

Increases in Human T Helper 2 Cytokine Responses to *Schistosoma mansoni* Worm and Worm-Tegument Antigens Are Induced by Treatment with Praziquantel

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Levels of *Schistosoma mansoni*-induced interleukin (IL)-4 and IL-5 and posttreatment levels of immunoglobulin E recognizing the parasite's tegument (Teg) correlate with human resistance to subsequent reinfection after treatment. We measured changes in whole-blood cytokine production in response to soluble egg antigen (SEA), soluble worm antigen (SWA), or Teg after treatment with praziquantel (PZQ) in a cohort of 187 individuals living near Lake Albert, Uganda. Levels of SWA-induced IL-4, IL-5, IL-10, and IL-13 increased after treatment with PZQ, and the greatest relative increases were seen in the responses to Teg. Mean levels of Teg-specific IL-5 and IL-10 increased ~10–15-fold, and mean levels of IL-13 increased ~5-fold. Correlations between the changes in cytokines suggested that their production was positively coregulated by tegumentally derived antigens. Levels of SEA-, SWA-, and Teg-induced interferon- γ were not significantly changed by treatment, and, with the exception of IL-10, which increased slightly, responses to SEA also remained largely unchanged. The changes in cytokines were not strongly influenced by age or intensity of infection and were not accompanied by corresponding increases in the numbers of circulating eosinophils or lymphocytes.

Schistosomiasis affects ~200 million people in the developing world and causes chronic morbidity in populations living in areas where the disease is endemic [1]. The disease is caused by infection with blood-dwelling worms of the genus *Schistosoma*, which infect humans after contact with water containing skin-penetrating parasite larvae. Epidemiological studies in areas of endemicity have shown that, in general, adults have lower intensities of infection than do children, sug-

gesting that resistance to infection develops with age, manifesting around the age of puberty [2]. Reinfection studies, in which the acquisition of new infections is monitored after treatment, have shown that adults become reinfected far less often than children, even in fishing communities where adults have the greatest exposure to the parasite [3]. The measurement of parasite-specific immune responses near the time of treatment has identified correlates of low intensities of reinfection. These include eosinophilia (*Schistosoma haematobium* [4]), parasite-specific IgE (*S. haematobium* [5], *Schistosoma mansoni* [6, 7], and *Schistosoma japonicum* [8]), and the production of interleukin (IL)-4 and IL-5 by peripheral blood mononuclear cells stimulated in vitro with parasite antigens (Ags) (*S. haematobium* [9] and *S. mansoni* [10]). Such reinfection studies have led to the consensus that schistosome-specific T helper 2 (Th2) responses are associated with, and perhaps directly involved in, human age-dependent immunity to reinfection after treatment.

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In areas of Kenya where transmission of *S. mansoni* is seasonal, immunological correlates of resistance to reinfection were strongest when parasite-specific immune responses were measured after treatment with praziquantel (PZQ) but before exposure to reinfection had occurred [7]. In that Kenyan study, posttreatment levels of adult worm-specific IgE correlated negatively with the intensity of reinfection [7], providing evidence for the hypothesis that drug-dependent killing of worms in situ might boost protective immunity against reinfection by immunizing with Ags released from dying worms [11, 12]. Adult *S. mansoni* worms in infected Kenyan populations have been estimated to have a mean life span of 6–10 years [13]; thus, in such areas of endemicity, exposure to Ags from naturally dying worms might be expected to be age dependent in untreated infected populations. It follows that the slow development of age-dependent immunity in such areas where schistosomiasis is endemic might arise as the result of the cumulative exposure to host-protective Ags derived from naturally dying worms.

The tissues of schistosome-infected individuals are simultaneously and chronically exposed to 2 distinct developmental stages of the parasite: the egg and the adult worm. Hundreds of eggs are released into the bloodstream of the host daily by each female worm. In studies in Kenya, posttreatment levels of soluble worm Ag (SWA)-specific IgE, but not soluble egg Ag (SEA)-specific IgE, were found to be associated with resistance to subsequent reinfection [7]. The interface between the adult worm and its environment occurs at its outer tegument (Teg), a highly specialized organelle with a syncytial structure that forms a highly responsive outer layer over the entire surface of the worm. The Teg can be isolated from live intact worms, and high posttreatment IgE responses against this worm substructure are also predictive of low levels of reinfection [7]. Western blots of parasite extracts, by use of serum samples obtained from chronically infected Kenyans, identified an ~22-kDa Ag located in the Teg of *S. mansoni* as the predominant target of *S. mansoni*-specific IgE responses in humans [7]. This tegumental Ag has been identified as a homologue of cytoplasmic dynein light chain [14], and IgEs recognizing either the purified native Ag (Sm22) [15] or a bacterially expressed recombinant form (rSm22.6) [16] were significantly correlated with resistance to reinfection after treatment. In addition, immunization with preparations of the Teg has been shown to induce some protection against experimental *S. mansoni* infections in mice [17, 18].

