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Downregulation of proinflammatory cytokines and dynamic expression of TGF-β1 molecules induced by anti-IL-17 mAb in murine schistosomiasis mansoni

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Abstract

Hepato-intestinal schistosomiasis is characterized by severe pathological changes at advanced chronic stages, including granulomatous lesions and liver fibrosis. The objective of our research was to assess the dynamic expression of profibrotic molecules, the transforming growth factor beta 1 (TGF-β1), and proinflammatory cytokines immunomodulation induced by interleukin 17 (IL-17) neutralization in murine *Schistosomiasis mansoni*. The study included 56 specific pathogen-free male C57BL/6 mice, divided into 3 main groups: GI uninfected normal controls, GII *S. mansoni* infected with 70±5 cercariae/non-treated, GIII *S. mansoni* infected and treated with anti-IL-17 monoclonal antibody (mAb), GIV *S. mansoni* infected and isotype-matched IgG2a mAb was given as a challenge. Mice were sacrificed at 6, 8, and 10 weeks after infection, then their liver enzymes and cytokines assessed, histopathological and immunohistochemical tested. The present study demonstrated a statistically significant elevation in serum levels of IL-17 ($p<0.01$), TGF-β1 ($p<0.01$), IL-1β ($p\leq0.001$), IL-4 ($p\leq0.003$), IL-6 ($p\leq0.05$), and liver enzymes (ALT: $p\leq0.001$; AST: $p\leq0.002$). Additionally, granulomatous lesions and TGF-β1 expression were significantly increased ($p<0.001$) in infected mice at 6, 8, and 10 weeks after infection. All showed significant reduction by neutralization with anti-IL-17 mAb. Finally, IL-17 exhibited potent profibrogenic activity, and anti-IL-17 mAb can be used to alleviate and counteract this impact in murine *S. mansoni.*

Keywords: Interleukin 17, transforming growth factor-beta 1, cytokines, *Schistosoma mansoni*.

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Introduction

Schistosomiasis is a neglected tropical disease that affected about 250 million people in 78 countries in 2021.[1] This disease has a significant detrimental effect on human health as well as on social and economic development and is caused mainly by three primary trematode

species: Schistosoma mansoni, S. japonicum causing hepato-intestinal schistosomiasis, and S. haematobium causing genitourinary schistosomiasis.[2]

Schistosome and chronic viral hepatitis infection, alcoholism, and autoimmune liver disorders are the main causes of liver fibrosis.[3, 4] Schistosome ova are the primary cause of the disease's morbidity; continuous stimulation by egg antigens causes conscription of immune cells and formation of granulomatous lesions and eventually fibrosis.[5]

Granuloma formation is initiated by cluster of differentiation 4 (CD4)+ T cells.[6] The modulation of the immunopathological response depends on switching from T helper (Th)1 to Th2 cells. About eight weeks after infection, the Th2 response peaks and is subsequently downregulated as the infection progresses to a chronic state.[7, 8] Activation of hepatic stellate cells (HSCs) is significantly influenced by certain soluble substances from inflammatory cells, including pro-inflammatory and profibrogenic cytokines and chemokines.[9-10]

The liver fibrosis is an inflammatory response induced by liver damage, which in turn activate macrophages to release transforming growth factor beta 1 (TGF-β1) and reactive oxygen species. Then, quiescent HSCs, responsible for the maintenance of the extracellular matrix and vitamin A storage, are activated and differentiated into myofibroblasts as a response to TGF-β1 in a way that is either Smads-dependent or Smads-independent. Ultimately, a significant amount of collagen is produced by the activated HSC, which causes excessive extracellular matrix deposition and liver fibrosis.[11, 12]

According to the study by Dooley and Dijke, 2012,[13] activated macrophages, T regulatory (T-reg) cells, and hepatic stellate cells secrete TGF-β1 in the liver in response to pro-inflammatory signals. TGF-β-belongs to the primary class of cytokines that stimulate fibrogenesis, hence facilitating enhanced matrix formation and HSC proliferation.[11]

Interleukin 17 A (IL-17A), produced by specific CD4+ Th cells (Th17) with pro-inflammatory properties, commonly known as IL-17, increases the secretions of collagen I, α-smooth muscle actin, and tissue inhibitors of metalloproteinases (TIMP)-I from HSCs in response to TGF-β1.[14] By stimulating HSCs to secrete more chemokines, it may potentially indirectly accelerate the progression of liver fibrosis by attracting macrophages. It subsequently induces the release of fibrogenic cytokines, such as TGF-β1, from these recently attracted macrophages.[15] The pro-inflammatory cytokine IL-17 was shown to be elevated in severe immunopathology and is likely to be a sign of advanced disease. 16 The objective of our research was to assess the dynamic expression of the profibrotic molecules, TGF-β1, and proinflammatory cytokines immunomodulation induced by IL-17 neutralization in *S. mansoni*-infected mice.

Materials and Methods

Animals and infection

The study was carried out at the Biological Unit of Theodor Bilharz Research Institute, Egypt. It included 56 specific pathogen-free male C57BL/6 mice, 6-8 weeks of age and weighted 18-22 g, obtained from the animal house. The mice were kept on balanced dry food containing 14% protein and sterile water in air-conditioned rooms at 26°C.

