

# Th2 Cytokines Are Associated with Persistent Hepatic Fibrosis in Human *Schistosoma japonicum* Infection

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We conducted a prospective cohort study in Leyte, the Philippines, among 611 *Schistosoma japonicum*-infected participants 7–30 years old, all of whom were treated with praziquantel at baseline. To detect hepatic fibrosis, abdominal ultrasound was performed at baseline and 12 months after treatment. Stool for assessment of *S. japonicum* infection was collected at baseline and at 3, 6, 9, and 12 months after treatment. Cytokines (interleukin [IL]–4, IL-5, IL-10, IL-13, tumor necrosis factor– $\alpha$ , and interferon– $\gamma$ ) produced by peripheral-blood mononuclear cells in response to soluble worm antigen preparation (SWAP), soluble egg antigen (SEA), and control medium were measured once 4 weeks after treatment. IL-4 to SWAP and IL-10 to both SWAP and SEA were associated with the presence of baseline fibrosis after adjustment for potential confounding variables ( $P < .03$ , for all). In participants with fibrosis at baseline, IL-4 to SWAP and IL-5 and IL-13 to both SWAP and SEA were associated with persistent fibrosis at 12 months after treatment ( $P < .05$ , for all). Males showed consistently stronger T helper 2 (Th2) cytokine responses to both SWAP and SEA than did females ( $P < .02$ , for all). These results suggest an independent role for Th2-biased cytokine responses to *S. japonicum* antigens in persistent hepatic fibrosis and indicate that Th2 cytokines may contribute to the male-biased prevalence of fibrosis.

Hepatic fibrosis is among the most serious consequences of chronic schistosomiasis and develops in a minority of those chronically infected. Parasite eggs trapped in hepatic sinusoids evoke a granulomatous inflammatory response that leads to excessive accumulation of extracellular matrix proteins and, subsequently, fibrosis [1, 2]. A dysregulated host immune response, leading to an imbalance between fibrogenesis and fibrolysis, is thought to be the main underlying mechanism [3, 4]. Treatment with praziquantel can lead

to the reversal of hepatic fibrosis within 7 months [5], especially in younger subjects, although this is uncommon once fibrosis is severe [6–8].

Despite the abundance of studies of the pathogenesis of schistosomiasis-associated hepatic fibrosis, our understanding of the immunological mechanisms involved remains incomplete. In both murine and human studies, Th2 cytokines (interleukin [IL]–4, IL-5, and IL-13) have been associated with an increased risk of fibrosis [3, 9–11], and IL-10 is thought to play a critical immunoregulatory role [3, 12]. However, human studies have suggested an important role for proinflammatory and Th1 cytokines: tumor necrosis factor (TNF)– $\alpha$  and interferon (IFN)– $\gamma$  have been associated with an increased and a decreased risk of fibrosis, respectively [10, 12–14]. Furthermore, different cytokine profiles may be involved in the development of fibrosis in children, males, and females [12]. Inconsistent criteria and measurement error in the diagnosis of hepatic fibrosis as well as methodological differences in study design, cytokine measurement, and analysis limit the

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comparability of these studies. In addition, it is unknown whether the results of these studies, performed in *Schistosoma mansoni*-infected subjects, can be generalized to hepatic fibrosis in *S. japonicum* infection, which may be more severe due to the higher egg production and clustered egg excretion [15].

Community-based studies indicate that schistosome-infected males are more likely to develop hepatic fibrosis than females [11, 13, 14, 16], possibly due to their higher prevalence and intensity of infection associated with physical, behavioral, and/or genetic factors [16–18]. Immunological differences between the sexes, resulting from the immunomodulatory effects of sex steroids, could also be involved in this increased risk of fibrosis in males [19]. Females typically produce more vigorous cellular and humoral immune responses, which may explain both the lower prevalence of several parasitic diseases and the higher prevalence of many autoimmune diseases in females than in males [19]. However, an opposite pattern would be expected if sex-specific immune responses play a role in the differential prevalence of schistosomiasis-associated hepatic fibrosis, which is induced and maintained by the host immune response to parasite eggs [3].