It is known that PZQ rapidly and specifically disrupts the schistosome Teg, exposing hitherto sequestered parasite Ags to the host immune system [19, 20]. In fact, the killing of schistosomes by PZQ in vivo depends, at least in part, on the integrity of the host immune system, and efficacy of PZQ may depend on host immune responses against newly exposed tegumental Ags [21, 22]. The Teg is crucial to the survival of schistosomes in the bloodstream, but, despite important in-

teractions between the Teg, the host immune system, and PZQ in vivo, human cellular responses with specificity for this structure have not been previously described. We therefore examined the effects of treatment with PZQ on the cytokine responses to the Teg of *S. mansoni* in a cohort of 187 Ugandans living in a fishing community who were exposed to high levels of parasite transmission. We assessed changes in the levels of IL-4, IL-5, IL-10, IL-13, and interferon (IFN)- γ in whole-blood cultures in response to stimulation in vitro with *S. mansoni* Teg before and 7 weeks after treatment and compared them with changes in responses to either homogenized SEA or SWA. We observed significant increases in several Th2 cytokines in response to Teg and SWA in this area where *S. mansoni* is endemic.

SUBJECTS, MATERIALS, AND METHODS

Study population. A study cohort was selected from the fishing village of Booma (in the parish of Butiaba), which is adjacent to Lake Albert, Masindi district, in northwestern Uganda. One hundred eighty-seven individuals (in equal numbers from all age groups and of both sexes) were selected on the following criteria: (1) they lived close to the lake shore, (2) they were 7–50 years old, (3) they had resided in the village for at least 10 years or since birth, and (4) they agreed to participate and to donate blood samples before and 7 weeks after treatment. Informed consent was obtained from all participants in this study, in accordance with the national guidelines of the Ugandan Ministry of Health, whose ethical review committees approved all the protocols used. Before treatment, 3 stool samples were obtained on consecutive days from each member of the study cohort. For each stool sample, 2 Kato slides (50 mg) were prepared and examined, and *S. mansoni* eggs were counted. The intensity of *S. mansoni* infection for the cohort members varied from 0 to 8100 eggs/gram of feces, with an estimated prevalence of infection of 94%. After donating a blood sample, each cohort member was treated immediately with PZQ (40 mg/kg of body weight) and was treated again 2 weeks later; an additional blood sample was obtained 7 weeks after the first treatment. At the parasitological examinations 7 weeks after treatment, 20% of the cohort members were excreting at least 1 egg. Overall, the burden of infection was reduced by 99.7%.

Whole-blood cultures. Venous blood was collected into heparin, at a concentration of 10 U/mL (Sigma), and was diluted immediately 1:4 in RPMI 1640 medium (Sigma) supplemented with penicillin (50 U/mL), streptomycin (50 μ g/mL), and L-glutamine (2 mmol/L) (Sigma). Whole-blood cultures were set up as described elsewhere [23], and parasite-derived Ags were added to the appropriate wells, to a final concentration of 10 μ g/mL. Cultures were stimulated with SEA, SWA, or Teg or were left unstimulated. Levels of IL-4 were measured in supernatants from 48-h cultures, and levels of IL-5, IL-10, IL-

13, and IFN- γ were measured in supernatants from 120-h cultures. The supernatants were immediately frozen at -20°C and were transported frozen to the United Kingdom. Subsequently, the culture supernatants were thawed and treated with inactive enveloped viruses, by incubation for 2 h in the presence of 0.3% tri-butyl phosphate (Sigma) and 1% Tween 80 (Sigma). Subsequently, the supernatants were either assayed for cytokines immediately or were stored at -70°C . None of the cytokine assays was affected by the viral inactivation procedure (data not shown). No supernatant sample went through >2 cycles of freezing and thawing before being assayed for cytokine content. Samples were diluted as required to ensure that the assay values were within the linear part of the standard curve for that assay.

Parasite Ags. The PBS-soluble fractions of *S. mansoni* SWA and SEA homogenates were prepared according to established protocols [16]. The schistosome outer Teg was stripped from freshly isolated worms by incubation in PBS for 1 h at room temperature and then was collected from the supernatant [23]. In the whole-blood cultures, all Ags were used at a final concentration of $10\ \mu\text{g}/\text{mL}$. Before use, the Ags were titrated in vitro by use of murine splenocytes from infected mice. Endotoxin content was measured by use of a limulus amoebocyte lysate kit (QCL-1000; BioWhittaker). The levels of endotoxin in the native Ag used in these studies were $1.44\ \text{ng}$ of endotoxin/mg of SEA, $1.23\ \text{ng}$ of endotoxin/mg of SWA, and $1.09\ \text{ng}$ of endotoxin/mg of Teg. This level of lipopolysaccharide did not induce detectable levels of any of the tested cytokines when added to our whole-blood cultures (data not shown).