All protocols and animal handling methods were reviewed and approved by the Ethics Committee of the Faculty of Medicine, Al-Azhar University, Cairo, Egypt (registration No. Para._15Med.Research._IL-17, TGF-β1, cytokines, *Schistosoma mansoni._0000032,* date June 2013).[30]

The *S. mansoni* cercariae were shed from infected *Biomphalaria alexandrina* snails, placed in 300 ml of distilled water, and left in artificial light for 2 hrs. Percutaneous infection of mice with 70±5 cercariae was performed, according to the method of Olivier and Stirewalt. [17]

Anti-IL-17 monoclonal antibody (mAb) was obtained commercially (Cat. # MBS2503506, eBiosource International, California, USA), and given intraperitoneally (62.5 µg/mouse) to study mice 3 weeks post-infection and repeated every four days until 2 days prior to mice scarification. [18]

Study design

In this study, 56 mice were included and divided into four groups: Group I (G1, normal mice), comprised 12 mice not infected with *S. mansoni*. GII (infected mice): 18 mice infected with *S. mansoni,* but not treated. GIII (Infected/anti-IL-17 monoclonal antibody-treated mice): 18 mice infected with *S. mansoni* and were given anti-IL17 mAbs. GIV (infected/ isotype-matched rat IgG2a mAb-treated mice): 8 mice infected with *S. mansoni* and were given isotype-matched rat IgG2a mAb (Cat. # 70-4321, BD/Pharmingen, San Diego, CA, USA.), administered intraperitoneally at the same dose and time, as the challenge control group. Mice were sacrificed at 6, 8, and 10 weeks after infection.

Samples collection

Animals were euthanized under isoflurane inhalation and then decapitated. Blood samples were collected, centrifuged at 1400 x g for five minutes to separate serum, which was stored in a freezer at -80°C until used.

Liver enzyme assessment

To determine serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes, commercial kits (Boehringer reagent kits, Mannheim, Germany), were used according to the manufacturer's instructions.

Immunological assessment

Cytokines including TGF-β1, IL-17, TNF-α, IL-1β, IL-4, and IL-6 were measured in mice serum using different commercial enzyme-linked immunosorbent assay (ELISA) kits (Biosource International, Camarillo, California, USA), according to the manufacturer's instructions. An ELISA reader (Bio-Rad mod. 680) was used to determine the optical density (OD) of the final ELISA products, measured at 450 nm.

Histopathological examination

Liver specimens were sectioned at a thickness of 5 µm, embedded in paraffin blocks, fixed in 10% phosphate buffered formalin, and stained with Masson's trichrome or hematoxylin and eosin. Under a microscope, granulomas diameter was measured using an ocular micrometer. Cross-sections with a visible center egg were only counted, and the mean area in µm2± SD was used to express the granuloma size. The percent reduction was calculated as follows:
Percent Reduction of granuloma diameter = (value of infected controls-value of treated mice)/(value of infected controls)*100.[19]

Immunohistochemical studies

Expression of TGF-β1 was analyzed immunohistochemically on unstained liver specimens. Liver tissue sections were deparaffinized, followed by blocking of the endogenous peroxidase. Then they were incubated for 1 hour at 37°C with mouse anti-TGF-β1 diluted 1:200 (Biotechnology, Santa Cruz, CA, USA) and then overnight at 4°C. Then the second antibody was added and incubated at 37°C for 1 hour and stained by diaminobenzidine. Then sections were stained with Mayer's hematoxylin for one minute and fixed with Aquatex liquid (Merck KGaA, Germany). For the negative controls, the main antibody was substituted with PBS. To evaluate the expression of TGF-β1, the mean percentage of positively stained cells in 10 granulomas ± SD was computed using an open-source platform for biological-image analysis (Imagej/software/Fiji). Immunoreactivity was described as weak, mild, moderate, and strong.

Statistical Analysis

Data were verified, coded, and analyzed using the Statistical Package for the Social Sciences (SPSS) IBM- version 21.0.[29] Descriptive statistics, including means, standard deviations, and percentages, were calculated. A test of significance, including Chi square, Fisher's exact, and Monte Carlo exact test was used to compare the difference in distribution of frequencies among different groups. For continuous variables with more than two categories, the one-way ANOVA test was calculated to test the mean differences between groups. The repeated measures ANOVA (RM-ANOVA) test was calculated to test the mean differences of the data that followed a normal distribution and had repeated

measures (between groups, within groups, and overall difference). In addition, a post-hoc test was calculated using the Bonferroni corrections for pairwise comparisons between the two study groups. Correlation analysis was used to test the association between variables (Pearson and Spearman's rank correlation, as appropriate). Significance was considered at *p*-value of ≤ 0.05.

Results

IL-17 and TGF-β1 cytokines were measured at 6- 8- and 10-weeks post infection and showed significantly increased levels in the *S. mansoni* infected mice group ($p<0.01$), but the levels of both cytokines showed significant decrease in anti-IL-17 mAb-treated mice compared to the untreated group ($p<0.03$) (Figure 1a & 1b).