The objectives of the present study were to (1) assess whether cytokines (IL-4, IL-5, IL-10, IL-13, TNF- $\alpha$ , and IFN- $\gamma$ ) produced by peripheral-blood mononuclear cells (PBMCs) in response to specific *S. japonicum* antigens were associated with hepatic fibrosis at baseline and/or at 1 year after treatment; (2) assess, in a subgroup of our cohort with hepatic fibrosis at baseline, whether these cytokine responses differentiated subjects in whom fibrosis reversed from those in whom it remained or progressed; and (3) detect potential sex differences in these cytokine responses that may be related to the higher prevalence of hepatic fibrosis in males.

## METHODS

**Study site and population.** This longitudinal treatment-reinfection study, designed to assess immunological mechanisms of resistance to reinfection, was conducted in 3 *S. japonicum*-endemic rice-farming villages in Leyte, the Philippines, where malaria is not endemic. In total, 74.3% (1262/1699) of individuals 7–30 years old residing in the study villages were screened for the presence of *S. japonicum* infection by duplicate examination of 3 stool specimens before enrollment. The prevalence of infection with *S. japonicum* in this age range was 60.0%. Participants were enrolled during 2 periods, in October 2002 and April 2003. For the main study, individuals were eligible if they were infected with *S. japonicum*; lived primarily in a study village; were between 7 and 30 years old; were not pregnant or lactating; had no severe fibrosis (grade II or III), severe wasting (defined as a body mass index less than the third percentile of a reference population of the same age and sex derived from the US National Health and Anthropometric Ex-

amination Survey), or severe anemia (hemoglobin level <7 g/dL); and provided adult consent if >18 years old or both child assent and parental consent otherwise. For the present analyses, ultrasound evaluation at the start of the study (baseline) was an additional participation criterion. Enrolled participants ( $n = 611$ ) were treated with praziquantel (60 mg/kg in a split dose) at baseline. The institutional review boards of Brown University and the Philippines Research Institute of Tropical Medicine approved the study. All *S. japonicum*-reinfected participants were treated after 18 months of follow-up, after completion of the longitudinal study.

**Stool examination.** At baseline and at 3, 6, 9, and 12 months after treatment, egg counts were determined by duplicate examination of 3 consecutive stool specimens obtained from each study participant by the Kato-Katz method. For each stool specimen, the average eggs per gram (epg) of the duplicate test was determined, and the overall mean eggs per gram was derived by averaging the egg count of the 3 individual specimens. Stool specimens negative for *S. japonicum* eggs after reinfection were considered to be misclassified and were imputed using the last-value-carried-forward method [20]. Intermittent missing stool specimens were imputed using the same method [20]. Cumulative egg count after treatment was defined as the sum of the egg counts at 3, 6, 9, and 12 months of follow-up. Intensity of infection was determined by use of World Health Organization criteria: low, moderate, and high intensity infections were defined as 1–99, 100–399, and  $\geq 400$  egg, respectively [21].

**Ultrasound evaluation.** At baseline and at 12 months of follow-up, study participants were evaluated by ultrasound by 2 observers (J.D.K. and R.M.O.) using a EUB-200 device with a 3.5-MHz probe (Hitachi). Both observers were blinded to the cytokine status of participants. Liver size was measured as the size of the left liver lobe in centimeters in the right parasternal line. Spleen size was measured in centimeters in the left intercostal oblique view. Reference measurements for liver and spleen size among healthy Filipinos were not available; hence, height-specific normal values from a healthy Chinese reference population were used [22]. Hepatomegaly and splenomegaly were defined as >2 SDs above the mean. Grading of hepatic fibrosis (grade I, II, or III) was based on a modification of the grading system described by Doehring-Schwerdtfeger et al. [23]. Severe fibrosis (grade II or III) was an exclusion criterion for participation in the study; thus, only participants with no or grade I fibrosis were included at baseline. Persistent fibrosis was defined as the presence of fibrosis both at baseline and follow-up. Reversible fibrosis was defined as the presence of fibrosis at baseline but not at follow-up.