Cytokine assays. Cytokines were measured using capture ELISAs and commercially available anticytokine antibodies (Abs) and standards from BD Biosciences, as described elsewhere [23]. All Abs were purchased from BD Biosciences and were titrated for optimal concentration before use. For the cytokine assays, the following Ab pairs were coated on microtiter plates to capture target cytokines or were used to detect captured cytokines (IL-4, coating 8D4-8 and detection MP4-25D2; IL-5, coating TRFK5 and detection JRS1-5A10; IL-10, coating JES3-9D7 and detection JES3-12G8; IL-13, coating JES10-5A2 and detection B69-2; and IFN- γ , coating NIB42 and detection 45B3).

Statistical analyses. All the analyses and graphs were performed in R, an open-source language and environment for statistical computing and graphics (available at: <http://www.r-project.org>). Paired *t* tests were used to assess whether responses had changed significantly between the 2 blood samples and were performed using nontransformed data. Correlations between variables were determined by calculating Spearman's rank correlation coefficient for each pair of immune responses, again using nontransformed data. The approximate percentage increase in responses after treatment with PZQ for all study subjects was determined using the following calculation: $100 \times (\exp\{\text{mean}[\ln(B+1) - \ln(A+1)]\} - 1)$. Effects of sex, age, and in-

tensity of infection on changes in cytokine responses were examined by analysis of variance on log-transformed cytokine responses, $\log(+0.1)$. The adequacy of the models was checked using variance ratios, which under the null hypothesis, follow an F distribution using appropriate *df*. The model fit was also checked by examining appropriate plots, and results were confirmed by Spearman's rank correlation coefficients on the raw data.

RESULTS

Effects of treatment with PZQ on production of cytokines.

Figure 1 shows the mean level of production of cytokines by diluted whole blood stimulated with SEA, SWA, or Teg or unstimulated, before and then again 7 weeks after treatment with PZQ. Treatment with PZQ had no significant effect on the mean levels of IL-4, IL-5, IL-10, IL-13, or IFN- γ produced in culture in the absence of Ag stimulation (figure 1A–1E). The mean levels of IL-4, IL-5, and IL-13 in response to SWA and Teg increased significantly after treatment, whereas the mean levels of these cytokines in response to SEA did not change significantly (figure 1A, 1B, and 1D). By contrast, the mean levels of IL-10 seen in response to all *S. mansoni*-derived Ags (SEA, SWA, and Teg) increased significantly during the 7 weeks after treatment (figure 1C). The mean levels of production of IFN- γ in response to SEA, SWA, and Teg were not significantly altered by treatment (figure 1E). The highest posttreatment levels of IL-5, IL-10, and IL-13 were seen in response to SWA, and the levels were significantly higher than the mean levels measured in response to Teg (figure 1B, 1C, and 1D). There was no significant difference in the mean posttreatment levels of IL-4 detected in whole-blood cultures in response to SWA and Teg (figure 1A), and these levels were higher than the corresponding responses to SEA.

Relative changes in cytokine response to SWA and Teg.

Of the responses that changed significantly after treatment, the mean response to Teg increased more (as a proportion of pretreatment levels) than did the response to SWA, with mean levels of IL-5 and IL-10 increasing ~ 10 – 15 -fold and mean levels of IL-13 increasing ~ 5 -fold in response to Teg, whereas mean levels of the same cytokines in response to SWA increased only ~ 2 – 4.5 -fold after treatment. The production of IL-4 did not show the same pattern, with similar relative increases seen (~ 2 – 4 -fold) in response to both SWA and Teg (table 1).

Correlations between the changes in specific cytokine responses.

The strongest correlations were between changes in SWA- and Teg-specific responses for each cytokine, and all such correlation coefficients were >0.675 and statistically significant (table 2). Changes in all SWA-specific responses were positively correlated with each other, as were all changes in Teg-specific responses (table 2). With the exception of Teg-specific IL-5 and IL-13, pairs of SWA-specific responses were always more strongly correlated than were the same responses to Teg