Figure (1a)

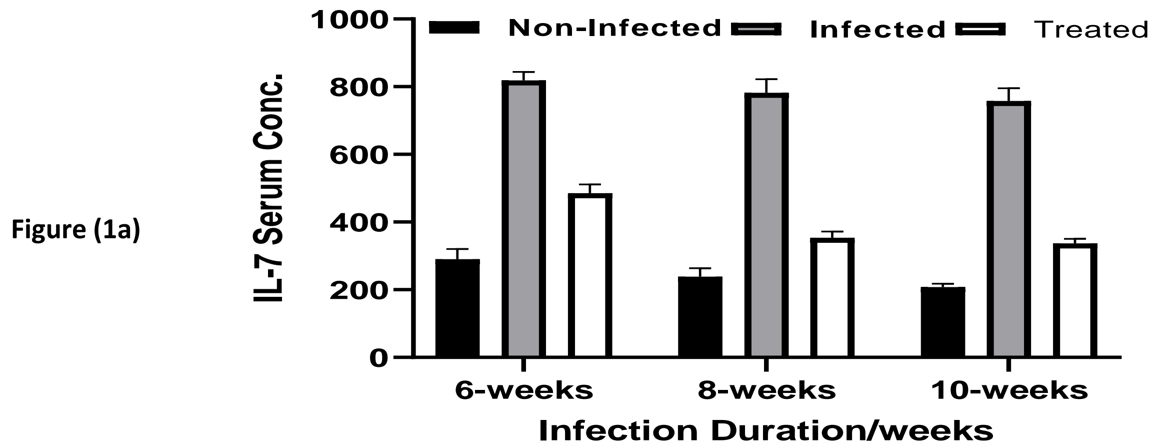

Figure (1b)

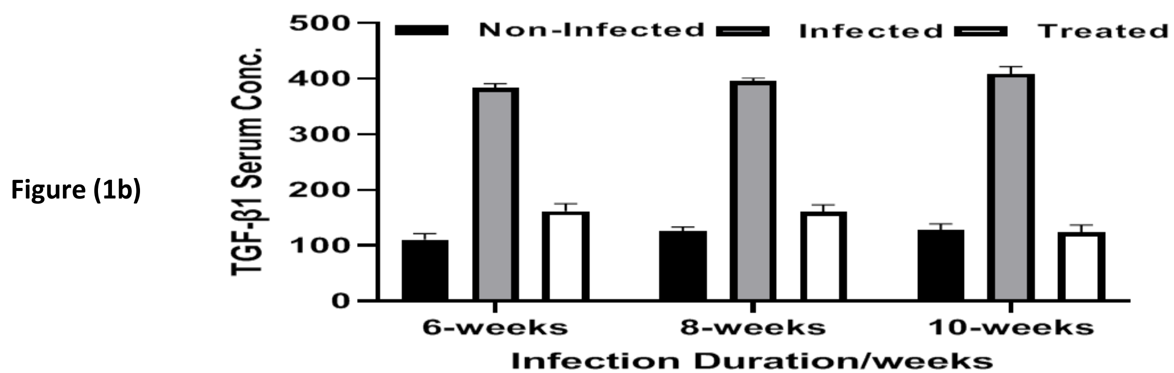

Figure 1. (a) IL-17A and (b) TGF-β1 production in response to *S. mansoni* infection.

There was significant increase in the liver enzymes AST ($p\leq0.002$), and ALT (ALT: $p\leq0.001$) in infected mice serum at 6-, 8-, and 10-weeks post-infection. However, the anti-IL-17 mAb-treated animals showed significantly lower serum ALT and AST ($p<0.03$) (Figure 2a & b).