**PBMC collection and *S. japonicum* antigens.** Four weeks after treatment, venipuncture was performed, and blood was collected into Vacutainer tubes (Becton Dickinson) containing

**Table 1. Descriptive characteristics of the cohort at baseline and at 12 months of follow-up.**

Variable	Baseline ( <i>n</i> = 611)	12 months ( <i>n</i> = 437)
Female sex	225 (36.8)	159 (36.4)
Age, mean (95% CI), years	15.6 (15.1–16.1)	16.0 (15.4–16.6)
<i>Schistosoma japonicum</i> egg count, geometric mean (95% CI), egg	42.9 (38.4–47.9)	15.4 (12.8–18.5)
<i>S. japonicum</i> intensity		
Uninfected	...	76 (17.4)
Low	431 (70.5)	288 (66.1)
Moderate	144 (23.6)	55 (12.6)
High	36 (5.9)	17 (3.9)
Presence of fibrosis	41 (6.7)	89 (20.4)
Fibrosis grade <sup>a</sup>		
I	41 (100)	79 (88.8)
II	...	9 (10.1)
III	...	1 (1.1)
Liver size, <sup>b</sup> mean (95% CI), cm	7.1 (7.1–7.2)	6.9 (6.8–7.0)
Hepatomegaly <sup>c</sup>	78 (12.9)	41 (10.0)
Splenomegaly <sup>c</sup>	2 (0.3)	2 (0.5)

**NOTE.** Data are no. (%) of participants, unless otherwise indicated. CI, confidence interval; egg, eggs per gram.

<sup>a</sup> Grading of hepatic fibrosis was based on a modification of the grading system described by Doehring-Schwerdtfeger et al. [23]. Percentages are of the no. of participants with fibrosis.

<sup>b</sup> Liver size was measured as size of the left liver lobe in centimeters in the right parasternal line.

<sup>c</sup> Hepatomegaly and splenomegaly were defined as >2 SDs above the mean of a healthy Chinese reference population.

heparin as anticoagulant. PBMCs were isolated and placed in culture within 4 h of collection, as described elsewhere [24]. Soluble egg antigen (SEA) and soluble worm antigen preparation (SWAP) were prepared as described elsewhere [25, 26]. PBMCs were stimulated with SWAP, SEA, phytohemagglutinin (PHA), and control medium, as described elsewhere [24]. All specimens produced detectable IFN- $\gamma$  in response to PHA.

**Cytokine assays.** All specimen identification and pipetting was performed by a bar-code-enabled, high-speed pipetting robot (Tecan). Cytokine assays were performed on culture supernatants by means of a multiplexed, bead-based platform and custom assay kits, as described elsewhere [24]. The assay kits demonstrated 4% interanalyte interference, and the median interassay coefficient of variation for all analytes, as assessed by use of 40 replicate high-concentration controls, was 14.3%. The lower limit of detection was consistently <2.4 pg/mL for all cytokines. In some analyses, cytokine levels were dichotomized into low and high responses on the basis of the mean of the distribution.

**Statistical analyses.** Variables that were not normally distributed (*S. japonicum* egg count and all cytokine responses) were log<sub>e</sub> transformed [ $\ln(n + 1)$ ]. Multilevel statistical analyses for dichotomous and continuous outcomes were performed using Proc GenMod and Proc Mixed, respectively, in SAS (ver-