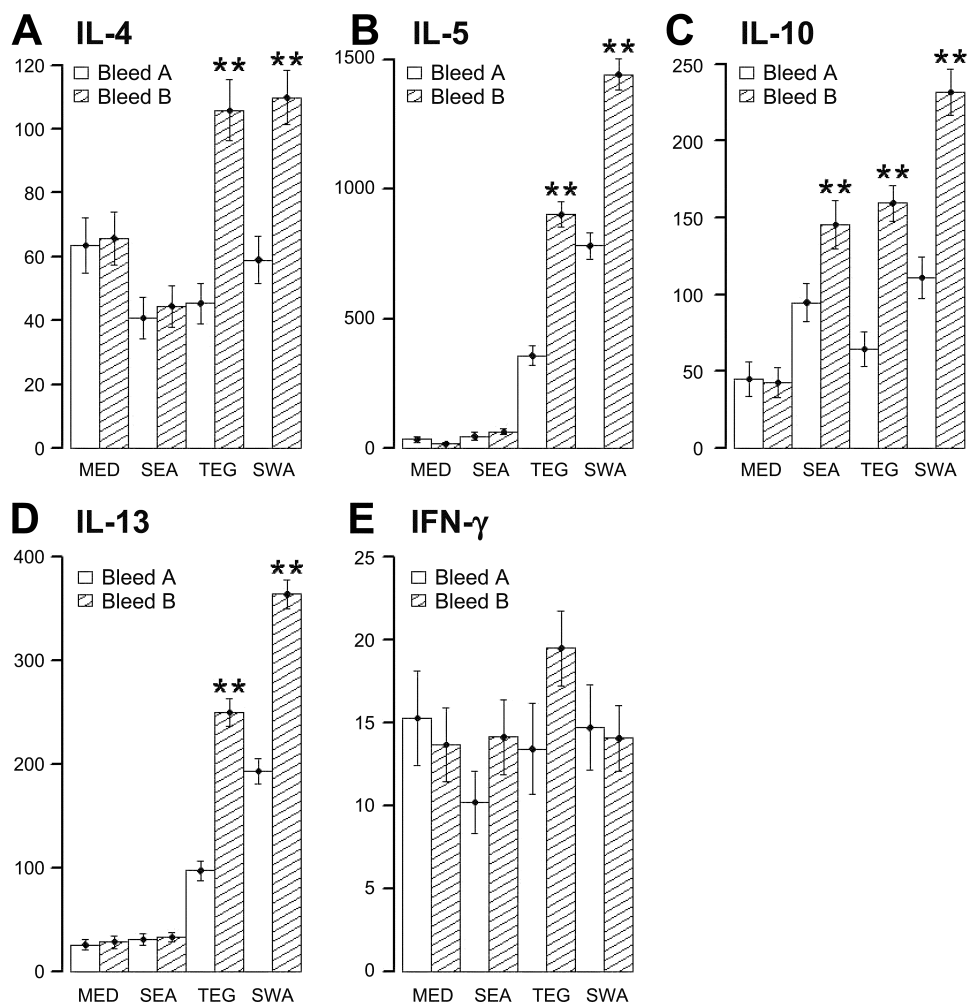


Figure 1. Effect of treatment with praziquantel (PZQ) on production of cytokines. Mean \pm SEM production of cytokines in the whole cohort was determined by use of whole-blood culture before (Bleed A) and 7 weeks after (Bleed B) treatment with PZQ. The cultures were left unstimulated (MED) or were stimulated with *Schistosoma mansoni* antigen (Ag), soluble egg Ag (SEA), adult worm outer tegument (TEG), or soluble adult worm Ag (SWA). A, Interleukin (IL)-4 ($n = 164$); B, IL-5 ($n = 186$); C, IL-10 ($n = 187$); D, IL-13 ($n = 186$); and E, interferon (IFN)- γ ($n = 187$). All cytokines are shown in picograms per milliliter, except IFN- γ , which is shown in units per milliliter. ** $P < .001$, for comparison of cytokine production before and after treatment for each individual (paired t test of the raw data).

(table 2). Changes in the levels of SEA-specific IL-10 were most strongly correlated with the changes in SWA- and Teg-specific IL-10. They were also weakly correlated with changes in SWA-specific IL-13, IL-5, and IL-4, but not with any Teg-specific cytokines other than IL-10. There were no responses that were negatively correlated.

Influence of age on the changes in cytokine production.

There was a very weak negative correlation between subject age and the change in production of IFN- γ in response to SEA ($r = -0.13$ and $P = .074$ [$n = 187$]), SWA ($r = -0.18$ and $P = .013$ [$n = 187$]), and Teg ($r = -0.20$ and $P = .006$ [$n = 187$]). The effect was stronger when nonresponders were removed from the analysis ($r = -0.18$ and $P = .039$ [$n = 137$]), $r = -0.24$ and $P = .003$ [$n = 156$], and $r = -0.23$ and $P =$

.003 [$n = 159$], respectively). Age was not significantly correlated with changes in the production of any other cytokines.

Influence of intensity of infection on the changes in cytokine production.

The changes in SEA IL-5 ($r = -0.193$ and $P = .008$ [$n = 186$]), SEA IL-10 ($r = +0.248$ and $P = .001$ [$n = 187$]), and Teg IL-10 ($r = +0.17$ and $P = .018$ [$n = 187$]) were positively correlated with pretreatment intensity of infection. Intensity of infection was not significantly correlated with changes in the production of any other cytokines.

Changes in cell populations after treatment.

The mean number of total white blood cells decreased significantly after treatment, from 6.72×10^6 cells/mL to 5.46×10^6 cells/mL ($P < .000$ [$n = 165$]), as did the mean number of circulating eosinophils, which decreased from 0.73×10^6 cells/mL to $0.44 \times$

Table 1. Changes in cytokine responses to *Schistosoma mansoni* soluble egg antigen (SEA), soluble worm antigen (SWA), and worm-tegument (Teg) as a percentage of pretreatment values.