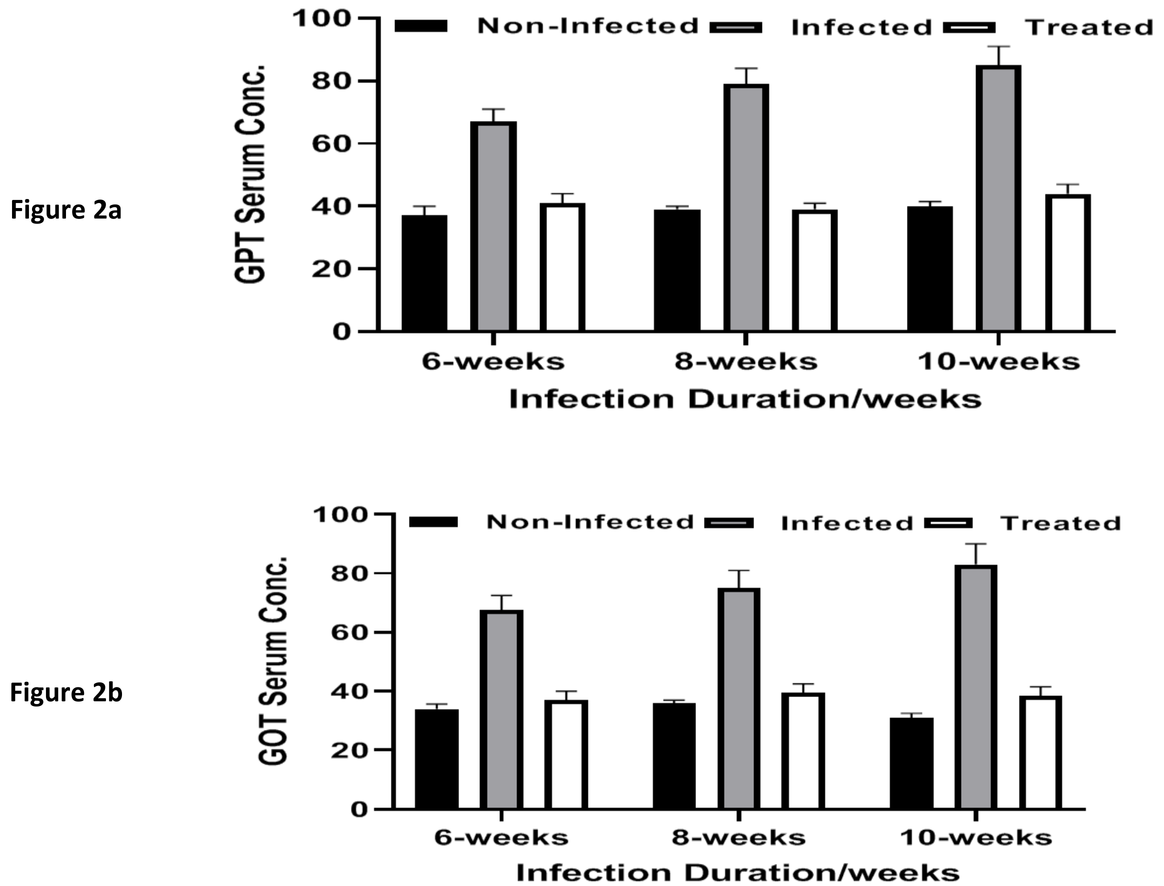

Figure 2. ALT and AST activities among studied groups.

Proinflammatory cytokines were measured at 6,8-, and 10-weeks post infection and showed significant increase in sera of *S. mansoni* infected-mice (IL-1β: $p≤0.001$, IL-4: $p≤0.003$, and IL-6: $p≤0.05$). The infected/anti-IL-17 mAb-treated group showed significant reduction in IL-1β (Figure 3a), IL-4 (Figure 3b), and IL-6 (Figure 3c), ($p<0.05$ for all). However, there was no effect on TNF-α levels during the infection (Figure 3d).