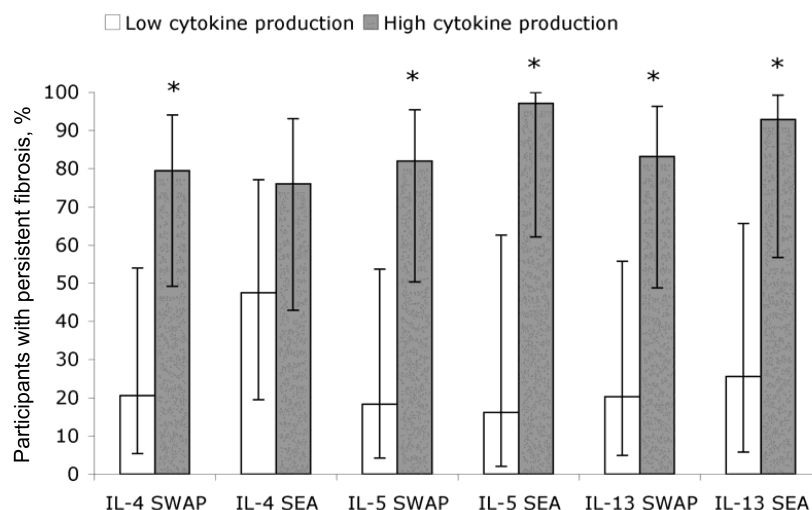
sion 9.1; SAS Institute).  $P < .05$  was considered to be statistically significant.

For dichotomous outcomes, we used generalized estimating equation models [27]; for continuous outcomes, we used random effect models. In both cases, we adjusted for clustering at the household level and used empirical SEs. For subgroup analyses of participants with baseline fibrosis, the small sample size precluded adjustment for household-level clustering.

All models evaluating the association between cytokine responses and fibrosis were adjusted for unstimulated cytokine production [24], sex, age, and baseline *S. japonicum* egg count. Models evaluating fibrosis at follow-up were also adjusted for the presence of fibrosis at baseline and cumulative egg count after treatment. Models evaluating the association between sex and cytokine responses were adjusted for baseline egg count and age.

## RESULTS

**Descriptive characteristics.** Table 1 presents descriptive characteristics of all participants at baseline ( $n = 611$ ) and of those available for ultrasound at 12 months of follow-up ( $n = 437$ ). Sex, age, mean *S. japonicum* egg count, liver size, and prevalence of fibrosis at baseline were not significantly different between



**Figure 1.** Adjusted prevalence of persistent fibrosis for participants with low and high cytokine responses. Cytokine levels were dichotomized into low and high responses in participants with baseline fibrosis and a follow-up ultrasound ( $n = 29$ ). Persistent and reversible fibrosis were present in 16 and 13 participants, respectively. White and gray bars represent low and high cytokine responses, respectively. Error bars represent 95% confidence intervals. An asterisk indicates a significant difference between low and high cytokine responses ( $P < .05$ ). Results are based on logistic regression analyses. All models were adjusted for sex, age, baseline *Schistosoma japonicum* egg count, cumulative egg count after treatment, and unstimulated cytokine response. IL, interleukin; SEA, soluble egg antigen; SWAP, soluble worm antigen preparation.

the participants in whom a follow-up ultrasound was performed (71.5%) and those in whom it was not (28.5%) (data not shown).

At baseline, 41 participants (6.7%) had grade I hepatic fibrosis. Of these participants, the fibrosis grade remained unchanged at 12 months of follow-up in 11 participants, progressed in 5, and reversed in 13. For 12 of the 41 participants, no follow-up ultrasound was available. Sex, age, mean *S. japonicum* egg count, and liver size at baseline were not significantly different between these participants and those with baseline fibrosis and a follow-up ultrasound. The prevalence of grade I fibrosis increased from 6.7% at baseline to 18.1% at 12 months of follow-up. Of the latter group, 68 (86.1%) of 79 had no fibrosis at baseline, and 82.3% were reinfected at 12 months.