Cytokine	SEA		SWA		Teg	
	Percentage boost (95% CI)	<i>P</i> ^a	Percentage boost (95% CI)	<i>P</i> ^a	Percentage boost (95% CI)	<i>P</i> ^a
IL-4	46 (−6 to 99)	.271	238 (179–29) ^b	<.001	401 (327–476) ^b	<.001
IL-5	133 (7–259)	.667	460 (344–576) ^b	<.001	1518 (1290–1747) ^b	<.001
IL-10	305 (202–408) ^b	.007	444 (355–533) ^b	<.001	1032 (901–1162) ^b	<.001
IL-13	84 (20–148)	.564	231 (166–296) ^b	<.001	478 (349–607) ^b	<.001
IFN- γ	68 (26–109)	.228	43 (−10 to 96)	.816	142 (84–199)	.051

NOTE. Percentage boost is the percentage change in cytokine responses averaged for all study subjects. Data were calculated as described in Subjects, Materials, and Methods. CI, confidence interval; IFN, interferon; IL, interleukin.

^a A paired *t* test was performed on raw cytokine data for pretreatment cytokine levels vs. posttreatment cytokine levels.

^b *P* ≤ .01.

10⁶ cells/mL (*P* < .000 [*n* = 151]). The mean number of lymphocytes was not significantly altered by treatment (3.71 × 10⁶ cells/mL before treatment and 3.5 × 10⁶ cells/mL 7 weeks after treatment [*n* = 155]).

DISCUSSION

The present study has described the effects of treatment with PZQ on the production of cytokines in response to SEA, SWA, and, for the first time, the Teg, in a cross-sectional cohort from an area where *S. mansoni* is endemic. Both the quantities and types of cytokines produced after treatment were influenced by the developmental stage of the parasite from which the Ags were derived. Before treatment, Th2 cytokines, including IL-4, IL-5, IL-10, and IL-13, were produced primarily in response to SWA and Teg, albeit at lower levels in response to the latter, whereas IL-10 was the only cytokine produced in comparable amounts in response to SEA. The effects of treatment were also polarized and Ag specific, with significant increases seen largely in Th2 cytokines in response to worm-derived Ags, with only small (and nonsignificant) increases seen in responses to SEA. After treatment, the greatest increases in IL-4, IL-5, IL-10, and IL-13 were seen in response to worm-derived Ags and, in particular, to Teg.

In contrast to what has been described for schistosome-specific Ab responses, particularly those that have been associated with resistance to reinfection, such as SWA- or Teg-specific IgE [24], age exerted little effect on the posttreatment levels of parasite-specific cytokines. Little association was seen between any cytokine response or change in such response and the pretreatment intensity of infection. Of all responses measured, only the changes in the mean SEA- or Teg-specific IL-10 were significantly positively associated with intensity of infection (albeit weakly).

Our data suggest that treatment with PZQ results in the release of Ags from adult worms in vivo and that the differential

effects of treatment on the responses to Teg and SWA perhaps reflect the fact that, before treatment, tegumental Ags are sequestered from the host immune system. According to this argument, Teg-specific responses might be specifically boosted when parasite Ags are synchronously released into the host circulation after treatment with drugs. It has been suggested elsewhere that infection with schistosomes causes the down-regulation of certain cellular immune responses [25–26] and that the increases in production of cytokines seen after treatment occur as a result of the removal of this immunosuppression. This down-regulation has been ascribed to IL-10, possibly via its direct action on Ag-presenting cells [27, 28]. IL-10 has been shown to play a role in the down-regulation of potentially harmful Th1 parasite-specific cytokines, such as IFN- γ and tumor necrosis factor- α , by cells from individuals chronically infected with *S. haematobium* [29, 30], and has been shown to be elicited by the onset of egg laying during murine infections [31], suggesting that egg-derived Ags might stimulate its production. The striking specificity of the increases in worm Ag-induced IL-4, IL-5, and IL-13 that we have described argues against the notion that such increases are the result of the removal of general immunosuppression. In the present study, the mean levels of SEA- and SWA-specific IL-10 increased significantly after treatment, suggesting that the specific increases in worm Ag-induced IL-4, IL-5, and IL-13 seen after treatment were not dependent on the removal of IL-10-mediated immunosuppression. Although IL-10 may be important in controlling responses, there does not appear to be a simple negative relationship between the levels of IL-10 and any of the other responses we measured.

The greater increases in responses to worm Ags, compared with egg Ags, seen after treatment may reflect variations in the exposure of the host to these Ags during the course of a chronic infection. Adult worms live for many years in the bloodstream

Table 2. Correlations between the cytokine responses to *Schistosoma mansoni* soluble egg antigen (SEA), soluble worm antigen (SWA), and worm-tegument (Teg) that changed significantly after treatment.

