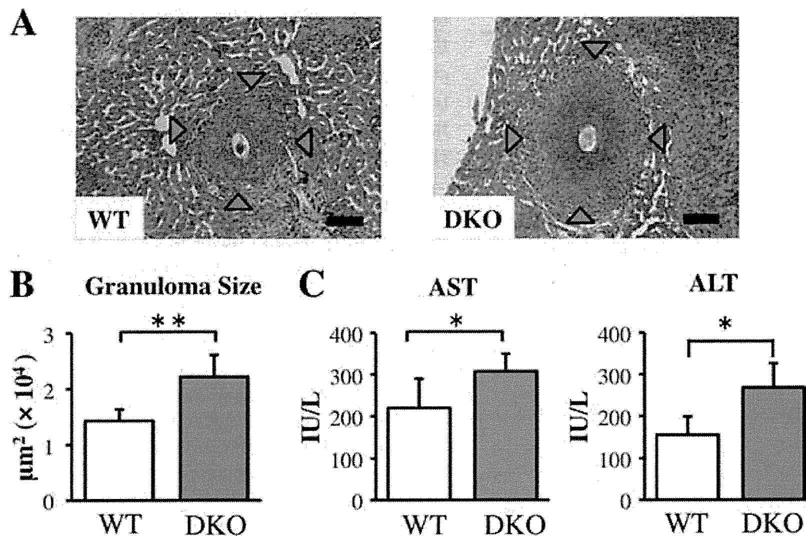

FIG 2 Severity of hepatic inflammation in infected DKO and WT mice at 6 weeks after *S. japonicum* infection. (A) Histopathology of *S. japonicum*-infected mice. Liver sections stained with hematoxylin-eosin show a granuloma surrounding a parasite egg. Original magnification, ×100; scale bar, 100 μm. Arrowheads indicate the margins of hepatic granulomatous inflammation. (B) Average granuloma sizes in DKO and WT mice. (C) Serum AST and ALT concentrations in infected WT and DKO mice. Means and SD are shown (n = 4 to 6). *, $P < 0.05$; **, $P < 0.01$.

Pure Chemical, Osaka, Japan) for 45 min at 37°C. This tissue was passed through 45-μm nylon mesh, and the cell pellet was resuspended in 35% Percoll and centrifuged at 2,000 rpm for 15 min at 4°C to remove hepatocytes and other debris (7). Erythrocytes were lysed with hemolysis buffer (0.017 M Tris, 0.16 M ammonium chloride, pH 7.4). The pellet was washed with phosphate-buffered saline (PBS), passed through 45-μm nylon mesh, centrifuged at 2,000 rpm for 3 min at 4°C, and resuspended in PBS. The dead cells were stained blue with trypan blue, and unstained living cells were counted on a hemocytometer.

Flow cytometry. Cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-CCR3 monoclonal antibody (MAb) (R&D Systems, MN) and phycoerythrin (PE)-conjugated anti-Gr-1 MAb (eBioscience, San Diego, CA), followed by forward-scatter (FSC)/side-scatter (SSC) plotting (3). The cells were analyzed on a FACSCalibur flow cytometer (BD Bioscience, NJ) equipped with Cell Quest software. Eosinophils were defined as cells that were Gr-1^{+} $CCR3^{+}$ SSC^{high} and neutrophils as cells that were Gr-1^{high} $CCR3^{-}$ SSC^{int} (16, 20, 36).

Pathology. Liver specimens were fixed in 10% formalin, embedded in paraffin, and sectioned at 3 μm. The sections were stained with hematoxylin and eosin to measure the sizes of granulomas containing a single egg. Granuloma size was quantitated using ImageJ software (NIH). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with the Wako transaminase CII test (Wako Pure Chemicals).

Cytokine ELISA. Spleen cells (5×10^6 cells/ml) were cultured in RPMI 1640 (Wako Pure Chemicals) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen, CA), and 50 μM 2-mercaptoethnaol (ME) (Invitrogen) and stimulated with 25 μg/ml soluble egg antigen (SEA) from *S. japonicum* at 37°C for 72 h in 5% CO_2. Supernatants were harvested after centrifugation and stored at −20°C until analyzed. The cytokines IL-4, IL-5, IL-13, IL-17A, and gamma interferon (IFN-γ) were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (eBioscience).

Real-time PCR. Liver samples were minced with a Biomusher (Nippi Research Institute of Biomatrix, Ibaraki, Japan), and total RNA was extracted using TRIzol reagent (Invitrogen). Aliquots of total RNA (5 μg) were reverse transcribed using Superscript III (Invitrogen) and random primers (Invitrogen), followed by real-time PCR using the primers shown in Table 1 (3). Relative quantities of PCR products were determined using a Kapa SYBR Fast quantitative PCR (qPCR) kit (Kapa Biosystems, MA) and a LightCycler 480 (Roche, Mannheim, Germany) and by the comparative threshold cycle method (LightCycler 480 SW1.5; Roche). The quantity of each mRNA in each sample was normalized relative to β-actin expression and expressed relative to controls. If control gene expression levels were below the detection limit, the y axis showed target per reference.

Statistical analyses. Groups were compared using the two-tailed Student t test and analysis of variance (ANOVA) with Statcel3 software (OMS Publishing Inc., Saitama, Japan). Results were considered significant at a P value of <0.05.

RESULTS

Recruitment of neutrophils is higher in DKO mice than in WT mice at 6 weeks after infection with *S. japonicum*. Hepatic granulomatous inflammation in murine schistosomiasis japonica is characterized by the infiltration of neutrophils and eosinophils. To examine whether the absence of IL-4/IL-13 affects neutrophil and eosinophil infiltration, we assayed liver cells of WT and IL-$4^{-/-}$ IL-$13^{-/-}$ (DKO) mice stained with antibodies to Gr-1 and CCR3, with Gr-1^{high} $CCR3^{-}$ SSC^{int} cells defined as neutrophils and Gr-1^{+} $CCR3^{+}$ SSC^{high} cells as eosinophils (Fig. 1A). At 6 weeks postinfection, the average ratio and number of neutrophils were significantly higher, and the ratio and number of eosinophils were lower, in infected DKO mice than in WT mice (Fig. 1B and C), indicating that IL-4/IL-13 suppressed excessive neutrophil recruitment in the liver during acute *S. japonicum* infection.

Hepatic granulomatous inflammation is more severe in infected DKO mice than in WT mice with acute schistosomiasis japonica. To determine whether excessive neutrophil infiltration in the livers of DKO mice correlated with hepatic inflammation, we analyzed tissue samples histologically. Granulomatous inflammation was more severe in DKO mice than in WT mice, with more severe necrosis observed in the granulomas of DKO mice (Fig. 2A). Moreover, the average granuloma size, including necrotic

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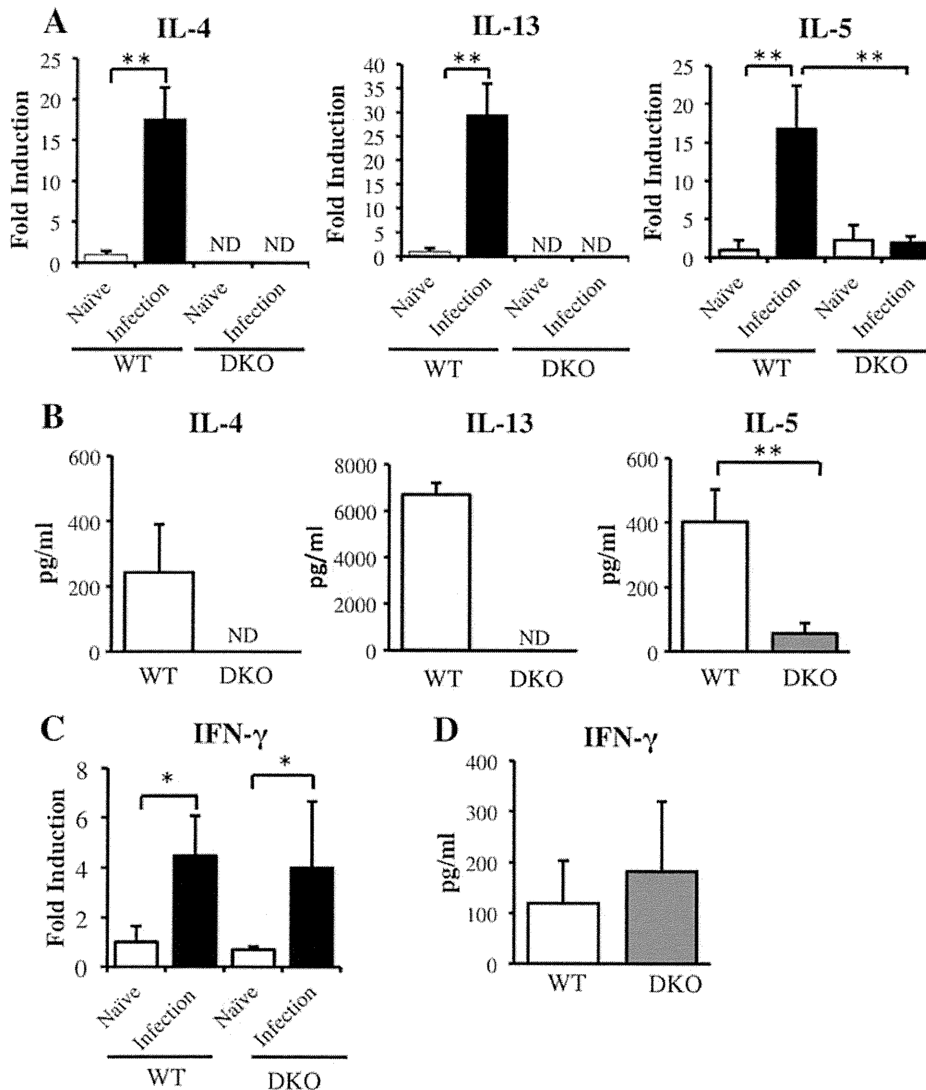

FIG 3 Expression of Th1 and Th2 cytokines in WT and DKO mice at 6 weeks postinfection. (A) Expression of mRNAs encoding the Th2 cytokines IL-4, IL-5, and IL-13 in livers of infected mice. (B) Production of IL-4, IL-5, and IL-13 by SEA-stimulated splenocytes collected from infected WT and DKO mice. (C and D) Expression of IFN-γ mRNA in livers (C) and IFN-γ production by SEA-stimulated splenocytes (D) from infected WT and DKO mice. Means and SD are shown (n = 4 to 6). Naïve, uninfected mice; infection, infected mice; ND, not detected. *, $P < 0.05$; **, $P < 0.01$.

areas, was larger in DKO than in WT mice (Fig. 2B), and serum concentrations of AST and ALT, both markers of hepatocyte damage, were higher in DKO than in WT mice (Fig. 2C). In contrast, although hepatic damage was more severe in DKO than in WT mice, their mortality rates did not differ (data not shown). In addition, the number of recovered worms and the hepatic egg burden were similar in the two strains (data not shown). These results suggested that IL-4/IL-13 suppressed excessive liver damage caused by hepatic inflammation following *S. japonicum* infection.

The Th2 response is decreased but the Th1 response is unaffected in liver and splenocytes from DKO mice during acute infection. To explain the differences in hepatic pathology observed in WT and DKO mice, we measured the concentrations of Th1 and Th2 cytokines in these animals at 6 weeks postinfection. We found that the expression of mRNAs encoding the Th2 cytokines IL-4, IL-5, and IL-13 was enhanced in the livers of *S. japonicum*-infected WT mice (Fig. 3A). In contrast, expression of IL-5 mRNA was impaired in infected DKO mice and was significantly lower than that in infected WT mice. Similarly, SEA-stimulated spleen cells from infected WT mice showed elevated production of IL-4, IL-5, and IL-13, but the production of IL-5 was lower in SEA-stimulated spleen cells from infected DKO mice than in those from infected WT mice (Fig. 3B).

To determine the effect of IL-4/IL-13 knockout on the Th1/Th2 balance in infected mice, we measured the levels of IFN-γ mRNA in their livers. Infection increased the levels of IFN-γ mRNA in both WT and DKO livers, but their expression levels did not differ (Fig. 3C). Moreover, splenocyte production of IFN-γ did not differ in infected WT and DKO mice (Fig. 3D).

Absence of IL-4/IL-13 promotes the production of IL-17A and expression of Th17-related cytokine mRNAs during acute

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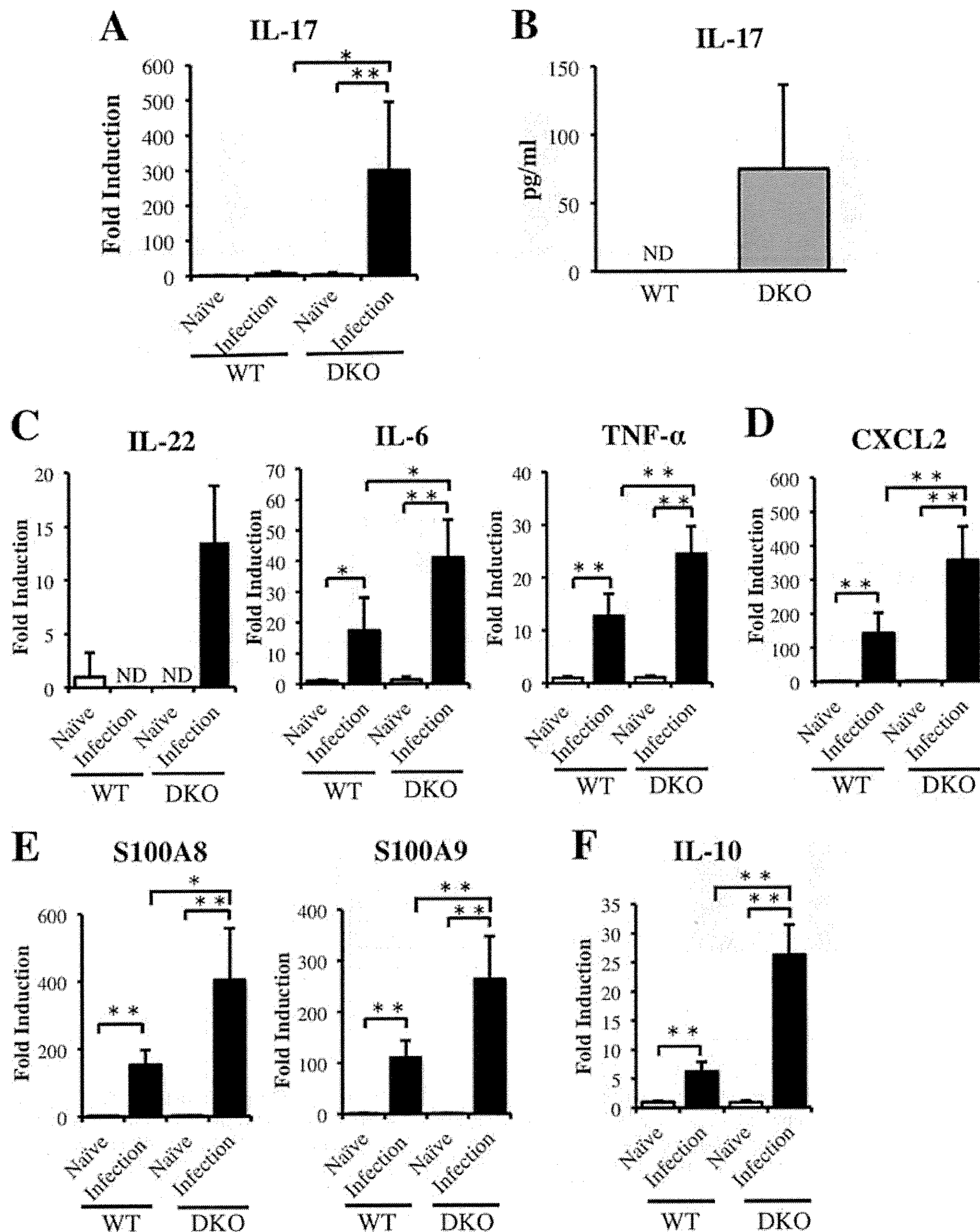