Figure 3a

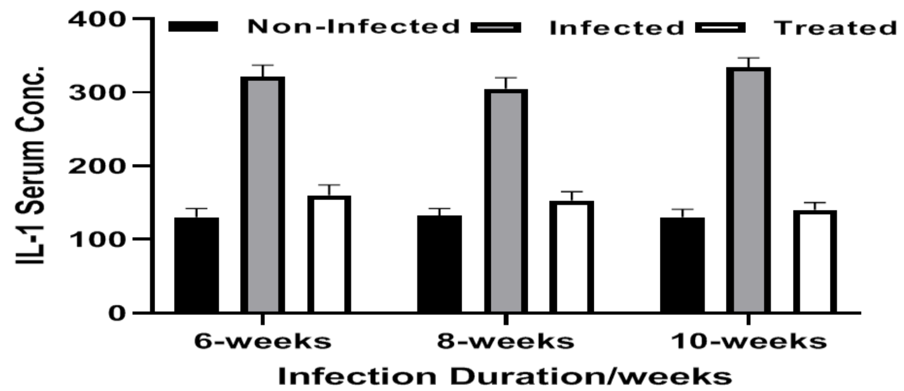

Figure 3b

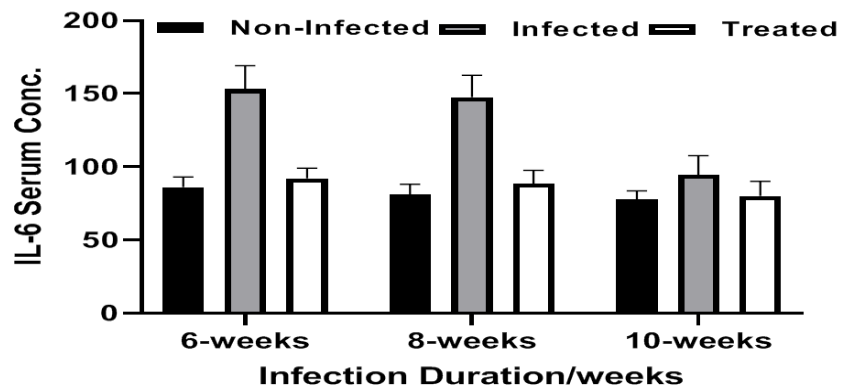

Figure 3c

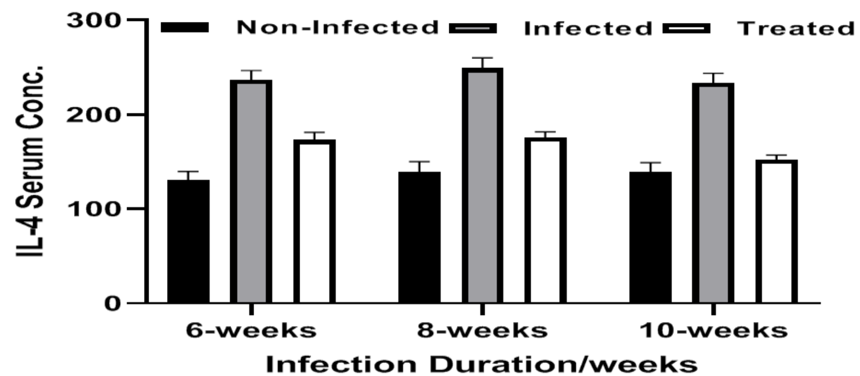

Figure 3d

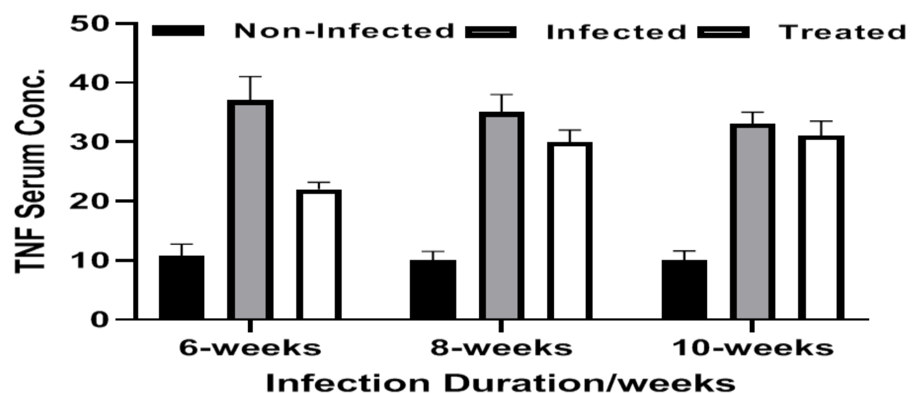

Figure 3. Effect of anti-IL-17 mAb on serum levels of IL-1β (a), IL-6 (b), IL-4 (c), TNF-α (d), among the studied groups.

Granuloma size increased over time in infected control group. However, the anti- IL-17 mAb treated mice showed non-significant reduction in granulomas size at 6-, 8-, and 10-weeks post-infection (p>0.05) (Table 1).

Table 1. Difference in Granuloma measurement between the studied Groups.

Granuloma Measurement	Group-II	Group-III	*p*-value
6-weeks	66.30 ± 43.7	43.20 ± 27.5	NS*
8-weeks	69.10 ± 34.6	41.90 ± 35.7	NS*
10-weeks	73.40 ± 23.9	39.80 ± 27.5	NS*
p-value***	= 0.701	= 0.694	NS$

Two-way RM-ANOVA test was used to compare the difference in the mean between groups

* Between Groups, **post-hoc test was used for pairwise comparison with Tukey's correction and *** Interaction analysis Within Group $. p > 0.05 is not significant (NS).

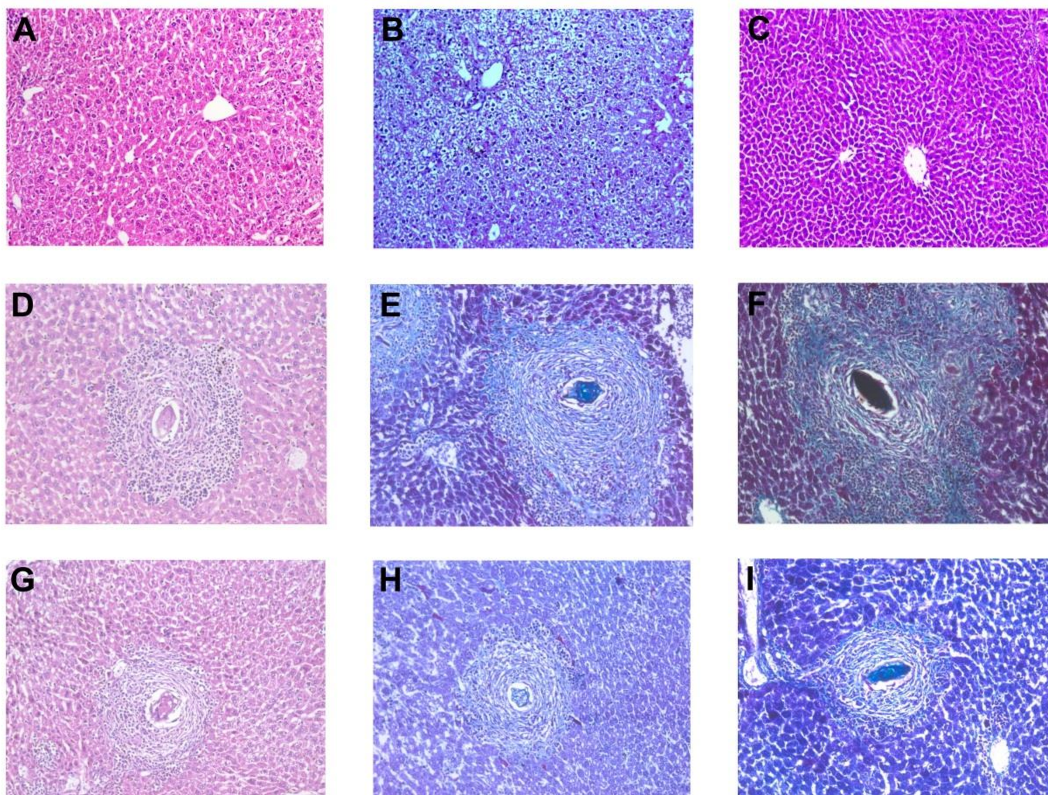

Figure 4. Granulomas measured at 6-, 8- and 10-weeks post infection among the studied groups. Sections of a mice liver: GI (A, B & C): showing normal hepatic architecture. GII (D, E, and F): D- showing large granuloma with central viable egg and massive cellular infiltration. E- showing large granuloma of fibro cellular type with viable egg. F- showing large granuloma with excessive fibrosis the egg is degenerated. GIII (G, H and I): G- showing large granuloma with central viable egg, massive cellular infiltration, H- showing medium-sized fibro cellular granuloma. I- showing a well-defined small fibro cellular granuloma, a degenerated egg and a decreased diameter (hematoxylin, eosin and Masson's trichrome stains figures at 200 x).

Table 2. Expression of TGF-β1 in liver of *S. mansoni*-infected mice and effect of IL-17A neutralization at 6-, 8- and 10-weeks post-infection.