	IL-4 SWA	IL-4 Teg	IL-5 SWA	IL-5 Teg	IL-10 SEA	IL-10 SWA	IL-10 Teg	IL-13 SWA	IL-13 Teg
IL-4 SWA	...	0.765 (164) ^a	0.476 (162) ^a	0.478 (162) ^a	0.213 (163) ^b	0.371 (163) ^a	0.483 (163) ^a	0.397 (163) ^a	0.438 (163) ^a
IL-4 Teg	0.377 (162) ^a	0.385 (162) ^a	NS (163)	0.184 (163) ^c	0.329 (163) ^a	0.234 (163) ^b	0.325 (163) ^a
IL-5 SWA	0.720 (185) ^a	NS (185)	0.380 (185) ^a	0.339 (185) ^a	0.524 (184) ^a	0.463 (184) ^a
IL-5 Teg	NS (187)	0.220 (185) ^b	0.360 (185) ^a	0.351 (184) ^a	0.569 (185) ^a
IL-10 SEA	0.529 (186) ^a	0.464 (186) ^a	0.205 (185) ^b	NS (185)
IL-10 SWA	0.678 (186) ^a	0.372 (185) ^a	0.294 (185) ^a
IL-10 Teg	0.287 (186) ^a	0.290 (186) ^a
IL-13 SWA	0.730 (186) ^a

NOTE. Data are Spearman's rank correlations between the specific cytokine responses that were significantly changed by treatment with praziquantel (no. of samples included in each correlation). Specific cytokine levels were calculated by subtracting the level of each cytokine produced in the absence of antigen from that produced in the presence of either SEA, SWA, or Teg for each individual. The change in specific responses was determined by subtracting these pretreatment from posttreatment levels for each individual. IL, interleukin.

^a $P < .001$.

^b $.001 < P < .01$.

^c $.01 < P < .05$.

and continuously produce large numbers of relatively short-lived eggs each day, many of which become trapped and die in host tissues. Thus, the human host is continuously exposed to the complete repertoire of egg-derived Ags, whereas worm-derived Ags are less likely to present a constant and chronic stimulus to the immune system, especially since adult worms exploit many strategies of immune evasion to preserve the immunological invisibility of crucial functional molecules [32]. As a result, even though eggs and worms are targets for PZQ, many worm Ags are only likely to be exposed to the host when worms die [33–35]. Posttreatment increases in cytokine production could not be explained simply by increases in the numbers of circulating lymphocytes or eosinophils, although it remains possible that particular subsets of cells proliferated in vivo after treatment.

Immune responses to schistosomes in chronically infected individuals are typically polarized toward Th2 cytokines and are characterized by eosinophilia, high levels of parasite-specific IgE, and the predominance of Th2 cytokines, such as IL-4 and IL-5, in vitro [2]. Previous studies have not specifically addressed the question of the differential effects of treatment on cytokine responses to SEA and SWA [10, 36, 37], although some have shown increases in certain Th2 cytokines in vitro after treatment for *S. haematobium* [36, 37]. The significant increases we observed were polarized toward Th2 cytokines and were largely specific for worm and, more particularly, Teg Ags. Regulation of cytokine production by *S. mansoni* Ags was determined by examining the extent to which the changes in different pairs of responses were correlated. The strongest correlations were seen between the changes seen in SWA- and Teg-specific responses for each cytokine, and these correlations were stronger than those between any pairs of different SWA- or Teg-specific cytokines. This result suggests that Teg and SWA Ags might coregulate the production of Th2 cytokines from a

common cellular source and that treatment with PZQ results in the release of worm-derived and, more particularly, tegumentally derived Ags that initiate this increased production. The changes in SWA- and Teg-specific IL-5, IL-13, IL-4, and (to a lesser extent) IL-10 were all positively correlated with each other, with the strongest correlations generally seen between the changes in pairs of SWA-specific cytokines. Such positive correlations again suggest that there is coregulation of these Th2 cytokines by *S. mansoni* worm Ags and argue against a negative regulatory role for IL-10. That there was no significant change in lymphocyte counts and that eosinophil counts decreased significantly after treatment suggest that either (1) the number of *S. mansoni*-specific cells had increased but was undetectable by the methods used or (2) the cellular production of SWA- and Teg-specific IL-5, IL-13, IL-4, and IL-10 and SEA-specific IL-10 had been up-regulated by treatment, which, considering the magnitude of the increases observed, we consider to be the most likely explanation.

Immune evasion, partially mediated via the maintenance of the integrity of the worm Teg, is vital to the long-term survival of the parasite in the bloodstream of the host [38]. Mice can be successfully immunized against schistosome infection with homogenized tegumental membranes from adult worms, and several of the targets of the protective humoral responses are located in the Teg [39], with levels of specific Abs correlating with the degree of protective immunity [17, 18]. In Western blot analysis, serum of infected humans detects a much more restricted set of Ags in preparations of tegumental membranes, compared with those detected in homogenized whole worms. In spite of this, the predominant human IgE response seen during both *S. mansoni* [7] and *S. japonicum* [40] infections is directed against a 22.6-kDa Ag located in the outer Teg. Teg-specific IgE [7] and parasite-specific IL-4 and IL-5 [9, 10] have been associated with resistance to reinfection with schistosomes, suggest-