FIG 4 Expression of mRNAs encoding Th17-related cytokines, proinflammatory cytokines, neutrophil chemokines, and S100 family proteins in infected DKO and WT mice at 6 weeks postinfection. (A) Expression of IL-17A mRNA in liver. (B) Production of IL-17A by SEA-stimulated splenocytes from infected mice. (C) Liver expression of mRNAs encoding the Th17-related and proinflammatory cytokines, IL-22, IL-6, and TNF-α. (D) Expression of mRNA encoding the neutrophil chemokine CXCL2 in liver. (E) Expression of S100A8 and S100A9 mRNAs in liver. (E) Expression of IL-10 mRNA in liver. Means and SD are shown ($n = 4$ to 6). Naïve, uninfected mice; infection, infected mice; ND, not detected. *, $P < 0.05$; **, $P < 0.01$.

infection. To determine the mechanism underlying the enhanced neutrophil infiltration and severe hepatic inflammation in DKO mice, we focused on IL-17A, since this cytokine is involved in the induction of neutrophils and the production of proinflammatory cytokines (4, 5, 29, 48). We found that the level of mRNA in liver and the splenocyte production of IL-17A protein were higher in infected DKO mice than in infected WT mice (Fig. 4A and B). We also found that liver expression of IL-22 mRNA, which is produced by Th17 cells (49), was higher in infected DKO mice than in infected WT mice (Fig. 4C). In addition, the expression of message encoding proinflammatory cytokines, such as IL-6 and tumor necrosis factor alpha (TNF-α) (Fig. 4C), CXCL2, a neutrophil chemokine enhanced by IL-17A (Fig. 4D) (48), and S100A8 and S100A9, which are enhanced by IL-17A and IL-22 (Fig. 4E), was greater in infected DKO mice than in infected WT mice (30). These results suggested that, at 6 weeks after infection, IL-4/IL-13 may suppress the Th17 response, which induces the expression in liver of mRNAs encoding the proinflammatory cytokines CXCL2,

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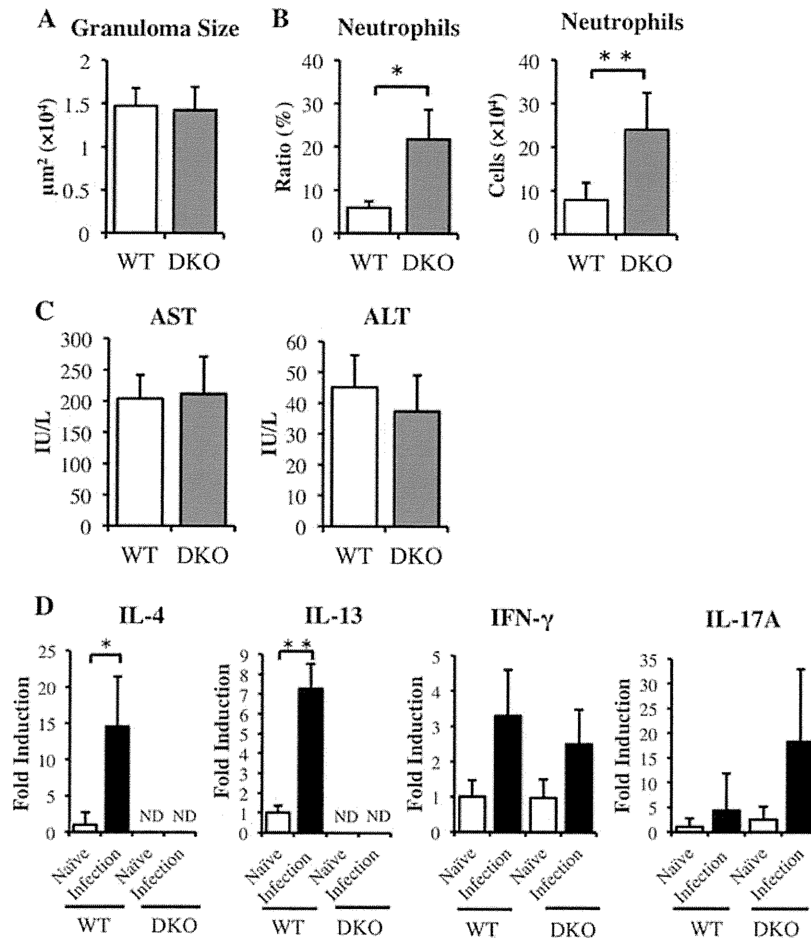

FIG 5 Hepatic granulomatous inflammation and mRNAs of Th1, Th2, and Th17 cytokines in WT and DKO mice at 8 weeks postinfection. (A) Average granuloma sizes in WT and DKO mice. (B) Average population and numbers of neutrophils from infected WT and DKO mice. (C) Serum concentrations of AST and ALT in infected WT and DKO mice. (D) Levels of expression of IL-4, IL-13, IFN-γ, and IL-17A mRNAs in WT and DKO mice. Means and SD are shown (n = 3 or 4). Naïve, uninfected mice; infection, infected mice; ND, not detected. *, $P < 0.05$; **, $P < 0.01$.

S100A8, and S100A9. Interestingly, mRNA encoding IL-10, which suppresses the secretion of Th17 cytokines, was more highly expressed in DKO than in WT mice (Fig. 4F) (15).

Hepatic damage at 8 weeks after infection does not differ in WT and DKO mice. Although hepatic damage was observed in both WT and DKO mice at 8 weeks after infection, their granuloma sizes did not differ (Fig. 5A), nor did their serum concentrations of AST and ALT (Fig. 5C). However, the neutrophil ratio and number were higher in DKO than in WT mice (Fig. 5B). Although IL-4 and IL-13 mRNA levels remained higher in WT mice, IFN-γ and IL-17A mRNA levels did not differ significantly (Fig. 5D). These findings therefore indicated that IL-4 and IL-13 do not suppress hepatic inflammation and damage at 8 weeks postinfection.

Induction of hepatic inflammation and neutrophil infiltration in DKO mice by IL-17A-dependent and -independent mechanisms during acute *S. japonicum* infection. To investigate the role of IL-17A in neutrophil recruitment and severe hepatic inflammation in the absence of IL-4 and IL-13, IL-4$^{-/-}$ IL-13$^{-/-}$ IL-17A$^{-/-}$ (TKO) mice were infected with *S. japonicum*. We found that hepatic inflammation in TKO mice was intermediate between those in WT and DKO mice (Fig. 6A and B). Moreover, serum AST and ALT concentrations and the number and ratio of neutrophils in TKO mice did not differ significantly from those in WT and DKO mice (Fig. 6C and D). Eosinophil infiltration was impaired in both DKO and TKO mice (data not shown), whereas the number of recovered worms and the hepatic egg burden did not differ in WT, DKO, and TKO mice (Table 2). These findings indicate that hepatic damage and neutrophil infiltration in DKO mice could not be explained solely by the effects of IL-17A.

Expression of mRNAs encoding proinflammatory cytokines, neutrophil chemoattractant, and S100 family proteins is independent of IL-17A. DKO mice infected with *S. japonicum* showed enhanced expression of mRNAs encoding IL-22, proinflammatory cytokines, CXCL2, and S100 family proteins. To determine whether the absence of IL-17A affects the expression of these mRNAs, we measured their expression in the livers of TKO mice. We found that the levels of mRNAs encoding the Th17-related cytokines IL-22 and IL-17F and the proinflammatory cytokines IL-6, TNF-α, and granulocyte colony-stimulating factor (G-CSF) did not differ in infected DKO and TKO mice (Fig. 7A and B), nor did the levels of CXCL2, S100A8, and S100A9 mRNAs, all of which are induced by IL17A (Fig. 7C and D). In addition, the expression

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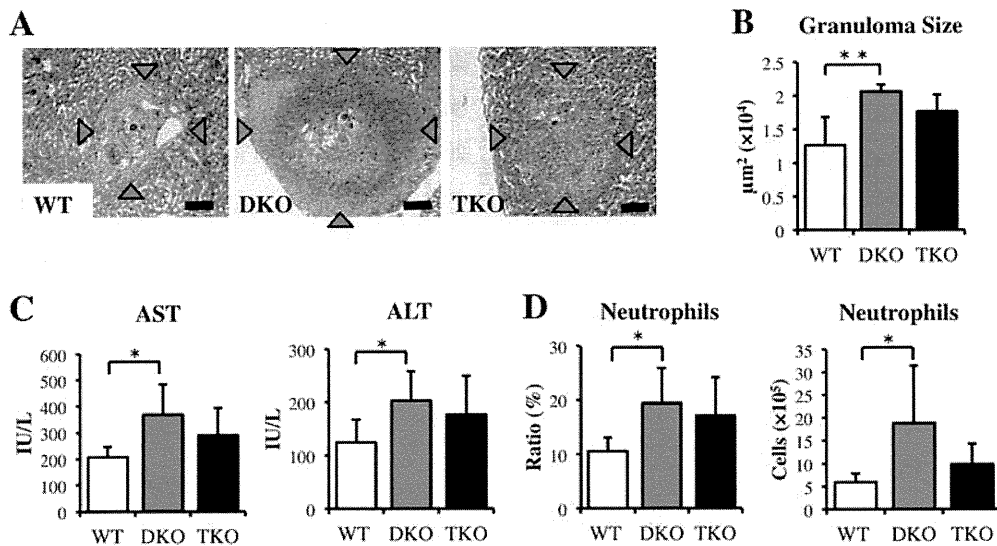

FIG 6 Hepatic inflammation in WT, DKO, and TKO mice at 6 weeks after infection with *S. japonicum*. (A) Histopathology of *S. japonicum*-infected mice. Liver sections stained with hematoxylin/eosin show granuloma surrounding parasite eggs. Original magnification, ×100; scale bar, 100 μm. (B) Average granuloma sizes in WT, DKO, and TKO mice. (C) Serum AST and ALT concentrations in infected WT, DKO, and TKO mice. (D) Ratio and number of neutrophils in the livers of naïve and infected WT, DKO, and TKO mice. Means and SD are shown ($n = 4$ to 6). *, $P < 0.05$; **, $P < 0.01$.

of mRNA encoding the Th1 cytokine IFN-γ did not differ among WT, DKO, and TKO mice (Fig. 7E), and the levels of mRNA encoding the anti-inflammatory cytokine IL-10 were similar in DKO and TKO mice (Fig. 7F). Taken together, these results suggested that IL-17A may not affect the production of other Th17-related cytokines and of the proinflammatory cytokines CXCL2, S100A8, and S100A9.

DISCUSSION

Less is known about the immunopathology of hepatic lesions in *S. japonicum* than about that in *S. mansoni* infection. Due to the importance of IL-4 and IL-13 in the development of hepatic lesions in *S. mansoni* infection (11), we analyzed the roles of IL-4 and IL-13 in *S. japonicum*-infected mice. Examination of hepatic lesions in infected DKO mice indicated that IL-4/IL-13 downregulates neutrophil infiltration and the production of proinflammatory cytokines. IL-4/IL-13 has been shown to suppress inflammation and neutrophil recruitment in a model of ovalbumin (OVA)-induced airway inflammation (18). We found that at 6 weeks after *S. japonicum* infection, the ratio and number of neutrophils in the liver were higher, while the ratio and number of eosinophils were lower, in DKO mice than in WT mice, suggesting the importance of IL-4/IL-13 in the suppression of excessive neutrophil recruitment and the induction of eosinophil recruitment in granulomatous inflammation of murine schistosomiasis japonica.

TABLE 2 Parasitological data[a]

Group	Worm burden	Fecundity (eggs/female, ×10⁴)	Tissue eggs (×10³) in: Liver	Intestine
WT	17.4 ± 4.3	23.9 ± 4.2	72.3 ± 43.7	59.3 ± 35.2
DKO	18.8 ± 3.9	23.8 ± 1.7	102.4 ± 22.2	65.0 ± 24.0
TKO	19.3 ± 4.3	24.0 ± 5.6	95.0 ± 25.2	57.1 ± 13.3

[a] All data are expressed as mean ± standard deviation.

It was reported that IL-4 and IL-13 share the IL-4 receptor α chain (IL-4Rα) and activate STAT6 via IL-4Rα (31, 50). In murine schistosomiasis mansoni, egg-granuloma formation is promoted by IL-4/IL-13 and the IL-4Rα-STAT6 pathway (11, 25, 27), whereas in the absence of IL-4/IL-13, hepatic granulomatous inflammation and eosinophil infiltration are impaired (11). Eosinophil infiltration in *S. japonicum*-infected DKO mice was also impaired, as shown by the reduced production of IL-5 (10). Unexpectedly, however, we found that hepatic inflammatory foci were more severe in DKO than in WT mice infected with *S. japonicum*, since granulomas accompanying broadly necrotic areas were larger in DKO mice. Moreover, hepatic lesions, including necrotic areas, were larger and serum AST and ALT concentrations were higher in DKO than in WT mice at 6 weeks after infection. Neutrophils have been shown to induce hepatic necrosis in mice at 2 weeks after implantation of *S. japonicum* eggs (21). Our results indicate that the more severe pathology observed in DKO than in WT mice may be related to the excess neutrophil recruitment in the former. Thus, IL-4/IL-13 may downregulate severe hepatic inflammation in schistosomiasis japonica by suppressing excessive neutrophil accumulation.