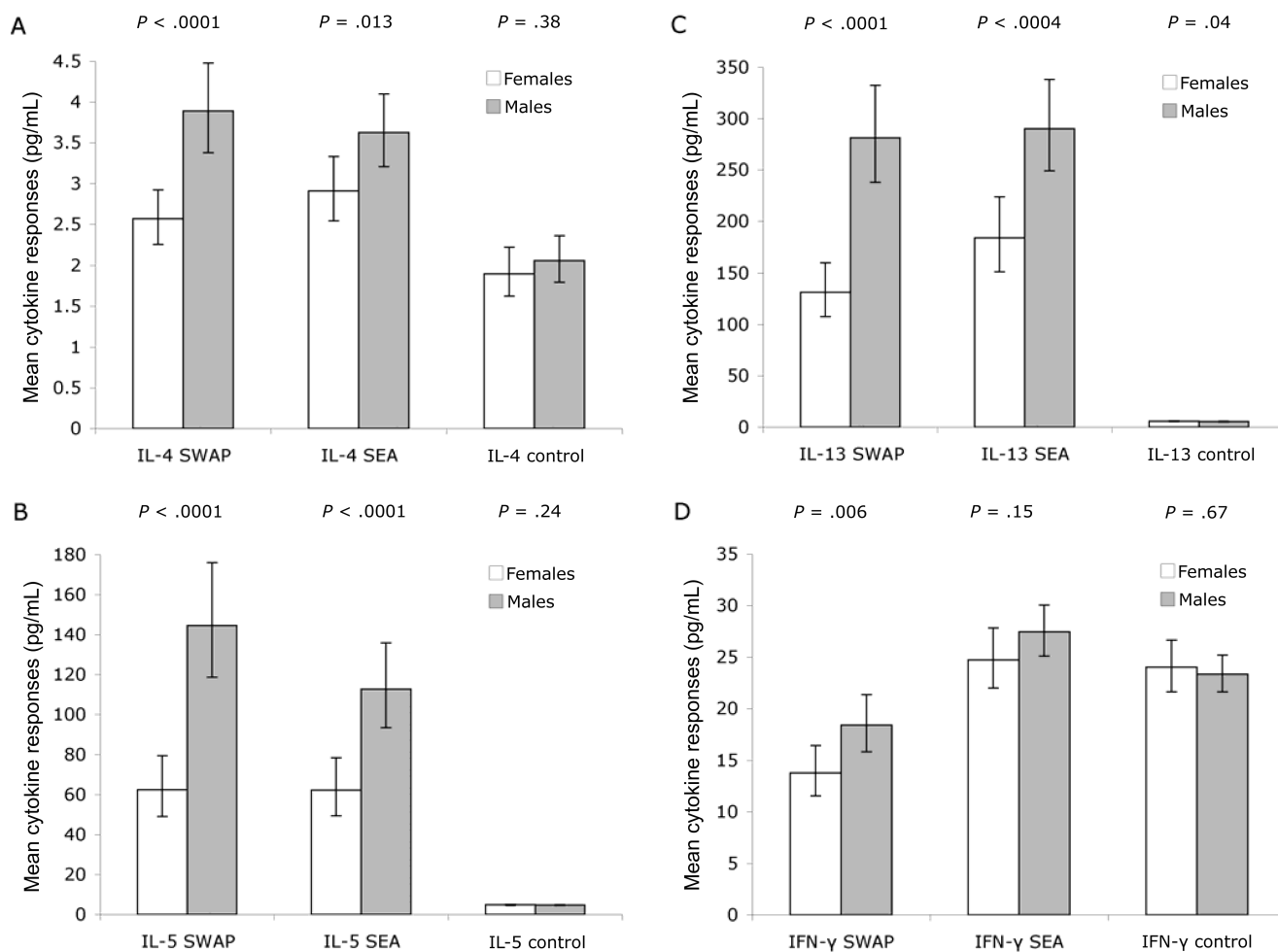
**Associations between cytokine responses and fibrosis in the baseline and follow-up cohorts.** We first evaluated whether cytokines produced by PBMCs in response to SEA and SWAP were associated with grade I fibrosis in the baseline cohort ( $n = 611$ ). Production of IL-4 in response to SWAP and of IL-10 in response to both SWAP and SEA were associated with the presence of fibrosis at baseline ( $\beta$  coefficient, 0.32 [ $P = .020$ ], 0.57 [ $P = .024$ ], and 0.76 [ $P = .026$ ], respectively), independent of sex, age, unstimulated cytokine production, and baseline *S. japonicum* egg count.