TGF Expression	Group-I	Group-II	Group-III	*p*-value
6-weeks	4.27 ± 2.1	65.37 ± 4.8	25.24 ± 7.2	< 0.001*
p-value**	I vs. II < 0.001	II vs. III < 0.001	I vs. II < 0.001	
8-weeks	5.11 ± 3.1	67.28 ± 6.8	19.45 ± 5.8	< 0.001*
p-value**	I vs. II < 0.001	II vs. III < 0.001	I vs. II = 0.015	
10-weeks	5.23 ± 3.3	72.10 ± 8.9	13.54 ± 5.6	< 0.001*
p-value**	I vs. II < 0.001	II vs. III < 0.001	I vs. II = 0.105	
p-value***	= 0.664	= 0.567	= 0.042	0.038$

Two-way RM-ANOVA test was used to compare the difference in the mean between groups. *Between Groups **post-hoc test was used for pairwise comparison with Tukey's correction. *** Interaction analysis Within Group $.
$p \leq 0.05$ is significant.

In the infected group, there was a substantial positive expression of TGF-β1 and high significant increase in IL-17 serum level ($p<0.01$). TGF-1 expression was reduced in the anti-IL-17 treated animals compared to the infected control group over the same weeks after infection ($p<0.001$) (Table 2).

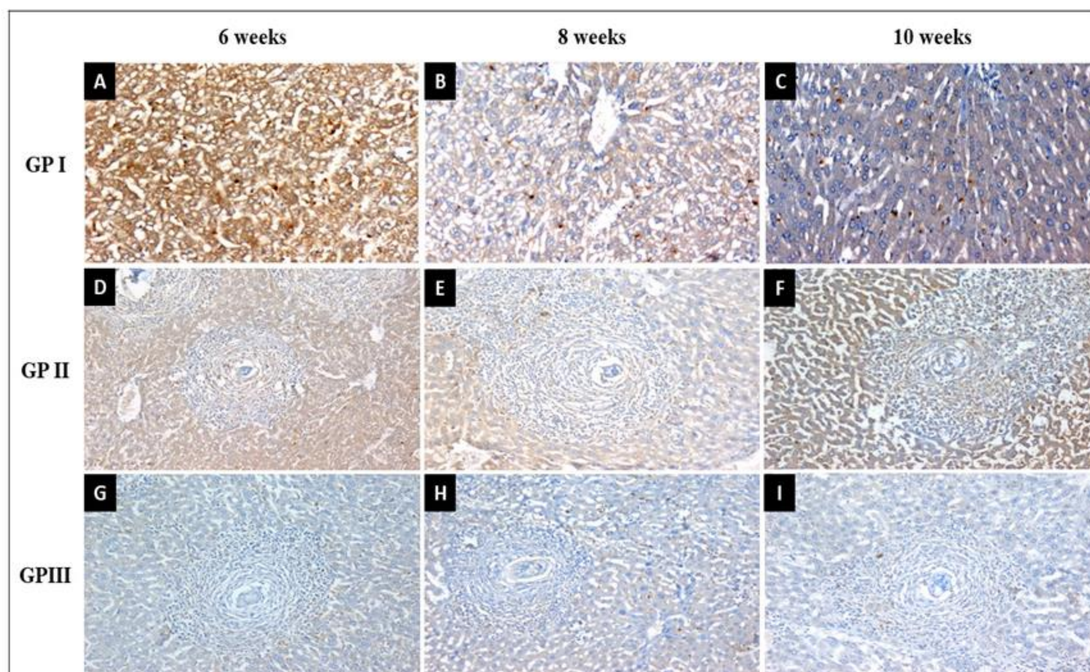

Figure 5. Immunohistochemical staining of TGF-β1 in liver of mice in the different studied groups. The TGF-1 expression showed a significant decrease in the anti-IL-17-treated group than in the infected control group over time. GI showing (A- B- C) weak immune reaction of hepatocytes for expression of TGF-β1 in normal control mice. GII showing (D- E- F) strong immune reaction of hepatocytes for expression of TGF-β1 in *Schistosoma* infected mice. GIII showing (G-H-I) moderate immune reaction of hepatocytes for expression of TGF-β1 in *Schistosoma* infected /anti-IL-17 treated mice (400x).

Discussion

TGF-β1 released from activated macrophages plays an important role in the pathogenesis of schistosomiasis-induced liver fibrosis and helps activate and differentiate quiescent HSCs into myofibroblasts that produce a significant amount of collagen, causing excessive extracellular matrix deposition and liver fibrosis. [11-12] Therefore, this research aimed to assess the dynamic expression of the profibrotic molecules, TGF-β1, and proinflammatory cytokines immunomodulation induced by IL-17 neutralization in *S. mansoni*-infected mice.

The present study demonstrated a statistically significant elevation of IL-17 in infected mice at 6, 8, and 10 weeks after infection in comparison to the normal control group. At the same weeks after infection, there was a significant decrease in IL-17 in group III compared to the infected controls ($p<0.001$). This is consistent with the findings of earlier reports that showed a positive correlation between the degree of liver pathology and activity of IL-17, as well as studies linking IL-17 to the inflammatory response during infections with *S. japonicum* and *S. mansoni*. [14,18,20,21] Also, there was a clear correlation between immunopathology and the IL-17 level. The study by El-Melegy et al., 2019, [22] revealed that the IL-17 level in the serum of infected mice grew progressively, reaching a significant peak at six weeks after infection.