Although neutrophil infiltration was higher in *S. japonicum*- and *S. mansoni*-infected DKO mice, granuloma formation was reduced in DKO mice infected with *S. mansoni* but not in those infected with *S. japonicum* almost 2 weeks after the commencement of egg laying (data not shown) (8, 11). In addition, unlike in *S. mansoni* infection, the mortality rates of *S. japonicum*-infected WT and DKO mice were similar (data not shown). Although IL-4/IL-13 suppressed excessive neutrophil infiltration during infection with both schistosomes, their activities largely differed, which may be partly attributed to differences in the roles of neutrophils in mice infected with these two schistosomes (11, 19, 21, 23). Thus, moderate neutrophil infiltration may be required in *S. japonicum* infection, since depletion of neutrophils causes high

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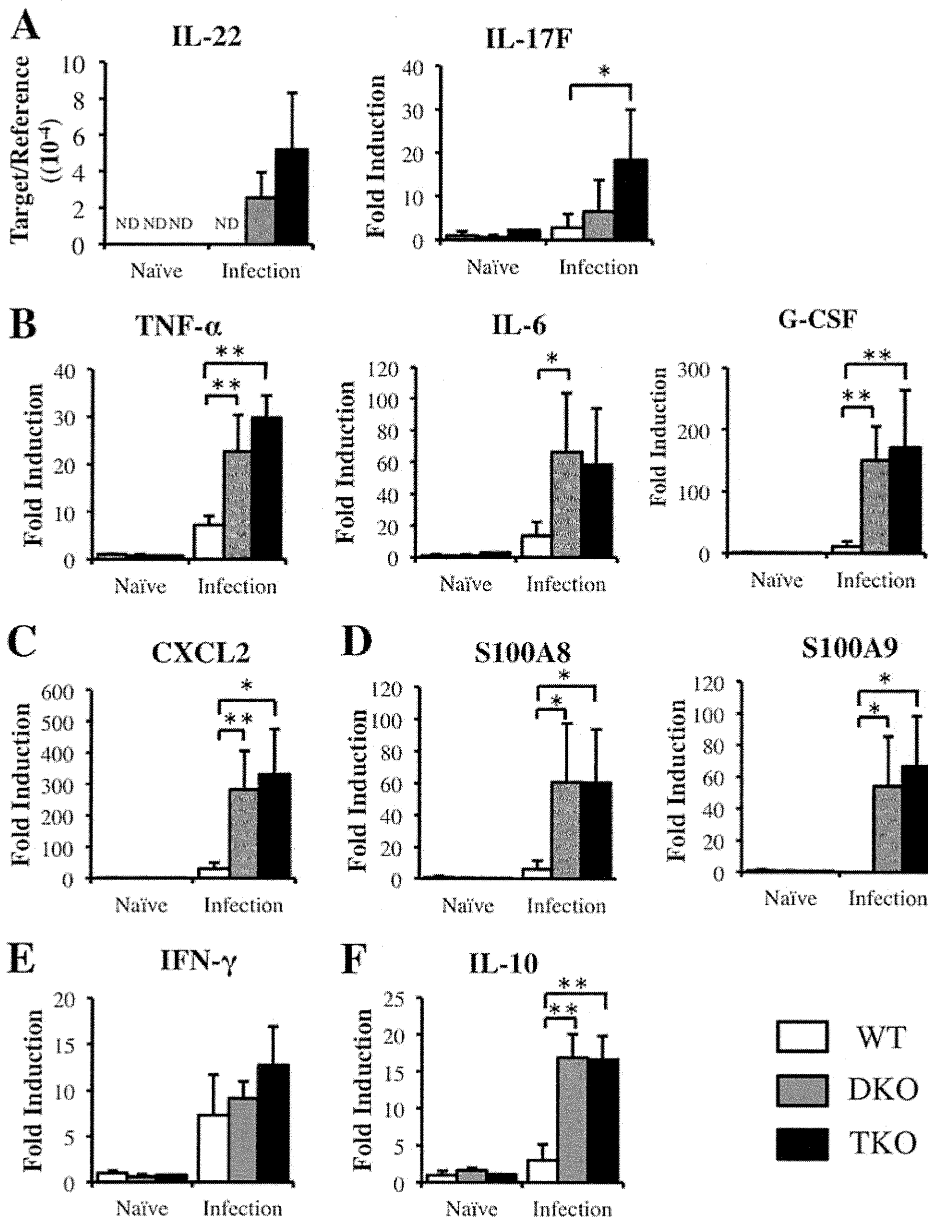

FIG 7 Effect of IL-17A on the expression of IL-22 and IL-17F (A), TNF-α, IL-6, and G-CSF (B), CXCL2 (C), S100A8 and S100A9 (D), IFN-γ (E), and IL-10 (F) mRNAs in the livers of WT, DKO, and TKO mice at 6 weeks postinfection, as determined by real-time PCR. Means and SD are shown (n = 4 to 6). *, $P < 0.05$; **, $P < 0.01$. Naïve, uninfected mice; infection, infected mice; ND, not detected. The y axis in the graph of IL-22 expression is the ratio of IL-22 mRNA to β-actin mRNA, since IL-22 mRNA in naïve controls was below the level of detection.

mortality and excessive infiltration of neutrophils may be associated with severe hepatic pathology (21).

Several mechanisms may be involved in neutrophil induction. The Th1 response has been reported to be involved in neutrophil induction in schistosomiasis japonica (6, 22). Although IFN-γ production was enhanced in *S. mansoni*-infected DKO mice (11), it did not differ in *S. japonicum*-infected DKO and WT mice, a finding contrary to our expectation and indicating that the increased neutrophil infiltration observed in *S. japonicum*-infected DKO mice was not due to increased IFN-γ production.

Th17 cells have been shown to produce IL-17A and IL-22 (9, 49) and to be involved in autoimmune diseases and the induction of proinflammatory cytokines such as IL-6 and TNF-α (5, 18, 21). IL-17A has been shown to induce the recruitment of neutrophils and the expression of CXCL2, a chemokine for neutrophil recruitment (9, 48). Since IL-4 has been found to downregulate Th17 differentiation *in vivo* and *in vitro* (17, 32), we investigated whether IL-17A production was enhanced in *S. japonicum*-infected DKO mice at 6 weeks postinfection. We found that IL-17A production by SEA-stimulated splenocytes and IL-17A mRNA expression in the liver were significantly higher in DKO than in WT mice. Moreover, the levels of mRNAs encoding IL-6, TNF-α, and CXCL2 were higher in the livers of

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DKO mice than in those of WT mice, as was the expression of mRNAs encoding the Ca^{2+}-binding proteins S100A8 and S100A9 (40). The expression of S100A8 and S100A9 is enhanced by IL-17A and/or IL-22 (30), with both of these proteins playing a role in neutrophil recruitment and the production of proinflammatory cytokines (42, 44). S100A8 has been reported to localize to areas of neutrophil accumulation in *S. japonicum*-infected livers (7), suggesting that IL-17A production was upregulated in the absence of IL-4/IL-13 and may be responsible, at least in part, for the excessive neutrophil infiltration, production of proinflammatory cytokines, and more severe inflammation observed in infected DKO mice than in infected WT mice at 6 weeks after infection. Interestingly, the mRNA level of IL-10, which suppresses the production of Th17 cytokines, was also higher in DKO than in WT mice (15), suggesting that elevated IL-17A production was not due to the anti-inflammatory activity of IL-10.

When we analyzed these mice at 8 weeks after infection, we found that although the ratio and number of neutrophils were higher in DKO than in WT mice, the two strains did not differ significantly in the number and severity of hepatic lesions or in their levels of IL-17A and other proinflammatory cytokines, such as IL-6 and TNF-α, (data not shown). These results suggested that IL-4/IL-13 may play an anti-inflammatory role against hepatic inflammation only during the acute stage of infection and that neutrophils may contribute to severe pathology in the acute stage, as in the egg implant model (21).

Since IL-17A has been shown to induce severe pathology in schistosomiasis mansoni and to exaggerate OVA-induced airway inflammation (6, 18, 41, 46), we analyzed the function of IL-17A in the absence of IL-4/IL-13 in acute murine schistosomiasis japonica. We found that the hepatic pathology in IL-4$^{-/-}$ IL-13$^{-/-}$ IL-17A$^{-/-}$ (TKO) mice infected with *S. japonicum* was intermediate between those in WT and DKO mice and that this moderate hepatic damage was coincident with moderate neutrophil infiltration. These results suggest that severe hepatic inflammation in DKO mice was related to excessive neutrophil infiltration, which was partially regulated by IL-17A.

Since IL-17A regulates the production of IL-6, TNF-α, G-CSF, CXCL2, S100A8, and S100A9, we hypothesized that the expression of mRNAs encoding these molecules would be downregulated in TKO mice infected with *S. japonicum* (5, 26, 30, 34, 43, 47). Our results for infected TKO mice suggested, however, that IL-17A may not induce their expression.

We then considered whether another factor could induce the production of proinflammatory cytokines, CXCL2, and S100 family proteins. Possible candidates included the proinflammatory cytokines IL-17F and IL-22, which are produced by Th17 cells (2, 12, 24, 30, 33, 47) and enhance the expression of G-CSF, CXCL2, S100A8, and S100A9, all of which induce neutrophils infiltration (4, 30, 38, 42). Moreover, S100A8 and S100A9 are secreted as a complex, and the S100A8/A9 heterodimer has been shown to enhance the production of proinflammatory cytokines (40, 44). We found that the expression of IL-17F and IL-22 mRNAs was similar in *S. japonicum*-infected TKO and DKO mice but was higher than that in infected WT mice. Although we did not analyze the function of IL-17F and IL-22, our results suggest that these two cytokines may be responsible for the inflammation and neutrophil recruitment observed in *S. japonicum*-infected DKO and TKO mice.

Unexpectedly, in the absence of IL-4/IL-13, the IL-10 level was increased in *S. japonicum* infection, but this result was opposite to that in the case of *S. mansoni* infection (11). It may be caused by the species difference of the parasites tested. Our findings indicated that in the absence of IL-4/IL-13, IL-10 did not seem to play an anti-inflammatory role in *S. japonicum* infection. However, drastic reduction of AAM induction in the absence of IL-4/IL-13 might result in increased hepatic pathology at 6 weeks after *S. japonicum* infection, as AAMs protect against hepatic damage in *S. mansoni* infection (19). Fizz-1, an AAM marker, was lower, whereas classically activated inducible nitric oxide synthase (iNOS), a macrophage (CAM) marker, was higher in DKO and TKO mice than in WT mice (data not shown) (19). Therefore, additional studies are needed to clarify the anti-inflammatory mechanism of IL-4/IL-13 in acute murine schistosomiasis japonica.

In conclusion, we found that IL-4 and IL-13 regulate excessive hepatic inflammation, proinflammatory cytokine production, and neutrophil recruitment in acute murine schistosomiasis japonica. Enhanced expression of mRNAs encoding IL-17A and proinflammatory cytokines may be involved in the more severe pathology observed in infected DKO mice than in WT mice. Although IL-17A has a proinflammatory role (5, 22, 25), it is not totally responsible for the excessive hepatic inflammation, neutrophil recruitment, and proinflammatory cytokine production observed in infected DKO mice.

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REFERENCES

1. **Abbas AK, Murphy KM, Sher A.** 1996. Functional diversity of helper T lymphocytes. Nature **383**:787–793.
2. **Andoh A, et al.** 2005. Interleukin-22, a member of the IL-10 subfamily, induces inflammatory responses in colonic subepithelial myofibroblasts. Gastroenterology **129**:969–984.
3. **Anyan WK, et al.** 2010. Schistosome eggs have a direct role in the induction of basophils capable of a high level of IL-4 production: comparative study of single- and bisexual infection of *Schistosoma mansoni in vivo*. Trop. Med. Health **38**:13–22.
4. **Aujla SJ, et al.** 2008. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat. Med. **14**:275–281.
5. **Bettelli E, Oukka M, Kuchroo VK.** 2007. T(H)-17 cells in the circle of immunity and autoimmunity. Nat. Immunol. **8**:345–350.
6. **Burke ML, et al.** 2009. Immunopathogenesis of human schistosomiasis. Parasite Immunol. **31**:163–176.
7. **Burke ML, et al.** 2010. Temporal expression of chemokines dictates the hepatic inflammatory infiltrate in a murine model of schistosomiasis. PLoS Negl. Trop. Dis. **4**:e598.
8. **Cheever AW, Poindexter RW, Wynn TA.** 1999. Egg laying is delayed but worm fecundity is normal in SCID mice infected with Schistosoma japonicum and S. mansoni with or without recombinant tumor necrosis factor alpha treatment. Infect. Immun. **67**:2201–2208.
9. **Dong C.** 2006. Diversification of T-helper-cell lineages: finding the family root of IL-17-producing cells. Nat. Rev. Immunol. **6**:329–333.
10. **Drazen JM, Arm JP, Austen KF.** 1996. Sorting out the cytokines of asthma. J. Exp. Med. **183**:1–5.
11. **Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN.** 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**:2585–2591.