Next, we evaluated whether cytokine responses, measured at 4 weeks after treatment, predicted the presence of any-grade fibrosis in the follow-up cohort ( $n = 437$ ). Models were adjusted for sex, age, unstimulated cytokine production, baseline

egg count, cumulative egg count after treatment, and the presence of fibrosis at baseline. No significant associations were found for continuous cytokine responses. After cytokine levels were dichotomized into low and high responses, low IFN- $\gamma$  responses to SEA and high TNF- $\alpha$  responses to SWAP showed a trend toward an association with the presence of fibrosis at follow-up ( $\beta$  coefficient, 0.53 [ $P = .063$ ] and 0.63 [ $P = .059$ ], respectively).

**Associations between cytokine responses and fibrosis in subgroup analyses.** In participants with grade I fibrosis at baseline, we evaluated the relationship between cytokine production and fibrosis status at follow-up. Models were adjusted for sex, age, unstimulated cytokine production, baseline egg count, and cumulative egg count after treatment. IL-4 responses to SWAP and IL-5 and IL-13 responses to both SWAP and SEA were associated with persistent fibrosis (all  $P$  values were  $>.01$  and  $<.05$ ). Cytokine levels in these participants ( $n = 29$ ) were dichotomized into low and high responses. Figure 1 shows the adjusted prevalence of persistent fibrosis among participants with low and high IL-4, IL-5, and IL-13 responses to both SWAP and SEA.

Because of the 2.7-fold increase in the prevalence of grade I fibrosis at follow-up, we also evaluated whether cytokine responses in participants with grade I fibrosis at follow-up who had no baseline fibrosis (new grade I fibrosis;  $n = 68$ ) were different than participants who had no fibrosis during the study period ( $n = 335$ ). Models were adjusted for sex, age, unstimulated cytokine production, baseline egg count, and cumulative egg count after treatment. No significant differences were found



**Figure 2.** Sex-specific adjusted means of cytokine responses to soluble worm antigen preparation (SWAP), soluble egg antigen (SEA), and control medium. Shown are the least square means of interleukin (IL)–4 (A), IL–5 (B), IL–13 (C), and interferon (IFN)– $\gamma$  (D) production. White and gray bars represent geometric mean cytokine levels in females and males, respectively. Error bars represent 95% confidence intervals. The no. of females/males for analyses with SWAP, SEA, and control medium were 195/341, 206/342, and 212/357, respectively. Results are based on multivariate linear regression analyses adjusted for age, baseline *Schistosoma japonicum* egg count, and household-level clustering.

for continuous cytokine responses. After cytokine levels were dichotomized into low and high responses, low IFN- $\gamma$  responses to SEA were associated with new grade I fibrosis ( $\beta$  coefficient, 0.63 [ $P = .044$ ]), and high IL-10 responses to SEA showed a trend toward an association with new grade I fibrosis ( $\beta$  coefficient, 0.70 [ $P = .066$ ]). Of note, neither the presence/absence of reinfection at 3 or 12 months after treatment nor the cumulative egg count after treatment was associated with the presence of new grade I fibrosis.

**Sex differences in the prevalence of fibrosis and cytokine responses.** Among participants with baseline and follow-up fibrosis, 7 (17.1%) of 41 and 17 (19.1%) of 89, respectively, were female. Severe fibrosis at follow-up was detected in 1 female and in 9 males. For males compared with females, the odds ratios for fibrosis at baseline and follow-up were 2.44 (95% confidence interval [CI], 1.05–5.67;  $P = .038$ ) and 2.49

(95% CI, 1.32–4.70;  $P = .005$ ), respectively, independent of *S. japonicum* egg count and age. To assess whether differences in cytokine responses play a role in this differential prevalence of fibrosis, we evaluated whether sex predicted cytokine responses to SWAP, SEA, and control medium, independent of baseline egg count and age. Males had significantly stronger Th2 responses to both SWAP and SEA than did females (figure 2). Surprisingly, the production of IFN- $\gamma$  in response to SWAP was 1.4-fold higher in males ( $P = .006$ ), but no significant sex difference was observed in the production of IFN- $\gamma$  in response to SEA or control medium.

