology*, 139: 523-32.
23. Gönnert, R., Andrews, P. (1977). Praziquantel, a New Broad-Spectrum Antischistosomal Agent. *Z Parasitenkd*, 52:129–50.
24. Reitman,, S., Frankel S.A. (1957). Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Amer J Clin Pathol.*, 28:56-63.
25. Von Lichtenberg, E.V. (1962). Host response to eggs of *Schistosomamansoni* Granuloma formation in the unsensitized laboratory mouse. *Am J Pathol.*, 41:711- 22.
26. Tsuchida, T., Friedman, S.L. (2017). Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol.*, 14:397–411.
27. Lemmers, A., Moreno, C., Gustot, T., *et al.* (2009). The interleukin-17 pathway is involved in human alcoholic liver disease. *Hepatology*, 49:646–657.
28. Crosby, A., Jones, F.M., Kolosionek, E., *et al.* (2011). Praziquantel reverses pulmonary hypertension and vascular remodeling in murine schistosomiasis. *Am J Resp Crit Care Med.*, 184, (4):467–473.
29. Andrade, Z.A. (2005). Regression of hepatic fibrosis. *Rev Soc Bras Med Trop*, 38, (6):514–520.
30. Wilson, M.S., Madala, S.K., Ramalingam, T.R., *et al.* (2001). Bleomycin and IL-1beta-mediated pulmonary fibrosis are IL-17A dependent. *The Journal of experimental medicine*, 207:535–552.
31. Chamberlain, J. (2001). Transforming growth factor-β1: a promising target for antistenosis therapy. *Cardiovasc. Drug Rev.,* 19:329.
32. Schnur, J., Ola´h, J., Szepesi, A., *et al.* (2004).Throacelamide induced hepatic fibrosis in transforming growth factor beta-1 transgenic mice. *Eur J Gastroenterol Hepatol.,* 16:127–133.
33. Bataller, R., Brenner, D.A. (2005). Liver fibrosis. *J Clin Invest.*, 115,(2):209–218.
34. Ruiz de Morales, J.M.G., Puig, L., *et al.* (2020). Critical role of interleukin (IL)-17 in inflammatory and immune disorders: An updated review of the evidence focusing on controversies. *Autoimmunity reviews*, 19:102429.