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12. **Geboes L, et al.** 2009. Proinflammatory role of the Th17 cytokine interleukin-22 in collagen-induced arthritis in C57BL/6 mice. Arthritis Rheum. **60**:390–395.
13. **Gordon S.** 2003. Alternative activation of macrophages. Nat. Rev. Immunol. **3**:23–35.
14. **Grzych JM, et al.** 1991. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. J. Immunol. **146**:1322–1327.
15. **Gu Y, et al.** 2008. Interleukin 10 suppresses Th17 cytokines secreted by macrophages and T cells. Eur. J. Immunol. **38**:1807–1813.
16. **Gurish MF, et al.** 2002. CCR3 is required for tissue eosinophilia and larval cytotoxicity after infection with Trichinella spiralis. J. Immunol. **168**: 5730–5736.
17. **Harrington LE, et al.** 2005. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat. Immunol. **6**:1123–1132.
18. **He R, et al.** 2009. Exaggerated IL-17 response to epicutaneous sensitization mediates airway inflammation in the absence of IL-4 and IL-13. J. Allergy Clin. Immunol. **124**:761–770.e761.
19. **Herbert DR, et al.** 2004. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. Immunity **20**:623–635.
20. **Hestdal K, et al.** 1991. Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. J. Immunol. **147**:22–28.
21. **Hirata M, Hara T, Kage M, Fukuma T, Sendo F.** 2002. Neutropenia augments experimentally induced Schistosoma japonicum egg granuloma formation in CBA mice, but not in C57BL/6 mice. Parasite Immunol. **24**:479–488.
22. **Hirata M, et al.** 2001. Schistosoma japonicum egg granuloma formation in the interleukin-4 or interferon-gamma deficient host. Parasite Immunol. **23**:271–280.
23. **Hsü SY, Hsü HF, Davis JR, Lust GL.** 1972. Comparative studies on the lesions caused by eggs of Schistosoma japonicum and Schistosoma mansoni in livers of albino mice and rhesus monkeys. Ann. Trop. Med. Parasitol. **66**:89–97.
24. **Ikeuchi H, et al.** 2005. Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. Arthritis Rheum. **52**:1037–1046.
25. **Jankovic D, et al.** 1999. Schistosome-infected IL-4 receptor knockout (KO) mice, in contrast to IL-4 KO mice, fail to develop granulomatous pathology while maintaining the same lymphokine expression profile. J. Immunol. **163**:337–342.
26. **Jovanovic DV, et al.** 1998. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. J. Immunol. **160**:3513–3521.
27. **Kaplan MH, Whitfield JR, Boros DL, Grusby MJ.** 1998. Th2 cells are required for the Schistosoma mansoni egg-induced granulomatous response. J. Immunol. **160**:1850–1856.
28. **King CH.** 2010. Parasites and poverty: the case of schistosomiasis. Acta Trop. **113**:95–104.
29. **Komiyama Y, et al.** 2006. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. J. Immunol. **177**: 566–573.
30. **Liang SC, et al.** 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J. Exp. Med. **203**:2271–2279.
31. **Lin JX, et al.** 1995. The role of shared receptor motifs and common Stat proteins in the generation of cytokine pleiotropy and redundancy by IL-2, IL-4, IL-7, IL-13, and IL-15. Immunity **2**:331–339.
32. **Lubberts E, et al.** 2000. IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion. J. Clin. Invest. **105**:1697–1710.
33. **Ma HL, et al.** 2008. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. J. Clin. Invest. **118**: 597–607.
34. **McAllister F, et al.** 2005. Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. J. Immunol. **175**:404–412.
35. **McKenzie GJ, Fallon PG, Emson CL, Grencis RK, McKenzie AN.** 1999. Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. J. Exp. Med. **189**: 1565–1572.
36. **Nagase H, et al.** 2001. Regulation of chemokine receptor expression in eosinophils. Int. Arch. Allergy Immunol. **125**(Suppl. 1):29–32.
37. **Nakae S, et al.** 2002. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity **17**:375–387.
38. **Oda N, et al.** 2005. Interleukin-17F induces pulmonary neutrophilia and amplifies antigen-induced allergic response. Am. J. Respir. Crit. Care Med. **171**:12–18.
39. **Pearce EJ, MacDonald AS.** 2002. The immunobiology of schistosomiasis. Nat. Rev. Immunol. **2**:499–511.
40. **Rammes A, et al.** 1997. Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. J. Biol. Chem. **272**: 9496–9502.
41. **Rutitzky LI, Lopes da Rosa JR, Stadecker MJ.** 2005. Severe CD4 T cell-mediated immunopathology in murine schistosomiasis is dependent on IL-12p40 and correlates with high levels of IL-17. J. Immunol. **175**: 3920–3926.
42. **Ryckman C, et al.** 2003. Role of S100A8 and S100A9 in neutrophil recruitment in response to monosodium urate monohydrate crystals in the air-pouch model of acute gouty arthritis. Arthritis Rheum. **48**:2310–2320.
43. **Schwarzenberger P, et al.** 2000. Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis. J. Immunol. **164**:4783–4789.
44. **Sunahori K, et al.** 2006. The S100A8/A9 heterodimer amplifies proinflammatory cytokine production by macrophages via activation of nuclear factor kappa B and p38 mitogen-activated protein kinase in rheumatoid arthritis. Arthritis Res. Ther. **8**:R69.
45. **Von Lichtenberg F, Erickson DG, Sadun EH.** 1973. Comparative histopathology of schistosome granulomas in the hamster. Am. J. Pathol. **72**: 149–178.
46. **Wilson MS, et al.** 2007. Immunopathology of schistosomiasis. Immunol. Cell Biol. **85**:148–154.
47. **Yang XO, et al.** 2008. Regulation of inflammatory responses by IL-17F. J. Exp. Med. **205**:1063–1075.
48. **Ye P, et al.** 2001. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. J. Exp. Med. **194**:519–527.
49. **Zheng Y, et al.** 2007. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature **445**:648–651.
50. **Zurawski SM, Vega F, Huyghe B, Zurawski G.** 1993. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. EMBO J. **12**:2663–2670.

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Review Article

Schistosome: Its Benefit and Harm in Patients Suffering from Concomitant Diseases

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Schistosomiasis is an important tropical disease affecting approximately 200 million people worldwide. Because of its chronicity and robust immunomodulatory activity, the effects of schistosomes on other diseases, such as allergies, autoimmunity, and infectious diseases, have been studied extensively in both epidemiological and experimental settings. In this paper, we summarize the beneficial and harmful effects of schistosomes. The importance of controlling schistosomiasis is also discussed.

1. Introduction

Schistosoma spp., blood flukes, are parasitic helminths found mainly in developing countries with a tropical or subtropical climate and affect 200 million people worldwide [1]. *Schistosoma mansoni, japonicum,* and *mekongi* harbor in veins of the portal system and lay eggs in the blood vessels. The deposition of numerous eggs in the intestines and liver results in intestinal and hepatic granulomatous lesions, fibrosis, portal hypertension, and hepatosplenomegaly. In contrast, *Schistosoma haematobium* mainly harbors in the venous plexus of the bladder and/or rectal venous plexus. This worm usually causes bloody urine but is also considered to have an etiological relationship with bladder cancer [2]. Because of the extensive distribution of schistosomes and morbidity due to egg deposition, researchers have been interested in the influences of schistosome infections on concomitant diseases [3]. As mentioned, *S. haematobium* is an important carcinogenic factor of bladder cancer although the worm itself does not have mutagenic activity [4]. This parasite is also suggested to be a risk factor for the transmission of HIV [5]. These effects are attributable to pathological lesions caused by the parasites. On the other hand, most helminthic parasites including schistosomes are known to induce robust Th2-polarization. Especially in schistosome infections, egg deposition in the host tissues was reported to be the major stimulus of Th2 responses [6], and egg proteins (e.g., omega-1, IPSE, and peroxiredoxin) are involved in the Th2-biasing activity [7–11]. In addition, schistosome eggs have immunomodulatory potential inducing the alternative activation of macrophages [12] and regulatory T-cell expansion [13]. Because of its robust systemic Th2-inducing and immunomodulatory ability, this worm has been studied most extensively for its bystander effects on various immunological phenomena *in vivo*. In this paper, we summarize the effects of a concurrent infection of schistosomes on immunological disorders and parasitic/microbial infections.

2. Effects of Schistosomes on Immunological Disorders or Infectious Diseases in Mice and Humans

2.1. Allergy. Effects of helminths (including schistosomes) on allergic diseases have been studied extensively in both experimental and epidemiological settings. In experimental asthma or airway hypersensitivity models, schistosome infections and antigen administrations have been consistently shown to protect the animals from the diseases (Table 1). In most studies on the antiasthmatic effects of schistosomes, cellular infiltration (including eosinophils) into bronchoalveolar lavage fluid (BALF) was diminished and simultaneously IL-4, IL-5, and IgE levels were reduced. In contrast, an increase in Treg cells and augmentation of IL-10 production

were observed. Fallon and Mangan [14] designated this kind of Th2 response (Treg dominant and IL-10 dominant) a "modified Th2 response" as opposed to the conventional "allergic Th2 response." They demonstrated that in infected mice, IL-10-producing CD1d^{high} B cells induce Treg cells and consequently ameliorate allergic airway inflammation [15]. In the study, IL-10 was indispensable to the effects of schistosomes. The authors also showed the importance of B cells, and IL-10 in the suppression of systemic anaphylaxis by schistosomes [16]. Smits et al. [17] found that in adoptive transfer experiments, spleen cells (especially B cells and CD4$^+$ T cells) from chronically infected mice could confer protection against pulmonary infiltration by white blood cells, especially eosinophils. In addition, administration of anti-IL-10R antibody cancelled out the effects of the cell transfer. These studies seem to support the critical importance of B cells, Treg cells and IL-10 in schistosome-induced protection against airway allergic inflammation. In studies by another group, however, IL-10 signaling was not essential for the antiallergic immunomodulation by schistosomes [18, 19]. The reason for this discrepancy is unclear, but as the authors point out, other immunomodulatory factors may be able to compensate the absence of IL-10 [19].

Antiallergic effects of schistosomes have also been demonstrated in humans. In a study in Brazil, both asthmatic symptoms and skin reactivity to indoor allergens were reduced in *S. mansoni*-infected asthmatics compared to non-infected individuals [42]. In another study, higher expression levels of HLA-DR, IL-10R (in monocytes), CTLA-4 and CD40L (in CD4$^+$ T cells) were observed in *S. mansoni*-infected asthmatics [43]. According to that paper, the main sources of IL-10 were monocytes and Treg cells. A meta-analysis of current parasite infections and atopy [44] revealed that schsitosomiasis was protective against allergic skin sensitization as well as some other helminths (*Ascaris lumbricoides*, *Trichuris trichiura*, hookworm). The same research group also reported a meta-analysis of interrelationships between helminths and asthma [45]. Hookworm infections were shown to be protective, but no beneficial effect was found for schistosome infections, probably due to the insufficient number of studies (only two). Collectively, anti-asthmatic effects of schistosomes have been confirmed in animal models and suppressive effects on allergic skin reactivity have been confirmed in humans. However, more epidemiological (especially intervention) studies are necessary to conclude whether schistosome infections have beneficial or detrimental effects on asthmatic patients.

2.2. Autoimmunity. Autoimmune disorders had been considered Th1-mediated diseases for a long time. As the Th2-biasing ability of parasitic helminths and consequent downregulation of Th1 responses have been well known, antiautoimmune effects of such parasites had been attributed to the downregulation of Th1 responses in infected animals. However, some major autoimmune diseases are now considered to be dependent on Th17, a newly found pathogenic T-cell subset that mainly produces IL-17 [46]. With this finding, the antiautoimmune properties of helminths have been revisited. In recent years, downmodulation of Th17 responses by parasitic helminths has been reported [24, 36, 47, 48]. If the suppressive activity on both Th1 and Th17 is common to parasitic helminths, helminths may become ideal sources of drug screening for treatment of autoimmune disorders. That is because both T-cell subsets are involved in the pathogenesis of some autoimmune diseases [49]. Moreover, the unstable nature of Th17 [49] reinforces this idea.

As summarized in Table 1, schistosomes suppress various autoimmunity models in rodents. An upregulation of IL-4 and downregulation of IFN-γ responses are almost commonly observed. In addition, responses of IL-17 and/or TNF-α, both of which play pathological roles in autoimmune arthritis [24, 26] and hapten-induced colitis [36, 40], were also downregulated by schistosome infections. Moreover, our study in mice with collagen-induced arthritis (CIA) revealed that the disease-associated local augmentation of bone-destructive cytokines (i.e., IL-6 and RANKL) was abrogated in infected animals [24]. These results indicate that schistosomes have suppressive effects on both Th1/Th17 and inflammatory cytokines. In addition, schistosomes suppress other pathogenic mediators such as autoreactive antibodies. Schistosomes decreased levels of anti-insulin IgG [30], anti-TSHR IgG2a [41], and anticollagen IgG [24, 25]. This effect was also observed in humans, as Mutapi et al. recently reported that *S. haematobium* infection intensity was inversely related to autoreactive antinuclear antibody (ANA) levels [50]. The authors also found that antihelminthic treatment increased ANA levels. However, Rahima et al. reported the presence of antinuclear antibodies in *S. mansoni*-infected mice and that sera from patients with systemic lupus erythematosus (SLE) reacted with cercarial antigens [51], suggesting that schistosomes trigger some kinds of autoimmunity. In conclusion, large-scale cross-sectional studies may be necessary to reveal the interrelationships between schistosomiasis and autoimmunity.

It is reasonable to hypothesize that the downmodulation of proinflammatory cytokines and pathogenic antibodies is involved in the antiautoimmune activity of schistosomes, at least partially. As regulatory cytokines are known to downregulate proinflammatory cytokines and pathogenic antibodies, it is important to determine the "essential" regulatory cytokines in each disease models for elucidation of suppressive mechanisms. For this purpose, it is necessary to perform experiments of cytokine neutralization with specific antibodies or experiments using gene-targeted animals. In Th1/Th17-dependent autoimmunity, IL-4, the key cytokine of Th2 responses, may be responsible for the alleviation of the disease symptoms. For instance, by using gene-targeted mice, STAT6 (a key signaling molecule in the response to IL-4 and IL-13) was shown to be indispensable to the *S. mansoni* egg-induced suppression of experimental autoimmune encephalomyelitis (EAE) [28] and of TNBS-induced colitis [35]. In contrast, IL-4 and IL-13 were dispensable to the suppression of DSS-induced colitis (Th2 cytokine dominant and macrophage-mediated colitis) by male worms of *S. mansoni* [39]. In the same study, authors demonstrated that IL-10 and TGF-β were also dispensable to the anticolitic effects of schistosome, by using specific antibodies against IL-10R and TGF-β. IL-10 was not a crucial regulatory cytokine

TABLE 1: Suppressive effects of schistosome on experimental allergy and autoimmunity in rodents.