The findings of the current study showed that *S. mansoni* infected mice had elevated levels of both AST and ALT, which indicate liver damage. Whereas, anti-IL-17 mAb-treated animals showed reduced liver damage, as demonstrated by significantly lower serum ALT and AST. These results are in accordance with the findings of a previous study which observed that infected mice had significantly lower ALT and AST levels following anti-IL-17 treatment [18].

Data of the present study for the effect of IL-17, on TNF-α, IL-1β, IL-4, and IL-6, showed that they were increased in the serum of S. *mansoni* infected mice. These increases peaked at 6, 8, and 10 weeks after infection. Cytokines IL-4, IL-6 and IL-1β showed significant decrease in anti-IL-17-treated mice, with no effect on the TNF-α level, which is indicative of downregulation during egg-induced immunopathology. This is in line with that reported in earlier research showing that anti-IL-17 mAb significantly reduces proinflammatory cytokines IL-1β, IL-4, and IL-6.[14,18,21] The inflammatory reactions triggered by *S. mansoni* eggs may be the reason for the serum elevation of these cytokines. Anti-IL-17 mAb partially inhibited this effect, which links proinflammatory cytokines upregulation to IL-17.

In the present study, according to the cytokine analysis results, at 6, 8, and 10 weeks after infection, there was a significant rise in TGF-β1 in the sera of infected mice compared to the normal control ($p<0.01$). Additionally, it was demonstrated that after 6, 8, and 10 weeks after infection, TGF-β1 showed significant decrease in anti-IL-17-treated group than in infected controls ($p<0.001$). This is in line with data reported in several previous studies, reported that anti-IL-17 mAb demonstrated its anti-fibrotic effects by reducing the expression of TGF-β and α-alpha-smooth muscle actin and promoting the de novo production of collagen type III. [22,23,24]

We observed that granulomatous lesions were significantly increased in the infected group compared to the normal and anti-IL-17A mAb-treated groups. These findings agree with data reported in several previous reports.[14, 18, 21] In the current study, when compared to the infected control mice, the mean granuloma diameter did not differ than in the anti-IL-17A mAb treated mice. In order to verify the direct contribution of IL-17 in the development of granulomatous inflammation, *S. mansoni*-infected mice were treated with anti-IL-17A mAb. There was a strong positive correlation between the size of the granuloma and the amount of IL-17 in the sera of the infected groups ($p<0.001$). Anti-IL-17 mAb administration significantly reduced hepatocyte damage and hepatic granulomatous inflammation. These effects were not seen in mice given an isotype-matched rat IgG2a mAb, suggesting that the specific neutralization of IL-17 was responsible for the reduction in granulomatous inflammation.

The current study showed that in the *S. mansoni* infected control group, there was a substantial positive expression of TGF-β1 and significant increase in serum IL-17 (*p*<0.01). TGF- β1 expression showed significant decrease in anti-IL-17 treated mice than in infected controls over the same weeks after infection (p<0.001). This was consistent with findings of the study by Huang et al., 2022,[23] that TGF-β1/Smad signaling was initiated in hepatic stellate cells at 4 weeks post infection, and that TGF-β1 expression dramatically increased with the progression of liver fibrosis in *S. japonicum*-infected animals, peaking at 7 or 9 wks after infection. The study by El-Melegy et al., 2019,[22] and Li et al., 2015,[25] demonstrated that TGF-β plays a role in the pathogenesis of human schistosomal hepatic fibrosis in addition to the inflammatory process. There is increasing evidence to show that TGF- β plays a role in activating dormant HSCs so they can transdifferentiate into fibro-genic, contractile and proliferative myofibroblasts.[26] Hepatic fibrosis development is effectively inhibited by blocking TGF-β signal transduction.[27] The study by Paquissi et al., 2017,[28] showed that IL-17 had a potent profibrogenic effect via a variety of pathways, one of which was inducing Kupffer cells to express TNF, IL-6, and IL-1 in addition to TGF- β1, the main fibro-genic cytokine.

In conclusion, IL-17 cytokine has strong profibrogenic activity, and anti-IL-17 monoclonal antibodies antagonize this effect and may be targeted in treatment of *Schistosoma* egg-induced immunopathology.

Author Contributions

MSHM, MRM, AAM, MAM, and FEL laid the plan, performed, analyzed, wrote and reviewed the manuscript. All authors read and approved of 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 study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Al-Azhar University, Cairo, Egypt (registration No. Para._15Med.Research._IL-17, TGF-β1, cytokines, *Schistosoma mansoni._0000032,* approval dated June 2023).