ing that a Th2 immune response to parasite tegumental Ags may contribute either directly or indirectly to immunity in humans. We speculate that the responses that we have observed are driven by the synchronized release of hitherto sequestered tegumentally derived Ags into the circulation. The polarized responses that follow may play a role in the generation and/or maintenance of the equally polarized Ab responses that have been shown to correlate with immunity to infection and reinfection in human populations. It is also interesting to note that the severe hepatosplenic form of human schistosomiasis, which is seen in a minority of infected individuals, has been attributed in experimental murine studies to parasite egg-specific IL-4 and IL-13 responses that give rise to granulomatous and fibrotic liver lesions [41]. If this notion is correct, the subdued responses to SEA and vigorous anti-SWA responses observed in the present study may be the combination of antiparasite responses that allow infected human populations in areas of endemicity to maintain protective responses to the threat of additional infection, while minimizing the risk of egg-induced, chronic, severe immunopathology.

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References

- World Health Organization (WHO) committee. The control of schistosomiasis. WHO technical report series 830. Geneva: WHO, 1993.
- Dunne DW, Mountford A. Resistance to infection in human and animal models. In: Mahmoud AAF, ed. Schistosomiasis. London: Imperial College Press, 2001:133–212.
- Kabatereine NB, Vennervald BJ, Ouma JH, et al. Adult resistance to *Schistosomiasis mansoni*: age-dependence of reinfection remains constant in communities with diverse exposure patterns. *Parasitology* 1999; 118:101–5.
- Hagan P, Wilkins HA, Blumenthal UJ, Hayes RJ, Greenwood BM. Eosinophilia and resistance to *Schistosoma haematobium* in man. *Parasite Immunol* 1985; 7:625–32.
- Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* 1991; 349:243–5.
- Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur J Immunol* 1991; 21:2679–86.
- Dunne DW, Butterworth AE, Fulford AJ, et al. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* 1992; 22:1483–94.
- Zhang Z, Wu H, Chen S, et al. Association between IgE antibody against soluble egg antigen and resistance to reinfection with *Schistosoma japonicum*. *Trans R Soc Trop Med Hyg* 1997; 91:606–8.
- Medhat A, Shehata M, Bucci K, et al. Increased interleukin-4 and interleukin-5 production in response to *Schistosoma haematobium* adult worm antigens correlates with lack of reinfection after treatment. *J Infect Dis* 1998; 178:512–9.
- Roberts M, Butterworth AE, Kimani G, et al. Immunity after treatment of human schistosomiasis: association between cellular responses and resistance to reinfection. *Infect Immun* 1993; 61:4984–93.
- Woolhouse ME, Hagan P. Seeking the ghost of worms past. *Nat Med* 1999; 5:1225–7.
- Karanja DMS, Hightower AW, Colley DG, et al. Resistance to reinfection with *Schistosoma mansoni* in occupationally exposed adults and effect of HIV-1 co-infection on susceptibility to schistosomiasis: a longitudinal study. *Lancet* 2002; 360:592–6.
- Fulford AJ, Butterworth AE, Ouma JH, Sturrock RF. A statistical approach to schistosome population dynamics and estimation of the lifespan of *Schistosoma mansoni* in man. *Parasitology* 1995; 110:307–16.
- Hoffmann KE, Strand M. Molecular identification of a *Schistosoma mansoni* tegumental protein with similarity to cytoplasmic dynein light chains. *J Biol Chem* 1996; 271:26117–23.
- Dunne DW, Webster M, Smith P, et al. The isolation of a 22 kDa band after SDS-PAGE of *Schistosoma mansoni* adult worms and its use to demonstrate that IgE responses against the antigen(s) it contains are associated with human resistance to reinfection. *Parasite Immunol* 1997; 19:79–89.
- Webster M, Fulford AJ, Braun G, et al. Human immunoglobulin E responses to a recombinant 22.6-kilodalton antigen from *Schistosoma mansoni* adult worms are associated with low intensities of reinfection after treatment. *Infect Immun* 1996; 64:4042–6.
- Smithers SR, Hackett F, Ali PO, Simpson AJ. Protective immunization of mice against *Schistosoma mansoni* with purified adult worm surface membranes. *Parasite Immunol* 1989; 11:301–18.
- Simpson AJG. Schistosome surface antigens: developmental expression and immunological function. *Parasitol Today* 1990; 6:40–5.
- Delgado VS, Suarez DP, Cesari IM, Incani RN. Experimental chemotherapy of *Schistosoma mansoni* with praziquantel and oxamniquine: differential effect of single or combined formulations of drugs on various strains and on both sexes of the parasite. *Parasitol Res* 1992; 78:648–54.
- Fallon PG, Smith P, Nicholls T, Modha J, Doenhoff MJ. *Schistosoma mansoni*: praziquantel-induced exposure of alkaline phosphatase: drug-antibody synergy which acts preferentially against female worms. *Parasite Immunol* 1994; 16:529–36.
- Fallon PG, Cooper RO, Probert AJ, Doenhoff MJ. Immune-dependent chemotherapy of schistosomiasis. *Parasitology* 1992; 105:S41–8.
- Fallon PG, Doenhoff MJ. Active-immunisation of mice with *Schistosoma* worm membranes antigens enhances efficacy of praziquantel treatment of *Schistosoma mansoni*. *Parasite Immunol* 1995; 17:261–7.
- Jeffs SA, Hagan P, Allen R, Correa-Oliveira R, Smithers SR, Simpson AJ. Molecular cloning and characterisation of the 22-kilodalton adult *Schistosoma mansoni* antigen recognised by antibodies from mice protectively vaccinated with isolated tegumental surface membranes. *Mol Biochem Parasitol* 1991; 46:159–67.
- Webster M, Libranda-Ramirez BD, Aligui GD, et al. The influence of sex and age on antibody isotype responses to *Schistosoma mansoni* and *Schistosoma japonicum* in human populations in Kenya and the Philippines. *Parasitology* 1997; 114:383–93.
- Zwingenberger K, Irschick E, Siqueira Vergetti JG, et al. Release of interleukin 2 and γ interferon by peripheral mononuclear cells in human *Schistosoma mansoni* infection normalizes after chemotherapy. *Scand J Immunol* 1989; 30:463–71.
- Colley DG, Barsoum IS, Dahawi HS, Gamil F, Habib M, El Alamy MA. Immune responses and immunoregulation in relation to human schistosomiasis in Egypt. III. Immunity and longitudinal studies of in vitro responsiveness after treatment. *Trans R Soc Trop Med Hyg* 1986; 80: 952–7.
- Grogan JL, Kremsner PG, Deelder AM, Yazdanbakhsh M. Antigen-specific proliferation and interferon- γ and interleukin-5 production are down-regulated during *Schistosoma haematobium* infection. *J Infect Dis* 1998; 177:1433–7.
- King CL, Medhat A, Malhotra I, et al. Cytokine control of parasite-specific anergy in human urinary schistosomiasis: IL-10 modulates lymphocyte reactivity. *J Immunol* 1996; 156:4715–21.
- Grogan JL, Kremsner PG, Deelder AM, Yazdanbakhsh M. The effect of anti-IL-10 on proliferation and cytokine production in human schis-