Category of animal models	Diseases	Schistosome	Treatment	Proposed mechanisms	Refs
Allergy	Asthma/Airway hypersensitivity or inflammation	Sm	Infection (male)	IL-5 ↓, IL-10↑	[20]
			Infection, Egg i.p.	IL-4 ↓, IL-5 ↓, IgE ↓, Treg↑, Independent of IL-10	[18]
			Infection (chronic)	B cells and $CD4^+$ T cells, Dependent on IL-10	[17]
			Infection and Adoptive transfer	IL-10-producing $CD1d^{high}$ B cells → Treg↑	[15]
			Sm22.6, PIII	IL-4 ↓, IL-5 ↓, IgE ↓, Treg ↑, independent of IL-10	[19]
		Sj	Infection (male, mixed)	IL-4 ↓, IL-5 ↓, IgE ↓, IL-10 ↑	[21]
			Infection and adoptive transfer	DC → IL-4 ↓, IL-5 ↓, IL-10 ↑	[22]
		Sj	SEA, Eggs (i.p., p.o.)	Treg↑	[23]
	Systemic anaphylaxis	Sm	Infection	IL-10-producing B cell	[16]
Autoimmunity	Collagen-induced arthritis (CIA)	Sm	Infection	IL-17 ↓, TNF-α ↓, IL-6 ↓, RANKL ↓, anticollagen IgG ↓	[24]
		Sj	Infection	IL-4 ↑, anticollagen IgG ↓	[25]
	Adjuvant-induced arthritis (AIA)	Sj	Sj16 i.p.	TNF-α ↓, IL-1β ↓, NO ↓, IL-10 ↑	[26]
	Experimental autoimmune encephalomyelitis (EAE)	Sm	Infection	IL-12p40 ↓, IFN-γ ↓, TNF-α ↓, IL-4 ↑	[27]
			Egg i.p.	IFN-γ ↓, IL-4 ↑, TGF-β ↑, IL-10 ↑, dependent on STAT6	[28]
		Sj	SEA i.p.	IFN-γ ↓, IL-4 ↑	[29]
	Type 1 diabetes in NOD mice	Sm	Infection, Egg i.p.	Inhibition of Ab class switch (anti-insulin IgG ↓)	[30]
			SEA, SWA i.p.	NKT ↑	[31]
			SEA i.p.	Treg ↑	[32]
	Streptozotocin-induced diabetes (multiple low dose)	Sm	Infection		[33]
	TNBS-induced colitis	Sm	Infection	IL-2 ↑, IL-4 ↑	[34]
			Egg i.p.	IFN-γ ↓, IL-4 ↑, dependent on STAT6	[35]
			SWA i.p.	IFN-γ ↓, IL-17 ↓, TGF-β ↑, IL-10 ↑	[36]
		Sj	Egg i.p.	IFN-γ ↓, IL-4 ↑, IL-10 ↑, Treg ↑	[37]
			Egg i.p.	IFN-γ ↓, IL-4 ↑, TLR4 ↓	[38]
	DSS-induced colitis	Sm	Infection (male)	Dependent on macrophages, independent of Treg, IL-10, IL-4, IL-13 and TGF-β	[39]
			Infection	TNF-α ↓	[40]
	Grave's hyperthyroidism	Sm	Infection	Anti-TSHR IgG2a ↓	[41]

↓: down-regulation, ↑: up-regulation, Sm: *S. mansoni*, Sj: *S. japonicum*.

also in other helminthic infections, that is, piroxicam-induced colitis was suppressed by *Heligmosomoides polygyrus* [48], and EAE was suppressed by *Fasciola hepatica* [47], both in IL-10-deficient mice. In the latter study, however, TGF-β was shown to be responsible for anti-EAE effects of the worms [47]. Taken together, the involvements of IL-4, IL-10, and TGF-β in antiautoimmune effects of helminths depend on the disease models and helminth species. Further investigations using various autoimmunity models and gene-targeted animals would be necessary for comprehensive elucidation of the suppressive mechanisms by regulatory cytokines in schistosome infections.

Regarding the participation of regulatory cell populations in schistosome-induced antiautoimmune effects, Treg cells, macrophages, and other types of cells (e.g., NKT cells) have been suggested. Although Treg cell population is known to expand by schistosome infection or SEA administration [13, 32], their involvement seems to depend on the disease

models. For instance, Cooke et al. have been studying antidiabetic effects of *S. mansoni* using a spontaneous T1D model (NOD mouse) [30], and they demonstrated that Treg cells were essential in the suppression of T1D by cell transfer experiments [32]. The authors (Zaccone et al.) showed that splenocytes from nontreated NOD mice successfully transmitted diabetes into NOD/SCID recipients, whereas splenocytes from SEA-treated NOD mice had a reduced capacity to transmit diabetes. They also showed that SEA had various immunomodulatory effects on dendritic cells (DCs), macrophages, and T cells of NOD mice [12, 32]: for example, increased expressions of TGF-β, galectins, PD-L1, and so forth. In contrast to this T1D model, Smith et al. showed that depletion of Treg cells did not influence the suppressive effect of schistosomes on DSS-induced colitis [39]. They demonstrated that macrophages (but NOT alternatively activated macrophages) played an essential role in the amelioration of the colitis.

In some of the studies in Table 1, the injection of eggs or SEA was not effective [27, 39, 40]. Likewise, in our study of CIA, SEA injection was not effective [52]. Taken together with findings that mice infected with male worms become resistant to DSS-induced colitis [39], egg-derived substances are not sufficient to explain all of the immunomodulatory activities of schistosomes. Indeed, injection of soluble worm antigens (SWAs) could prevent T1D in NOD mice [31] and TNBS-induced colitis [36]. Therefore, differential effects of worms and eggs should be further elucidated for a precise understanding of the immunomodulatory mechanisms of schistosomes. In addition, further studies on differences between single-sex infections and mixed-sex infections may be necessary.

2.3. Parasitic Infections. In tropical developing countries, infections with multiple microbes/parasites are common. Consequently, the immune responses and/or pathological lesions caused by one pathogen may affect the outcome of other infections. Therefore, influences of parasitic infections on concomitant diseases have been studied. In this section, we focus on the effects of schistosomes on other parasitic infections. Malaria is the world's deadliest and most widely distributed parasitic disease. Consequently, there are more than a few experimental studies on the interrelationships between schistosomiasis and malaria (Table 2). The influence of schistosome infections depends on the species of the malarial parasites and mouse strains used in the experiments. For instance, in CBA/Ca mice, *S. mansoni* protected against *P. chabaudi* infection, worsened *P. yoelii* infection, and had no effect on *P. berghei* infection [53]. Even when the same parasite, *P. chabaudi*, was used, schistosomes exacerbated the parasitemia and mortality in C57BL/6 mice but ameliorated the outcome in A/J mice [54, 55]. Although these complicated outcomes should be taken into consideration, in general, schistosome infections seem to exacerbate rodent malaria, that is, increase of parasitemia, mortality, and hepatosplenomegaly [56]. Detrimental effects of schistosome infections on malarial outcome are also reported in humans [57, 58]. In Kenyan school children, even a light *S. mansoni* infection was shown to exacerbate hepatosplenomegaly of malarial patients [57]. In a study in Senegal, heavy *S. mansoni* infections significantly increased the incidence of malarial attacks [58]. However, the modification of antibody responses by concomitant schistosomiasis is controversial [59, 60]. The mechanism responsible for the exacerbation (or amelioration) of malaria is clear in neither mice nor humans. Helmby et al. [54] suggested diminished production of TNF-α in schistosome-infected mice to have contributed to the increase in parasitemia of *P. chabaudi*. Yoshida et al. extensively analyzed possible mechanisms of protective effects of schistosomes against *P. chabaudi* in A/J mice [55]. They observed an enhanced Th1 response to *P. chabaudi* in schistosome-infected mice and that an anti-IFN-γ antibody abrogated the schistosome-induced protective effect against *P. chabaudi*. As monoinfection with *S. mansoni* induced a Th2-dominant response, upregulation of IFN-γ seemed to derive from the mixed infections of both parasites. They also observed the upregulation of iNOS gene expression in spleen from mice with mixed infections, suggesting an increase of splenic nitric oxide production to have contributed to the protection from *P. chabaudi*. Although the mechanism of the exacerbation of rodent malaria by schistosome is yet to be sufficiently analyzed, Treg cells have been shown to play an important role in the exacerbation of *P. yoelii* infection by preceding *H. polygyrus* infection [61]. Likewise, the induction/expansion of Treg cells by schistosome might be involved in the increased susceptibility to rodent malaria. The Th1 immune response protects against malarial parasites, but it also plays pathological roles in malaria, especially cerebral malaria [62]. Thus, schistosome as a representative Th2-biasing helminth is expected to suppress brain pathology. Indeed, in a model of cerebral malaria using mice infected with *P. berghei* ANKA, *S. mansoni* reduced the incidence of cerebral malaria and delayed death [63]. Moreover, the administration of IPSE/alpha-1, a Th2-inducing schistosomal egg protein [9, 10], also delayed death. Bucher et al. reported that brain pathology was reduced in schistosome-infected mice although they did not observe any beneficial effect on mortality [64].

Influence of schistosomes on other protozoan infections has also been investigated (Table 2). Regarding *Leishmania major*, reports vary, with Yoshida et al. finding no effects of schistosome on the outcome of *L. major* infection [67], and La Flamme et al. reporting an exacerbation of experimental leishmaniasis [68]. The reason for the discrepancy is not clear but might be intensity of the *S. mansoni* infection (20 cercariae in the former versus 70 cercariae in the latter). In the case of *L. donovani*, schistosome-preinfected mice failed to control growth of the protozoa in the liver [70]. In the coinfected mice, hepatic egg granulomas were shown to provide a favorable microenvironment for the growth of the amastigotes. Likewise, *S. mansoni* exacerbated *Trypanosoma cruzi* infection [71] and *Toxoplasma gondii* infection [72]. In general, schistosome infections seem to be detrimental to animals infected with protozoan parasites.

In marked contrast to that for protozoan parasites, protective immunity against intestinal helminths is usually Th2 dependent. Therefore, the Th2-dominant environment

Table 2: Effects of schistosome on other parasitic infections in rodents.

Category	Parasites	Schistosome	Mouse strain	Effects	Refs.
Malaria	*Plasmodium berghei*	Sm	CBA/Ca	No effect	[53]
	Plasmodium berghei ANKA	Sm	Swiss albino	Parasitemia ↑, mortality ↑	[65]
	Plasmodium berghei NK65	Sm	BALB/c	Parasitemia ↑, mortality ↑	[66]
	Plasmodium chabaudi	Sm	CBA/Ca	Parasitemia ↓	[53]
	Plasmodium chabaudi AS	Sm	A/J	Mortality ↓	[55]
			C57BL/6	Parasitemia ↑, mortality ↑	
		Sm	C57BL/6	Parasitemia ↑	[54]
	Plasmodium yoelii	Sm	CBA/Ca	Parasitemia ↑	[53]
		Sm	BALB/c	Parasitemia ↑, mortality ↑	[56]
Cerebral Malaria	*Plasmodium berghei* ANKA	Sm	ICR HSD	Cerebral malaria ↓	[63]
		Sm	C57BL/6	Brain pathology ↓, no effect on mortality	[64]
Other protozoan infections	*Leishmania major*	Sm	BALB/c	No effect	[67]
			C57BL/6	No effect	
		Sm	C57BL/6	Parasitemia ↑, lesion resolution delayed	[68]
	Leishmania mexicana	Sm	Outbred	Incubation period shortened	[69]
	Leishmania donovani	Sm	C57BL/6	Parasitemia ↑	[70]
	Trypanosoma cruzi	Sm	Albino	Parasitemia ↑, mortality ↑	[71]
	Toxoplasma gondii	Sm	Albino	Mortality ↑	[72]
Helminthic infections	*Hymenolepis diminuta*	Sm	NMRI	Expulsion ↑	[73]
	Strongyloides venezuelensis	Sm	C57BL/6	Migration ↓	[67]
		Sj	C57BL/6	Migration ↓, expulsion ↑	[69]
	Trichuris muris	Sm	AKR	Expulsion ↑	[74]

↓: downregulation, ↑: upregulation; Sm: *S. mansoni*, Sj: *S. japonicum*.

produced by schistosomes can be expected to protect against intestinal helminths. Indeed, as summarized in Table 2, schistosome infections protected mice from intestinal helminths. Likewise, in a human study in Brazil [75], *S. mansoni* egg counts were inversely correlated with *A. lumbricoides* and *T. trichiura*. (One exception was *Ancylostoma*, which was positively correlated with schistosome infections). Although protection against intestinal helminths is commonly observed in experimental settings, the mechanisms differ in each case. For instance, protection against a lung-migratory parasite, *Strongyloides venezuelensis*, mainly involved eosinophil-mediated killing of larvae in the lungs [76]. In addition, intestinal mucosal mastocytosis induced by *S. japonicum* infection rendered mice resistant to harboring of adult worms [76]. Antigen cross-reactivity between schistosomes and *S. venezuelensis* [67, 76] may have also contributed to the protection. In contrast, in the case of *Hymenolepis diminuta* and *Trichuris muris* (nonmigratory intestinal helminths), the protection seems to be dependent on accelerated expulsion from the intestines [73, 74]. Overall, schistosome infections appear to be beneficial to animals infected with intestinal helminths.

2.4. Bacterial Infections and Vaccinations. Prolonged bacteremia in schistosomiasis patients was first reported more than 50 year ago, and the relationship between enterobacteria infections and schistosomiasis has been long studied [3]. The prolonged enterobacterial infection is referred to "prolonged septicemic enterobacteriosis." The schistosome-induced exacerbation of enterobacterial infections has also been observed in experimental settings [77, 78]. As most studies were conducted before the "molecular immunology age," the mechanisms of exacerbation of bacterial infections have not been sufficiently elucidated. However, the mechanisms are likely similar to those responsible for the schistosome-induced increase in susceptibility to protozoan parasites, that is, a reduction in Th1-dependent protective immunity as a consequence of augmented Th2 responses. Indeed, a Th1-inducing protozoan parasite, *L. donovani*, did not affect the growth of *Salmonella paratyphi* A in infected hamsters [78]. Moreover, impairment of the bactericidal function of macrophages from schistosome-infected mice was reported [79]. In addition to the immunomodulation by schistosomes, there are direct schistosome-bacteria interactions providing worm bodies as foci for bacterial multiplication [3, 80, 81]. Another considerable influence of schistosomes on bacterial infections is a reduction in vaccine efficacy. The *Mycobacterium bovis* BCG-induced protective response against *Mycobacterium tuberculosis* in mice was reduced by *S. mansoni* infection [82]. The authors also reported increased susceptibility to intravenous BCG

inoculations and lung pathology in *S. mansoni*-infected mice [83]. In these studies, decrease in IFN-γ and nitric oxide in response to PPD were observed.

2.5. Viral Infections and Vaccinations. As hepatotropic viruses, that is, hepatitis B virus (HBV) and hepatitis C virus (HCV), cause liver cirrhosis during chronic infections, the synergistic exacerbation of hepatic pathology is expectable outcome of concurrent infections of HBV/HCV and schistosomes. Because of a lack of suitable animal models for infections of these hepatotropic viruses, major findings in schistosome-HBV/HCV coinfections have been obtained from epidemiological studies. Regarding HBV, schistosomiasis (especially the severe hepatosplenic form) was correlated with a higher frequency of HBV infection [3, 84, 85]. This observation could be explained by an increased susceptibility to HBV caused by schistosome infections. On the other hand, there are reports of no relationship between schistosomiasis and HBV [86, 87]. In addition, experiments with animals do not support increased susceptibility to HBV in schistosomiasis. Some evidence comes from an experiment using woodchucks infected with both schistosomes and woodchuck hepatitis virus (WHV) [88]. As HBV and WHV belong to the same family (family Hepadnaviridae), a concurrent infection by schistosomes and WHV in woodchucks is a good model of concurrent infections of schistosomes and HBV in humans. The authors reported no impact of the schistosome infection on WHV serum markers. Another paper on HBV transgenic mice [89] reported an inhibition of HBV replication during schistosome infection. In that study, the antiviral effects of schistosomes were attributed to IFN-γ and nitric oxide. Overall, it seems premature to conclude the presence of certain positive or negative effects of schistosomes on HBV infection. Regarding HBV vaccines (both serum derived and recombinant), they were immunogenic in schistosomiasis patients although reduced responses to vaccination were observed in hepatosplenic schistosomiasis [90–92].