References

1. WHO. Fact Sheet. (2023). Available online: https://www.who.int/news-room/fact-sheets/detail/schistosomiasis (accessed on 16 July 2023).
2. McManus, D.P.; Dunne, D.W.; Sacko, M.et al. (2018). Schistosomiasis. *Nat. Rev. Dis. Primers*, 4; 13-21.
3. Rosenthal SB, Liu X, Ganguly S, et al. (2021). Heterogeneity of HSCs in a mouse model of NASH. *Hepatology*,74:667–85.
4. Beringer A, Miossec P. (2018). IL-17 and IL-17-producing cells and liver diseases, with focus on autoimmune liver diseases. *Autoimmun Rev.*, 17:1176–85.
5. Gieseck RL, Wilson MS, Wynn TA. (2018). Type 2 immunity in tissue repair and fibrosis. *Nat Rev Immunol.*, 18:62–76.
6. Rutitzky LI. and Stadecker MJ. (2011). Exacerbated egg-induced immunopathology in murine *Schistosoma mansoni* infection is primarily mediated by IL-17 and restrained by IFN-c. *Eur. J. Immunol.*, 41: 2677–2687.
7. Pearce EJ & MacDonald AS. (2002). The Immunobiology of Schistosomiasis. *Nat Rev Immunol*, 2: 499–511.
8. Wilson MS, Mentink-Kane MM, Pesce JT, et al. (2007). Immunopathology of schistosomiasis. *Immunol Cell Biol.*, 85: 148–154.
9. Liu Y, Meyer C, Muller A, et al. (2011). IL- 13 induces connective tissue growth factor in rat hepatic stellate cells via TGF-beta-independent Smad signaling. *J Immunol.,* 187:2814–23.
10. Mann DA, Marra F. (2010). Fibrogenic signalling in hepatic stellate cells. *J Hepatol.,* 52:949–50. doi: 10.1016/j.jhep.2010.02.005).
11. Xu F, Liu C, Zhou D, Zhang L. (2016). TGF-β/SMAD Pathway and its regulation in hepatic fibrosis. *J Histochem Cytochem*., 64:157–67.

12. Kisseleva T, Brenner D. (2021). Molecular and cellular mechanisms of liver fibrosis and its regression. *Nat Rev Gastroenterol Hepatol.,* 18:151–66.
13. Dooley S, Dijke P. (2012), TGF-beta in progression of liver disease. *Cell Tissue Res.*, 347(1):245-56.
14. Abd Allah HM, Zaalouk KH. T, Abo-Sheishaa, AG. Et al. (2022). Role of IL-17A in enhancing liver fibrosis induced by TGF-β1 and IL-13 in *Schistosoma mansoni* infected mice. *The Egyptian Journal of Immunology*, 29 (4), 2022: 174–183.
15. 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.
16. Lundy S. and Lukacs NW. (2013). Chronic *schistosome* infection leads to modulation of granuloma formation and systemic immunesuppression. *frontiers in immunol*.,4 (39):1-18.
17. Olivier, L, Stirewalt, MA, (1952). An efficient method for exposure of mice to cercariae of *Schistosoma mansoni*. *J. Parasitol*., 38:19-23.
18. Y. Zhang, L. Chen, W.Gao, et al. (2012). IL-17 neutralization significantly ameliorates hepatic granulomatous inflammation and liver damage in Schistosoma japonicum infected mice Eur. *J. Immunol*., 42: 1523–1535.
19. Von Lichtenberg, E.V. (1962). Host response to eggs of *Schistosoma mansoni* Granuloma formation in the unsensitized laboratory mouse. *Am J Pathol.*, 41:711- 22.
20. 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.
21. Zaalouk KT, Abo-Sheishaa AG, Shalash, RI. (2020). Regulation of Liver Fibrosis during Murine *Schistosomiasis Mansoni*. *The Egyptian Journal of Hospital Medicine,* 81 (1), Page 1275-1280.
22. El-Melegy A. N, Badawy S. N, Serag El-ddin M. M. (2019). Interlukin -17 Promotes Granuloma Formation and Epithelial- Mesenchymal Transition (EMT) of Hepatic Cells in Experimental Schistosomiasis *mansoni. Egyptian Journal of Medical Microbiology*, 28(3): 111-21.
23. Huang P, Huihui Ma, Yun Cao. Et al. (2022). Activation of primary hepatic stellate cells and liver fibrosis induced by targeting TGF-β1/ Smad signaling in schistosomiasis in mice. *Parasites & Vectors,* 15:*456*-466.
24. Fabre V, Wu H, PondTor S, et al. (2011). Tissue inhibitor of matrix-metalloprotease- 1 predicts risk of hepatic fibrosis in human *Schistosoma japonicum* infection. *J Infect Dis.,* 203(5):707-14.
25. Li L, Wu T, Huang J, et al. (2015). Expression of heat shock protein 47, transforming growth factor-beta 1, and connective tissue growth factor in liver tissue of patients with *Schistosoma japonicum*-induced hepatic fibrosis. *Parasitology*., 142(2):341-51.
26. Wang Q, Chou X, Guan F, et al. (2017). Enhanced Wnt Signalling in Hepatocytes is Associated with *Schistosoma japonicum* Infection and Contributes to Liver Fibrosis. *Scientific Reports.*, 7 (1):230.
27. Sobhy MMK, Mahmoud SS, El-Sayed SH, et al. (2018). Impact of treatment with a Protein Tyrosine Kinase Inhibitor (Genistein) on acute and chronic experimental *Schistosoma mansoni* infection. *Experimental Parasitology.*, https://doi.org/10.1016/j.exppara.2018.01.013.
28. Paquissi FC. (2017). Immunity and fibrogenesis: the role of Th17/IL-17 Axis in HBV andHCV-induced chronic hepatitis and progression to cirrhosis. *Front. Immunol*., 8, 1195.
29. IBM_SPSS (2012). Statistical Package for Social Science. *IBM Corp. Released*. *IBM SPSS Statistics for Windows*, Version 21.0. Armonk, NY: IBM Corp.
30. World Medical Association. Declaration of Helsinki (2013): Ethical principles for medical research involving human subjects. *JAMA*., Nov 27; 310 (20):2191-4. doi: 10.1001/jama.2013.281053. PMID: 24141714.