## DISCUSSION

In the present study of *S. japonicum*-infected children, adolescents, and young adults, we evaluated associations between

*S. japonicum*-specific cytokine responses (measured 4 weeks after treatment with praziquantel) and hepatic fibrosis. The production of IL-4 in response to SWAP and of IL-10 in response to both SWAP and SEA were positively associated with grade I fibrosis at baseline. In addition, in participants with grade I fibrosis at baseline, the production of IL-4 in response to SWAP and of IL-5 and IL-13 in response to both SWAP and SEA were positively associated with persistent fibrosis 1 year after treatment with praziquantel. This is the first human study to assess associations between antigen-specific cytokines and hepatic fibrosis in *S. japonicum* infection. Similar associations have been described in human *S. mansoni* infection [9, 11]. Our findings were independent of potential confounding variables of the relationship between cytokine responses and fibrosis: sex, age, constitutive cytokine response, and *S. japonicum* egg count. Importantly, this suggests that the host cytokine response to *S. japonicum* is an important determinant of fibrosis and its potential reversibility with treatment, independent of egg burden.

Consistently higher Th2 responses to *S. japonicum* antigens were found in males compared with females. This finding is in accord with those of human studies in *S. mansoni*- and *S. japonicum*-infected subjects, which showed higher IgG4 and IgE antibody responses to worm antigen in males than in females, independent of age and intensity of infection [18, 28]. Furthermore, our finding supports an immunological basis for the higher prevalence of hepatic fibrosis in males. In naive mice, schistosome eggs trapped in the liver typically induce a shift from an initial Th1 response to a Th2 response with chronic infection. Dysfunctional regulation of this Th2 response contributes to the development of hepatic fibrosis [3]. In murine schistosomiasis, myeloid differentiation factor 88-deficient mice, which are incapable of mounting an antigen-specific Th1 response, have smaller but more fibrotic hepatic granulomas, and a trend toward elevated hepatic IL-13 levels is also observed [29]. IL-13, which directly and indirectly stimulates collagen production by fibroblasts [4], has been shown to be particularly important in the development and progression of hepatic fibrosis [2–4, 11]. Moreover, a recent study found that IL-13 receptor  $\alpha$ , a decoy receptor that blocks IL-13 function, is a critical mediator of immune down-modulation in chronic schistosomiasis. Elevated serum levels of this receptor have been found in *S. mansoni*-infected subjects relative to those in uninfected control subjects, with higher levels in subjects with higher intensity infections [30]. Taken together, our findings indicate that the profibrotic cytokines IL-4, IL-5, and IL-13 play an independent role in the persistence of *S. japonicum*-associated hepatic fibrosis and may explain the higher prevalence of fibrosis in males.

Human studies have linked IFN- $\gamma$  with protection against fibrosis [13, 14], and, in line with this, we found an association

between low egg-induced IFN- $\gamma$  production and the development of grade I fibrosis at follow-up. Surprisingly, males had higher levels of worm-induced IFN- $\gamma$ , compared with those in females, yet had 2.4-fold increased odds of having fibrosis at baseline than females. We speculate that the potential protective effect of IFN- $\gamma$  was attenuated by the strong parasite-specific profibrotic Th2 response in males. The positive association between both egg- and worm-induced IL-10 and grade I fibrosis in the present study, which has also been described for severe fibrosis in *S. mansoni* infection [9], may reflect a counterregulatory mechanism; IL-10 has been implicated in the control of both Th1 and Th2 responses and is considered to be a host-protective cytokine during schistosomiasis [3]. In contrast to our findings, Booth et al. [12] found an association between low IL-10 production and hepatic fibrosis in children and suggested this may be related to the immunoregulatory role played by IL-10. These conflicting results may reflect differences in study populations and designs as well as in methods of fibrosis and cytokine assessment, or they may represent distinct regulatory mechanisms operating in *S. mansoni*- versus *S. japonicum*-infected individuals.