In contrast to the controversial effects on HBV infections, detrimental effects of schistosomes on HCV infections have been clearly demonstrated, that is, schistosomes weaken anti-HCV immune responses and worsen liver disease. According to studies in Egypt, patients with coinfections were characterized by a more advanced liver pathology, greater viral burden, higher levels of anti-HCV antibodies, and progression to chronic hepatitis [93–95]. Moreover, schistosomiasis was shown to be inversely correlated with HCV-specific $CD4^+$ T cells, $CD8^+$ T cells, and/or Th1 cytokine responses [95–100]. In addition to the modulatory effects of schistosomes on HCV-specific immune responses, SEA of *S. mansoni* [101] and *S. haematobium* [102] were shown to enhance *in vitro* viral replication in a hepatoblastoma cell line (HepG2) and peripheral blood mononuclear cells (PBMCs), respectively. Likewise, in other viral infections, schistosomes were shown to suppress specific CTL and cytokine responses and to prevent viral clearance [103–107]. It is interesting that the granulomas in *S. mansoni*-infected mice provide a microenvironment suitable for viral expansion [107], as in the case of a hepatic infection with the protozoan parasite *L. donovani* [70].

3. Concluding Remarks

Based on the experimental and epidemiological findings reviewed here, it can be concluded that schistosome infections are generally beneficial to patients with intestinal helminth infections and detrimental to patients with bacterial, viral, or protozoan infections. In most tropical or subtropical countries where schistosomiasis is endemic, more serious infectious diseases (e.g., HIV/AIDS, tuberculosis, and malaria) are also endemic. Therefore, control of schistosomiasis (especially infections of *S. mansoni* and *S. haematobium*) has been given low priority compared to control of such infectious diseases. However, if the detrimental bystander effects of schistosomes on concomitant bacterial, viral, or protozoan infections are properly considered, the importance of controlling schistosomiasis should be more emphasized.

In 2002, J. F. Bach summarized epidemiological trends of allergic and autoimmune diseases during recent several decades in developed countries [108]. According to the paper, the prevalence of the immunological disorders such as asthma, T1D, multiple sclerosis (MS), and Crohn's disease (CD) was increasing, whereas the prevalence of infectious diseases such as rheumatic fever, hepatitis A, tuberculosis, mumps, and measles was decreasing. These phenomena may be explained by the "hygiene hypothesis" in which microbial and helminthic infections prevent immunological disorders. Along with this hypothesis, schistosome infections are expected to prevent or alleviate symptoms of immunological disorders. According to the experimental studies until now, antiallergic and antiautoimmune effects of schistosomes are also plausible in humans. However, schistosomes could not be directly used for therapeutic treatment because of their pathogenicity. Instead, purified immunomodulatory products or recombinant proteins could be tested for clinical use. Indeed, considerable numbers of helminths' products have been shown to protect against experimental immunological disorders [109]. The immunomodulators exert their effects via Toll-like receptors (TLRs) and/or C-type lectin receptors (CLRs) [109]. Taken together with the finding that systemic administration of TLR agonists could prevent experimental autoimmunity and allergy [110], appropriately synthesized TLR agonists may be able to mimic or replace the prophylactic or therapeutic effects of schistosomes.

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References

[1] P. J. Hotez, D. A. P. Bundy, K. Beegle et al., "Helminth infections: soil-transmitted helminth infections and schistosomiasis," in *Disease Control Priorities in Developing Countries,*

D. T. Jamison, J. G. Breman, A. R. Measham et al., Eds., chapter 24, World Bank, Washington, DC, USA, 2nd edition, 2006.

[2] B. J. Vennervald and K. Polman, "Helminths and malignancy," *Parasite Immunology*, vol. 31, no. 11, pp. 686–696, 2009.

[3] P. P. Chieffi, "Interrelationship between schistosomiasis and concomitant diseases," *Memorias do Instituto Oswaldo Cruz*, vol. 87, pp. 291–296, 1992.

[4] Y. Osada, T. Kumagai, K. Masuda, T. Suzuki, and T. Kanazawa, "Mutagenicity evaluation of *Schistosoma* spp. extracts by the *umu*-test and V79/HGPRT gene mutation assay," *Parasitology International*, vol. 54, no. 1, pp. 29–34, 2005.

[5] E. F. Kjetland, P. D. Ndhlovu, E. Gomo et al., "Association between genital schistosomiasis and HIV in rural Zimbabwean women," *AIDS*, vol. 20, no. 4, pp. 593–600, 2006.

[6] J. M. Grzych, E. Pearce, A. Cheever et al., "Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistomiasis mansoni," *Journal of Immunology*, vol. 146, no. 4, pp. 1322–1327, 1991.

[7] B. Everts, G. Perona-Wright, H. H. Smits et al., "Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses," *Journal of Experimental Medicine*, vol. 206, no. 8, pp. 1673–1680, 2009.

[8] S. Steinfelder, J. F. Andersen, J. L. Cannons et al., "The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1)," *Journal of Experimental Medicine*, vol. 206, no. 8, pp. 1681–1690, 2009.

[9] G. Schramm, F. H. Falcone, A. Gronow et al., "Molecular characterization of an interleukin-4-inducing factor from *Schistosoma mansoni* eggs," *Journal of Biological Chemistry*, vol. 278, no. 20, pp. 18384–18392, 2003.

[10] G. Schramm, K. Mohrs, M. Wodrich et al., "Cutting edge: IPSE/ alpha-1, a glycoprotein from *Schistosoma mansoni* eggs, induces IgE-dependent, antigen-independent IL-4 production by murine basophils in vivo," *Journal of Immunology*, vol. 178, no. 10, pp. 6023–6027, 2007.

[11] S. Donnelly, C. M. Stack, S. M. O'Neill, A. A. Sayed, D. L. Williams, and J. P. Dalton, "Helminth 2-Cys peroxiredoxin drives Th2 responses through a mechanism involving alternatively activated macrophages," *FASEB Journal*, vol. 22, no. 11, pp. 4022–4032, 2008.

[12] P. Zaccone, O. T. Burton, S. Gibbs et al., "Immune modulation by *Schistosoma mansoni* antigens in NOD mice: effects on both innate and adaptive immune systems," *Journal of Biomedicine and Biotechnology*, vol. 2010, Article ID 795210, 2010.

[13] J. J. Taylor, M. Mohrs, and E. J. Pearce, "Regulatory T cell responses develop in parallel to Th responses and control the magnitude and phenotype of the Th effector population," *Journal of Immunology*, vol. 176, no. 10, pp. 5839–5847, 2006.

[14] P. G. Fallon and N. E. Mangan, "Suppression of TH2-type allergic reactions by helminth infection," *Nature Reviews Immunology*, vol. 7, no. 3, pp. 220–230, 2007.

[15] S. Amu, S. P. Saunders, M. Kronenberg, N. E. Mangan, A. Atzberger, and P. G. Fallon, "Regulatory B cells prevent and reverse allergic airway inflammation via Foxp3-positive T regulatory cells in a murine model," *Journal of Allergy and Clinical Immunology*, vol. 125, no. 5, pp. 1114.e8–1124.e8, 2010.

[16] N. E. Mangan, R. E. Fallon, P. Smith, N. Van Rooijen, A. N. McKenzie, and P. G. Fallon, "Helminth infection protects mice from anaphylaxis via IL-10-producing B cells," *Journal of Immunology*, vol. 173, no. 10, pp. 6346–6356, 2004.

[17] H. H. Smits, H. Hammad, M. van Nimwegen et al., "Protective effect of *Schistosoma mansoni* infection on allergic airway inflammation depends on the intensity and chronicity of infection," *Journal of Allergy and Clinical Immunology*, vol. 120, no. 4, pp. 932–940, 2007.

[18] L. G. G. Pacífico, F. A. V. Marinho, C. T. Fonseca et al., "*Schistosoma mansoni* antigens modulate experimental allergic asthma in a murine model: a major role for CD4+ $CD25^+$ $Foxp3^+$ T cells independent of interleukin-10," *Infection and Immunity*, vol. 77, no. 1, pp. 98–107, 2009.

[19] L. S. Cardoso, S. C. Oliveira, A. M. Góes et al., "*Schistosoma mansoni* antigens modulate the allergic response in a murine model of ovalbumin-induced airway inflammation," *Clinical and Experimental Immunology*, vol. 160, no. 2, pp. 266–274, 2010.

[20] N. E. Mangan, N. Van Rooijen, A. N. J. McKenzie, and P. G. Fallon, "Helminth-modified pulmonary immune response protects mice from allergen-induced airway hyperresponsiveness," *Journal of Immunology*, vol. 176, no. 1, pp. 138–147, 2006.

[21] H. M. Mo, J. H. Lei, Z. W. Jiang et al., "*Schistosoma japonicum* infection modulates the development of allergen-induced airway inflammation in mice," *Parasitology Research*, vol. 103, no. 5, pp. 1183–1189, 2008.

[22] P. Liu, J. Li, X. Yang et al., "Helminth infection inhibits airway allergic reaction and dendritic cells are involved in the modulation process," *Parasite Immunology*, vol. 32, no. 1, pp. 57–66, 2010.

[23] J. Yang, J. Zhao, Y. Yang et al., "*Schistosoma japonicum* egg antigens stimulate CD4+ CD25 + T cells and modulate airway inflammation in a murine model of asthma," *Immunology*, vol. 120, no. 1, pp. 8–18, 2007.

[24] Y. Osada, S. Shimizu, T. Kumagai, S. Yamada, and T. Kanazawa, "*Schistosoma mansoni* infection reduces severity of collagen-induced arthritis via down-regulation of pro-inflammatory mediators," *International Journal for Parasitology*, vol. 39, no. 4, pp. 457–464, 2010.

[25] Y. He, J. Li, W. Zhuang et al., "The inhibitory effect against collagen-induced arthritis by *Schistosoma japonicum* infection is infection stage-dependent," *BMC Immunology*, vol. 11, article 28, 2010.

[26] X. Sun, Y. H. Liu, Z. Y. Lv et al., "rSj16, a recombinant protein of *Schistosoma japonicum*-derived molecule, reduces severity of the complete Freund's adjuvant-induced adjuvant arthritis in rats' model," *Parasite Immunology*, vol. 32, no. 11-12, pp. 739–748, 2010.

[27] A. C. La Flamme, K. Ruddenklau, and B. T. Bäckström, "Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis," *Infection and Immunity*, vol. 71, no. 9, pp. 4996–5004, 2003.

[28] D. Sewell, Z. Qing, E. Reinke et al., "Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization," *International Immunology*, vol. 15, no. 1, pp. 59–69, 2003.

[29] X. Zheng, X. Hu, G. Zhou et al., "Soluble egg antigen from *Schistosoma japonicum* modulates the progression of chronic progressive experimental autoimmune encephalomyelitis via Th2-shift response," *Journal of Neuroimmunology*, vol. 194, no. 1-2, pp. 107–114, 2008.

[30] A. Cooke, P. Tonks, F. M. Jones et al., "Infection with *Schistosoma mansoni* prevents insulin dependent diabetes mellitus in non-obese diabetic mice," *Parasite Immunology*, vol. 21, no. 4, pp. 169–176, 1999.

[31] P. Zaccone, Z. Feheérvári, F. M. Jones et al., "*Schistosoma mansoni* antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes," *European Journal of Immunology*, vol. 33, no. 5, pp. 1439–1449, 2003.

[32] P. Zaccone, O. Burton, N. Miller, F. M. Jones, D. W. Dunne, and A. Cooke, "*Schistosoma mansoni* egg antigens induce Treg that participate in diabetes prevention in NOD mice," *European Journal of Immunology*, vol. 39, no. 4, pp. 1098–1107, 2009.

[33] H. S. El-Wakil, T. S. Aboushousha, O. El Haddad, N. B. Gamil, T. Mansour, and H. El-Said, "Effect of *Schistosoma mansoni* egg deposition on multiple low doses streptozotocin induced insulin dependent diabetes," *Journal of the Egyptian Society of Parasitology*, vol. 32, no. 3, pp. 987–1002, 2002.

[34] T. G. Moreels, R. J. Nieuwendijk, J. G. De Man et al., "Concurrent infection with *Schistosoma mansoni* attenuates inflammation induced changes in colonic morphology, cytokine levels, and smooth muscle contractility of trinitrobenzene sulphonic acid induced colitis in rats," *Gut*, vol. 53, no. 1, pp. 99–107, 2004.

[35] D. E. Elliott, J. Li, A. Blum et al., "Exposure to schistosome eggs protects mice from TNBS-induced colitis," *American Journal of Physiology*, vol. 284, no. 3, pp. G385–G391, 2003.

[36] N. E. Ruyssers, B. Y. De Winter, J. G. De Man et al., "Therapeutic potential of helminth soluble proteins in TNBS induced colitis in mice," *Inflammatory Bowel Diseases*, vol. 15, no. 4, pp. 491–500, 2009.

[37] H. M. Mo, W. Q. Liu, J. H. Lei, Y. L. Cheng, C. Z. Wang, and Y. L. Li, "*Schistosoma japonicum* eggs modulate the activity of CD4+ CD25+ Tregs and prevent development of colitis in mice," *Experimental Parasitology*, vol. 116, no. 4, pp. 385–389, 2007.

[38] Y. Zhao, S. Zhang, L. Jiang, J. Jiang, and H. Liu, "Preventive effects of *Schistosoma japonicum* ova on trinitrobenzenesulfonic acid-induced colitis and bacterial translocation in mice," *Journal of Gastroenterology and Hepatology*, vol. 24, no. 11, pp. 1775–1780, 2009.

[39] P. Smith, N. E. Mangan, C. M. Walsh et al., "Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism," *Journal of Immunology*, vol. 178, no. 7, pp. 4557–4566, 2007.

[40] P. Bodammer, G. Waitz, M. Loebermann et al., "*Schistosoma mansoni* infection but not egg antigen promotes recovery from colitis in outbred NMRI mice," *Digestive Diseases and Sciences*, vol. 56, no. 1, pp. 70–78, 2011.

[41] Y. Nagayama, K. Watanabe, M. Niwa, S. M. McLachlan, and B. Rapoport, "*Schistosoma mansoni* and α-galactosylceramide: prophylactic effect of Th1 immune suppression in a mouse model of Graves' hyperthyroidism," *Journal of Immunology*, vol. 173, no. 3, pp. 2167–2173, 2004.

[42] M. Medeiros, J. P. Figueiredo, M. C. Almeida et al., "*Schistosoma mansoni* infection is associated with a reduced course of asthma," *Journal of Allergy and Clinical Immunology*, vol. 111, no. 5, pp. 947–951, 2003.

[43] R. R. Oliveira, K. J. Gollob, J. P. Figueiredo et al., "*Schistosoma mansoni* infection alters co-stimulatory molecule expression and cell activation in asthma," *Microbes and Infection*, vol. 11, no. 2, pp. 223–229, 2009.

[44] J. Feary, J. Britton, and J. Leonardi-Bee, "Atopy and current intestinal parasite infection: a systematic review and meta-analysis," *Allergy*, vol. 66, no. 4, pp. 569–578, 2011.

[45] J. Leonardi-Bee, D. Pritchard, and J. Britton, "Asthma and current intestinal parasite infection: systematic review and meta-analysis," *American Journal of Respiratory and Critical Care Medicine*, vol. 174, no. 5, pp. 514–523, 2006.

[46] H. Park, Z. Li, X. O. Yang et al., "A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17," *Nature Immunology*, vol. 6, no. 11, pp. 1133–1141, 2005.

[47] K. P. Walsh, M. T. Brady, C. M. Finlay, L. Boon, and K. H. G. Mills, "Infection with a helminth parasite attenuates autoimmunity through TGF-β-mediated suppression of Th17 and Th1 responses," *Journal of Immunology*, vol. 183, no. 3, pp. 1577–1586, 2009.

[48] D. E. Elliott, A. Metwali, J. Leung et al., "Colonization with *Heligmosomoides polygyrus* suppresses mucosal IL-17 production," *Journal of Immunology*, vol. 181, no. 4, pp. 2414–2419, 2008.

[49] M. El-Behi, A. Rostami, and B. Ciric, "Current views on the roles of Th1 and Th17 cells in experimental autoimmune encephalomyelitis," *Journal of Neuroimmune Pharmacology*, vol. 5, no. 2, pp. 189–197, 2010.

[50] F. Mutapi, N. Imai, N. Nausch et al., "Schistosome infection intensity is inversely related to auto-reactive antibody levels," *PLoS ONE*, vol. 6, no. 5, article e19149, 2011.

[51] D. Rahima, R. Tarrab-Hazdai, M. Blank, R. Arnon, and Y. Shoenfeld, "Anti-nuclear antibodies associated with schistosomiasis and anti-schistosomal antibodies associated with SLE," *Autoimmunity*, vol. 17, no. 2, pp. 127–141, 1994.

[52] Y. Osada and T. Kanazawa, "Parasitic helminths: new weapons against immunological disorders," *Journal of Biomedicine & Biotechnology*, vol. 2010, Article ID 743758, 9 pages, 2010.

[53] M. Lwin, C. Last, G. A. T. Targett, and M. J. Doenhoff, "Infection of mice concurrently with *Schistosoma mansoni* and rodent malarias: contrasting effects of patent *S. mansoni* infections on *Plasmodium chabaudi*, *P. yoelii* and *P. berghei*," *Annals of Tropical Medicine and Parasitology*, vol. 76, no. 3, pp. 265–273, 1982.

[54] H. Helmby, M. Kullberg, and M. Troye-Blomberg, "Altered immune responses in mice with concomitant *Schistosoma mansoni* and *Plasmodium chabaudi* infections," *Infection and Immunity*, vol. 66, no. 11, pp. 5167–5174, 1998.

[55] A. Yoshida, H. Maruyama, T. Kumagai et al., "*Schistosoma mansoni* infection cancels the susceptibility to *Plasmodium chabaudi* through induction of type 1 immune responses in A/J mice," *International Immunology*, vol. 12, no. 8, pp. 1117–1125, 2000.

[56] D. Sangweme, C. Shiff, and N. Kumar, "*Plasmodium yoelii*: adverse outcome of non-lethal *P. yoelii malaria* during co-infection with *Schistosoma mansoni* in BALB/c mouse model," *Experimental Parasitology*, vol. 122, no. 3, pp. 254–259, 2009.

[57] S. Wilson, B. J. Vennervald, H. Kadzo et al., "Hepatosplenomegaly in Kenyan schoolchildren: exacerbation by concurrent chronic exposure to malaria and *Schistosoma mansoni* infection," *Tropical Medicine and International Health*, vol. 12, no. 12, pp. 1442–1449, 2007.

[58] C. Sokhna, J. Y. Le Hesran, P. A. Mbaye et al., "Increase of malaria attacks among children presenting concomitant infection by *Schistosoma mansoni* in Senegal," *Malaria Journal*, vol. 3, p. 43, 2004.

[59] T. O. Diallo, F. Remoue, L. Gaayeb et al., "Schistosomiasis co-infection in children influences acquired immune response against *Plasmodium falciparum* Malaria antigens," *PLoS ONE*, vol. 5, no. 9, Article ID 12764, pp. 1–7, 2010.

[60] D. Courtin, A. Djilali-Saïah, J. Milet et al., "*Schistosoma haematobium* infection affects *Plasmodium falciparum-specific* IgG responses associated with protection against malaria," *Parasite Immunology*, vol. 33, no. 2, pp. 124–131, 2011.

[61] K. Tetsutani, K. Ishiwata, H. Ishida et al., "Concurrent infection with *Heligmosomoides polygyrus* suppresses anti-*Plasmodium yoelii* protection partially by induction of CD4 +CD25+Foxp3+ Treg in mice," *European Journal of Immunology*, vol. 39, no. 10, pp. 2822–2830, 2009.

[62] N. H. Hunt and G. E. Grau, "Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria," *Trends in Immunology*, vol. 24, no. 9, pp. 491–499, 2003.

[63] J. H. Waknine-Grinberg, D. Gold, A. Ohayon et al., "*Schistosoma mansoni* infection reduces the incidence of murine cerebral malaria," *Malaria Journal*, vol. 9, no. 1, article 5, 2010.

[64] K. Bucher, K. Dietz, P. Lackner et al., "*Schistosoma* co-infection protects against brain pathology but does not prevent severe disease and death in a murine model of cerebral malaria," *International Journal for Parasitology*, vol. 41, no. 1, pp. 21–31, 2011.

[65] M. Legesse, B. Erko, and F. Balcha, "Increased parasitaemia and delayed parasite clearance in *Schistosoma mansoni* and *Plasmodium berghei* co-infected mice," *Acta Tropica*, vol. 91, no. 2, pp. 161–166, 2004.

[66] R. F. Laranjeiras, L. C. C. Brant, A. C. L. Lima, P. M. Z. Coelho, and E. M. Braga, "Reduced protective effect of Plasmodium berghei immunization by concurrent *Schistosoma mansoni* infection," *Memorias do Instituto Oswaldo Cruz*, vol. 103, no. 7, pp. 674–677, 2008.

[67] A. Yoshida, H. Maruyama, Y. Yabu, T. Amano, T. Kobayakawa, and N. Ohta, "Immune responses against protozoal and nematodal infection in mice with underlying *Schistosoma mansoni* infection," *Parasitology International*, vol. 48, no. 1, pp. 73–79, 1999.

[68] A. C. La Flamme, P. Scott, and E. J. Pearce, "Schistosomiasis delays lesion resolution during *Leishmania* major infection by impairing parasite killing by macrophages," *Parasite Immunology*, vol. 24, no. 7, pp. 339–345, 2002.

[69] P. M. Z. W. Coelho Mayrink, M. Dias, and L. H. Pereira, "Susceptibility to *Leishmania mexicana* of mice infected with *Schistosoma mansoni*," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 74, no. 1, p. 141, 1980.

[70] M. F. Hassan, Y. Zhang, C. R. Engwerda, P. M. Kaye, H. Sharp, and Q. D. Bickle, "The *Schistosoma mansoni* hepatic egg granuloma provides a favorable microenvironment for sustained growth of *Leishmania donovani*," *American Journal of Pathology*, vol. 169, no. 3, pp. 943–953, 2006.

[71] K. Kloetzel, J. Faleiros, S. R. Mendes, C. T. Stanley, and H. S. Arias, "Concomitant infection of albino mice by *Trypanosoma cruzi* and *Schistosoma mansoni* parasitological parameters," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 67, no. 5, pp. 652–658, 1973.

[72] K. Kloetzel, P. P. Chieffi, J. J. Faleiros, and T. J. Merluzzi Filho, "Mortality and other parameters of concomitant infections in albino mice: the *Schistosoma-Toxoplasma* model," *Tropical and Geographical Medicine*, vol. 29, no. 4, pp. 407–410, 1977.

[73] J. Andreassen, A. B. Odaibo, and N. O. Christensen, "Antagonistic effects of *Schistosoma mansoni* on superimposed *Hymenolepis diminuta* and *H. microstoma* infections in mice," *Journal of Helminthology*, vol. 64, no. 4, pp. 337–339, 1990.

[74] A. J. Curry, K. J. Else, F. Jones, A. Bancroft, R. K. Grencis, and D. W. Dunne, "Evidence that cytokine-mediated immune interactions induced by *Schistosoma mansoni* alter disease outcome in mice concurrently infected with *Trichuris muris*," *Journal of Experimental Medicine*, vol. 181, no. 2, pp. 769–774, 1995.

[75] M. Chamone, C. A. Marques, G. S. Atuncar, A. L. A. Pereira, and L. H. Pereira, "Are there interactions between schistosomes and intestinal nematodes?" *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 84, no. 4, pp. 557–558, 1990.

[76] H. Maruyama, Y. Osada, A. Yoshida et al., "Protective mechanisms against the intestinal nematode *Strongyloides venezuelensis* in *Schistosoma japonicum*-infected mice," *Parasite Immunology*, vol. 22, no. 6, pp. 279–286, 2000.

[77] A. L. Njunda and J. P. Oyerinde, "*Salmonella typhi* infection in *Schistosoma mansoni* infected mice," *West African Journal of Medicine*, vol. 15, no. 1, pp. 24–30, 1996.

[78] I. A. Mikhail, G. I. Higashi, N. S. Mansour et al., "*Salmonella paratyphi* A in hamsters concurrently infected with *Schistosoma mansoni*," *American Journal of Tropical Medicine and Hygiene*, vol. 30, no. 2, pp. 385–393, 1981.

[79] M. I. Muniz-Junqueira, A. Prata, and C. E. Tosta, "Phagocytic and bactericidal function of mouse macrophages to *Salmo nella typhimurium* in schistosomiasis mansoni," *American Journal of Tropical Medicine and Hygiene*, vol. 46, no. 2, pp. 132–136, 1992.

[80] I. A. Mikhail, G. I. Higashi, D. C. Edman, and S. H. Elwan, "Interaction of *Salmonella paratyphi* A and *Schistosoma mansoni* in hamsters," *American Journal of Tropical Medicine and Hygiene*, vol. 31, no. 2, pp. 328–334, 1982.

[81] R. F. Melhem and P. T. LoVerde, "Mechanism of interaction of *Salmonella* and *Schistosoma* species," *Infection and Immunity*, vol. 44, no. 2, pp. 274–281, 1984.

[82] D. Elias, H. Akuffo, A. Pawlowski, M. Haile, T. Schön, and S. Britton, "*Schistosoma mansoni* infection reduces the protective efficacy of BCG vaccination against virulent *Mycobacterium tuberculosis*," *Vaccine*, vol. 23, no. 11, pp. 1326–1334, 2005.

[83] D. Elias, H. Akuffo, C. Thors, A. Pawlowski, and S. Britton, "Low dose chronic *Schistosoma mansoni* infection increases susceptibility to *Mycobacterium bovis* BCG infection in mice," *Clinical and Experimental Immunology*, vol. 139, no. 3, pp. 398–404, 2005.

[84] H. A. Hammad, M. M. B'd El Fattah, M. Moris, E. H. Madina, A. A. El Abbasy, and A. T. M. Soliman, "Study on some hepatic functions and prevalence of hepatitis B surface antigenaemiain Egyptian children with schistosomal hepatic fibrosis," *Journal of Tropical Pediatrics*, vol. 36, no. 3, pp. 126–127, 1990.

[85] H. M. Al-Freihi, "Prevalence of hepatitis B surface antigenemia among patients with *Schistosoma mansoni*," *Annals of Saudi Medicine*, vol. 13, no. 2, pp. 121–125, 1993.

[86] B. Larouze, M. C. Dazza, C. Gaudebout, M. Habib, M. Elamy, and B. Cline, "Absence of relationship between *Schistosoma mansoni* and hepatitis B virus infection in the Qalyub Governate, Egypt," *Annals of Tropical Medicine and Parasitology*, vol. 81, no. 4, pp. 373–375, 1987.

[87] X. P. Ye, Y. L. Fu, R. M. Anderson, and D. J. Nokes, "Absence of relationship between *Schistosoma japonicum* and hepatitis B virus infection in the Dongting lake region, China," *Epidemiology and Infection*, vol. 121, no. 1, pp. 193–195, 1998.

[88] Z. A. Andrade, P. Berthillon, R. Paraná, J. A. Grimaud, and C. Trépo, "Schistosomiasis mansoni and viral B hepatitis in woodchucks," *Journal of Hepatology*, vol. 34, no. 1, pp. 134–139, 2001.

[89] H. McClary, R. Koch, F. V. Chisari, and L. G. Guidotti, "Inhibition of hepatitis B virus replication during *Schistosoma mansoni* infection in transgenic mice," *Journal of Experimental Medicine*, vol. 192, no. 2, pp. 289–294, 2000.

[90] S. Bassily, K. C. Hyams, N. M. El-Ghorab, M. M. Mansour, N. A. El-Masry, and M. A. Dunn, "Immunogenicity of hepatitis B vaccine in patients infected with *Schistosoma mansoni*," *American Journal of Tropical Medicine and Hygiene*, vol. 36, no. 3, pp. 549–553, 1987.

[91] S. Bassily, K. C. Hyams, N. El-Ghorab, and N. A. El-Masry, "Safety and immunogenicity of a recombinant hepatitis B vaccine in patients infected with *Schistosoma mansoni*," *American Journal of Tropical Medicine and Hygiene*, vol. 42, no. 5, pp. 449–452, 1990.

[92] S. Bassily, G. T. Strickland, M. F. Abdel-Wahab et al., "Efficacy of hepatitis B vaccination in primary school children from a village endemic for *Schistosoma mansoni*," *Journal of Infectious Diseases*, vol. 166, no. 2, pp. 265–268, 1992.