Although hepatic fibrosis is induced by the host response to schistosome eggs [1, 3], we also found consistent associations between worm-induced cytokine production and fibrosis. Treatment results in the exposure of previously masked worm proteins to the host immune system [1]. We measured specific cytokine responses 4 weeks after treatment; thus, cross-reactivity between worm and egg antigens, which display significant similarity [31], may explain these findings.

Surprisingly, a 2.7-fold increase in the prevalence of grade I fibrosis was found at follow-up relative to baseline; 86% of participants with fibrosis at follow-up did not have fibrosis at baseline. A potential explanation for this observation may be rebound morbidity—the exacerbation of morbidity after chemotherapy and rapid reinfection, as has been described for hepatomegaly [32]. This would suggest a potential adverse effect of treatment if rapid reinfection occurs. Paradoxically, neither reinfection (including within 3 months after treatment) nor cumulative egg count after treatment was associated with the presence of new grade I fibrosis. Insufficient sensitivity of egg counts despite duplicate examination of 3 stool specimens may be responsible for this lack of association. Alternatively, persistence of egg antigen in cured individuals may lead to continued stimulation of profibrotic cytokines.

The limitations of the present study need to be addressed. First, we did not collect data on viral hepatitis or alcohol use, both of which may influence the progression of hepatic fibrosis [33, 34]. Approximately 12% of the rural population of the Philippines is a carrier of hepatitis B virus [35], and hepatitis C antibodies were found in 2.2% of Filipino blood donors [36]. Alcohol intake in rural communities in the Philippines is high,

but it is currently unknown to what extent this contributes to the development of liver disease (R.M.O., unpublished data). Nonetheless, it is unlikely that these factors are related to both hepatic fibrosis and production of *S. japonicum*-specific cytokines, which limits the possibility of residual confounding. Second, our study did not include data on genetics, an important determinant of hepatic fibrosis. Genetic polymorphisms in the genes for IFN- $\gamma$  and IFN- $\gamma$  receptor 1 as well as in the gene for IL-13 have been associated with severe hepatic fibrosis in *S. mansoni* and *S. japonicum* infection, respectively [14, 37, 38]. In addition, specific HLA class II alleles have been identified that are associated with both increased and decreased risk of developing hepatic fibrosis in *S. japonicum* infection [38, 39]. Importantly, adjusting our analyses for clustering at the household level likely reduced the variation in fibrosis that can be explained by inherited factors. Third, participants with severe fibrosis at baseline were excluded for ethical reasons, reducing the variability of our outcome (fibrosis) but possibly also that of our predictor variables (cytokine responses). This may have introduced selection bias and resulted in a type II error, limiting our ability to detect differences in cytokine responses. Finally, our analyses of persistent fibrosis were based on a small number of individuals. Nevertheless, we did find significant differences in Th2 cytokine production between participants whose fibrosis remained and those whose fibrosis regressed at follow-up.

Recently, we described a protective effect of Th2-biased host responses to specific *S. japonicum* antigens in limiting the intensity of reinfection after praziquantel treatment in this cohort [24]. These data were broadly concordant with earlier observations in *S. mansoni* and *S. hematobium* infection [40, 41]. Taken together, these results, along with evidence implicating IgE responses in mediating protective immunity [42], have focused vaccine-development efforts on generating Th2 responses to limit the intensity of infection. In the context of our present findings implicating Th2 responses to crude worm and egg antigens in mediating hepatic fibrosis, concern regarding enhanced hepatic morbidity accompanying a Th2-boosting vaccine is warranted. Careful selection of vaccine candidates and adjuvants to avoid these deleterious consequences will require an assessment of hepatic morbidity in vaccinated infected animals before phase 2 trials in humans.

In conclusion, our results suggest an independent role for Th2-biased cytokine responses to *S. japonicum* antigens in the persistence of hepatic fibrosis and indicate that Th2 cytokines may play a role in the higher prevalence of fibrosis in males.

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