[93] S. Kamal, M. Madwar, L. Bianchi et al., "Clinical, virological and histopathological features: long-term follow-up in patients with chronic hepatitis C co-infected with *S. mansoni*," *Liver*, vol. 20, no. 4, pp. 281–289, 2000.

[94] A. R. el-Zayadi, O. Selim, E. H. Ibrahim et al., "Does schistosomiasis play a role in the high sero prevalence of HCV antibody among Egyptians?" *Tropical Gastroenterology*, vol. 18, no. 3, pp. 98–100, 1997.

[95] S. M. Kamal, J. W. Rasenack, L. Bianchi et al., "Acute hepatitis C without and with schistosomiasis: correlation with hepatitis C-specific CD4+ T-cell and cytokine response," *Gastroenterology*, vol. 121, no. 3, pp. 646–656, 2001.

[96] S. M. Kamal, C. S. Graham, Q. He et al., "Kinetics of intrahepatic hepatitis C virus (HCV)-specific CD4+ T cell responses in HCV and *Schistosoma mansoni* coinfection: relation to progression of liver fibrosis," *Journal of Infectious Diseases*, vol. 189, no. 7, pp. 1140–1150, 2004.

[97] S. M. Kamal, L. Bianchi, A. Al Tawil et al., "Specific cellular immune response and cytokine patterns in patients coinfected with hepatitis C virus and *Schistosoma mansoni*," *Journal of Infectious Diseases*, vol. 184, no. 8, pp. 972–982, 2001.

[98] A. Farid, M. Al-Sherbiny, A. Osman et al., "*Schistosoma* infection inhibits cellular immune responses to core HCV peptides," *Parasite Immunology*, vol. 27, no. 5, pp. 189–196, 2005.

[99] I. M. El-Kady, M. Lotfy, G. Badraf, S. El-Masry, and I. Waked, "Interleukin (IL)-4, IL-10, IL-18 and IFN-γ cytokines pattern in patients with combined hepatitis C virus and *Schistosoma mansoni* infections," *Scandinavian Journal of Immunology*, vol. 61, no. 1, pp. 87–91, 2005.

[100] M. Elrefaei, N. El-Sheikh, K. Kamal, and H. Cao, "HCV-specific CD27- CD28- memory T cells are depleted in hepatitis C virus and *Schistosoma mansoni* co-infection," *Immunology*, vol. 110, no. 4, pp. 513–518, 2003.

[101] M. M. Bahgat, M. A. E. El-Far, A. A. Mesalam et al., "*Schistosoma mansoni* soluble egg antigens enhance HCV replication in mammalian cells," *Journal of Infection in Developing Countries*, vol. 4, no. 4, pp. 226–234, 2010.

[102] M. K. El-Awady, S. S. Youssef, M. H. Omran, A. A. Tabll, W. T. El Garf, and A. M. Salem, "Soluble egg antigen of *Schistosoma haematobium* induces HCV replication in PBMC from patients with chronic HCV infection," *BMC Infectious Diseases*, vol. 6, article 91, 2006.

[103] J. K. Actor, M. Shirai, M. C. Kullberg, R. M. L. Buller, A. Sher, and J. A. Berzofsky, "Helminth infection results in decreased virus-specific CD8+ cytotoxic T- cell and Th1 cytokine responses as well as delayed virus clearance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 3, pp. 948–952, 1993.

[104] M. A. Marshall, D. Jankovic, V. Ellen Maher, A. Sher, and J. A. Berzofsky, "Mice infected with *Schistosoma mansoni* develop a novel non-T-lymphocyte suppressor population which inhibits virus-specific CTL induction via a soluble factor," *Microbes and Infection*, vol. 3, no. 13, pp. 1051–1061, 2001.

[105] M. J. Edwards, O. Buchatska, M. Ashton, M. Montoya, Q. D. Bickle, and P. Borrow, "Reciprocal immunomodulation in a schistosome and hepatotropic virus coinfection model," *Journal of Immunology*, vol. 175, no. 10, pp. 6275–6285, 2005.

[106] A. A. Da'Dara, N. Lautsch, T. Dudek et al., "Helminth infection suppresses T-cell immune response to HIV-DNA-based vaccine in mice," *Vaccine*, vol. 24, no. 24, pp. 5211–5219, 2006.

[107] J. K. Actor, M. A. Marshall, I. A. Eltoum, R. M. L. Buller, J. A. Berzofsky, and A. Sher, "Increased susceptibility of mice infected with *Schistosoma mansoni* to recombinant vaccinia virus: association of viral persistence with egg granuloma formation," *European Journal of Immunology*, vol. 24, no. 12, pp. 3050–3056, 1994.

[108] J. F. Bach, "The effect of infections on susceptibility to autoimmune and allergic diseases," *New England Journal of Medicine*, vol. 347, no. 12, pp. 911–920, 2002.

[109] W. Harnett and M. M. Harnett, "Helminth-derived immunomodulators: can understanding the worm produce the pill?" *Nature Reviews Immunology*, vol. 10, no. 4, pp. 278–284, 2010.

[110] A. Aumeunier, F. Grela, A. Ramadan et al., "Systemic toll-like receptor stimulation suppresses experimental allergic asthma and autoimmune diabetes in NOD mice," *PLoS ONE*, vol. 5, no. 7, Article ID e11484, 2010.

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Cerebral Schistosomiasis Due to *Schistosoma haematobium* Confirmed by PCR Analysis of Brain Specimen∇

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The case of a 25-year-old Japanese male who had cerebral schistosomiasis caused by *Schistosoma haematobium* is reported here. Although serum antibody tests showed a cross-reaction with other helminths and no ova were excreted in urine or feces, the existence of *Schistosoma haematobium* in the brain was confirmed by PCR analysis.

CASE REPORT

In October 2009, a 25-year-old Japanese man was admitted to a local community hospital in Japan with a 1-week history of mild headache and sporadic paraphasia. He had worked as an agricultural consultant in the Republic of Malawi from April 2007 to June 2009. During his stay, he lived with local residents, consumed water from a well, and had swum in a lake at least twice. He had been in excellent health until October 2009, except for a *Giardia lamblia* infection in 2008. At the community hospital, a computed tomography (CT) scan of the patient's head showed four hyperdense and edematous lesions in the left parietal lobe, and these lesions were suspected to be related to tropical infectious diseases due to the fact that the onset of his symptoms appeared soon after his return from the Republic of Malawi. Subsequently, the patient was referred to our institution for further workup.

Upon presentation to our institute, the patient's temperature was 36.8°C, his pulse was 60 beats per minute (bpm), and his blood pressure was 120/70 mm Hg. Although the patient was alert and appropriate at a glance, verbal paraphasia was occasionally observed. Laboratory evaluation revealed the following: white blood cell count, 8,780/μl (67.5% neutrophils, 25.0% lymphocytes, 1.5% eosinophils); serum C-reactive protein, 0.03 mg/dl; IgE, 18 U/ml; HIV antibody negative; toxoplasma IgM and IgG negative; and *Entamoeba* antibody negative. A magnetic resonance imaging (MRI) scan of the brain with gadolinium enhancement showed a couple of ill-defined, heterogeneously enhancing lesions. They were each approximately 10 mm in diameter, in the left parietal lobe, with increased intensity of the signal on the T1-weighted image (Fig. 1). A lumbar puncture was not performed. The patient's headache and nausea worsened rapidly, and we were obliged to relieve his symptoms as soon as possible. Based on the clinical presentation and characteristic imaging finding, we clinically concluded that the cerebral lesions were neurocysticercosis. Albendazole (15 mg/kg of body weight per day) was administered with dexamethasone (0.1 mg/kg per day) for a total of 8 days. The patient's headache and nausea then subsided, and the verbal paraphasia disappeared. The findings from an MRI scan of the brain were improved but still remained.

One week after the initiation of treatment, the results of the commercially available serum enzyme-linked immunosorbent assay (ELISA; SRL, Tokyo, Japan), which can detect IgG antibody for 12 helminthic diseases as a screening (22), were reported: *Spirometra erinacei* (also known as *Spirometra mansoni*) antibody on admission was positive, whereas *Taenia solium* antibody was negative. *Schistosoma* species are not included in this screening ELISA. Repeated microscopic examination of urine and stool specimens disclosed no ova or parasites. An enhanced CT scan from the neck to the pelvis was unremarkable, without evidence of subcutaneous nodules. From these findings, cerebral sparganosis, which is due to *Spirometra* species, was strongly suspected as the cause of the cerebral lesions. Cerebral sparganosis responds best to surgical excision of the parasite, because praziquantel has limited success or no effect on adult worms (14, 17). Eleven days after admission, subtotal excision of the nodules at left parietal lobe was achieved by a craniotomy. No live worms or degenerative worms were observed in the surgical field. Pathological examination of the specimen revealed gliosis and multiple necrotizing granulomas scattered within the parenchyma of the brain,

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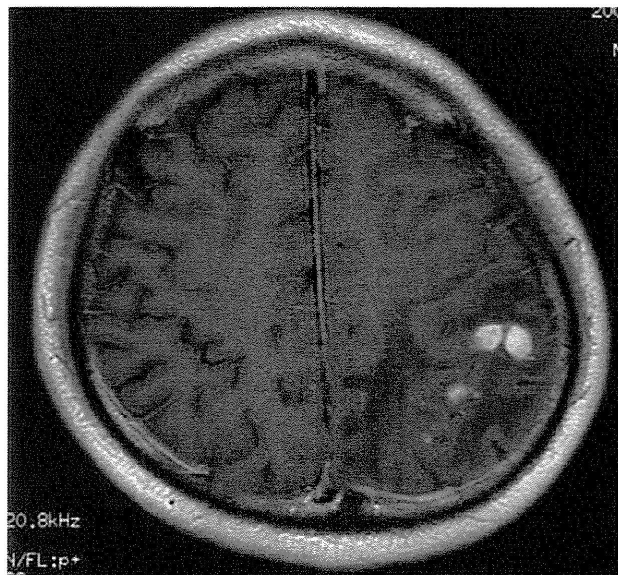

FIG. 1. T1-weighted MRI scan with enhancement, obtained at admission, showed tumor-like lesions in the left parietal lobe with the presence of edema.

with deposits of helminth ova in the center of these granulomas (Fig. 2a). Granulomas had multinucleated giant cells around these ova, which seem to have a prominent terminal spine (Fig. 2b). These morphological characteristics suggested that the helminth ova were eggs of the *Schistosoma* species, particularly *S. haematobium*. To identify *Schistosoma* species, we performed serological tests in our laboratory in the Section of Environmental Parasitology, Tokyo Medical and Dental University (Tokyo, Japan). The result of ELISA revealed increases in serum IgG antibodies against the ova of *S. haematobium* and *Schistosoma mansoni* (*S. mansoni*) and against the larvae of *Spirometra erinacei*. The serum IgG antibodies against *S. haematobium* and *S. mansoni* increased to a level higher than those against *S. erinacei*. For the treatment for the residual lesion, oral praziquantel was commenced at a dose of 20 mg/kg twice a day for a total of 3 days. An MRI scan of the brain with gadolinium enhancement 3 months after the excision and the

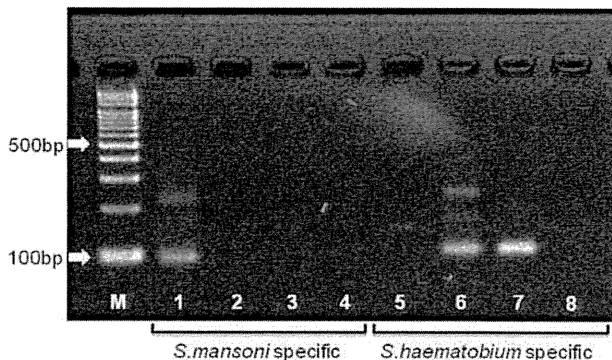

FIG. 3. PCR assay for *Schistosoma haematobium* and *S. mansoni*. M, marker; lanes 1 and 5, *S. mansoni* DNA; lanes 2 and 6, *S. haematobium* DNA; lanes 3 and 7, patient DNA; lanes 4 and 8, no DNA; lanes 1 to 4, *S. mansoni*-specific 121-bp tandem repeat sequence; lanes 5 to 8, *S. haematobium*-specific DraI sequence.

chemotherapy showed a significant reduction in the high signal change. The patient remained in stable condition without clinical complications 4 months after completion of the therapy.

In order to make the definitive diagnosis, the brain specimen was tested by PCR assays. DNA extraction from a paraffin-embedded section of the brain specimen was carried out by using a PCR template preparation kit (TaKaRa DEXPAT Easy; TaKaRa, Shiga, Japan). DNA was amplified with two PCR assays utilizing distinct primer pairs. The first primer targeted to the 97-bp repeated DNA sequence, DraI, which is specific to *S. haematobium* (10). The second primer targeted to the 121-bp tandem repeated DNA sequence, which is specific to *S. mansoni* (11, 25). These specific DNA sequences are not contained in the DNA of *S. japonicum*. As shown in Fig. 3, the PCR amplification using the brain specimen showed an intense band of *S. haematobium* DNA; however, there was no band specific to *S. mansoni* DNA. Therefore, we finally diagnosed the patient's cerebral lesions as cerebral schistosomiasis due to *S. haematobium*.

Diagnosis of a focal lesion in the brain of patients with a

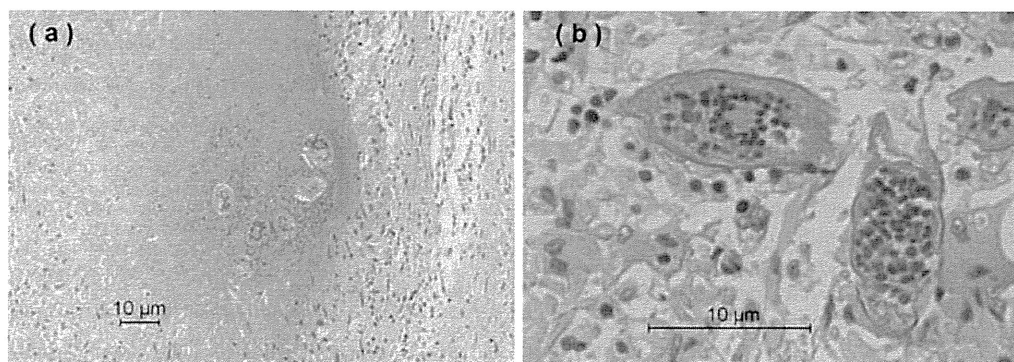

FIG. 2. (a) Photomicrograph showing nodular granulomas within the parenchyma of the brain containing deposits of *S. haematobium* ova in the center of the granulomas (hematoxylin-eosin stained; magnification, ×100). (b) Ova of *S. haematobium* with a characteristic prominent terminal spine (hematoxylin-eosin stained; magnification, ×400).