- tosomiasis: fresh versus cryopreserved cells. *Parasite Immunol* **1998**; 20:345–9.
30. King CL, Malhotra I, Mungai P, et al. *Schistosoma haematobium*-induced urinary tract morbidity correlates with increased tumor necrosis factor- α and diminished interleukin-10 production. *J Infect Dis* **2001**; 184:1176–82.
 31. Grzych JM, Pearce E, Cheever A, et al. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *J Immunol* **1991**; 146:1322–7.
 32. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol* **2003**; 3: 733–44.
 33. Harnett W, Kusel R. Increased exposure of parasite antigens at the surface of adult male *Schistosoma mansoni* exposed to praziquantel in vitro. *Parasitology* **1986**; 93:401–5.
 34. Redman CA, Robertson A, Fallon PG, et al. Praziquantel: an urgent and exciting challenge. *Parasitol Today* **1996**; 12:14–20.
 35. Giboda M, Smith JM. *Schistosoma mansoni* eggs as a target for praziquantel: efficacy of oral application in mice. *J Trop Med Hyg* **1994**; 97: 98–102.
 36. Grogan JL, Kremsner PG, Deelder AM, Yazdanbakhsh M. Elevated proliferation and interleukin-4 release from CD4⁺ cells after chemotherapy in human *Schistosoma haematobium* infection. *Eur J Immunol* **1996**; 26:1365–70.
 37. van den Biggelaar AHJ, Borrmann S, Kremsner P, Yazdanbakhsh M. Immune responses induced by repeated treatment do not result in protective immunity to *Schistosoma haematobium*: interleukin (IL)-5 and IL-10 responses. *J Infect Dis* **2002**; 186:1474–82.
 38. Abath FGC, Werkhauser RC. The tegument of *Schistosoma mansoni*: functional and immunological features. *Parasite Immunol* **1996**; 18:15–20.
 39. Jeffs SA, Hagan P, Allen R, Correa-Oliveira R, Smithies SR, Simpson AJ. Molecular cloning and characterisation of a 22 kilodalton adult *Schistosoma* antigen recognised by antibodies from mice protectively vaccinated with isolated tegumental surface membranes. *Mol Biochem Parasitol* **1991**; 46:159–67.
 40. Santiago ML, Hafalla JC, Kurtis JD, et al. Identification of the *Schistosoma japonicum* 22.6-kDa antigen as a major target of the human IgE response: similarity of IgE-binding epitopes to allergen peptides. *Int Arch Allergy Immunol* **1998**; 117:94–104.
 41. Hoffmann KE, Wynn TA, Dunne DW. Cytokine-mediated host responses during schistosome infections: walking the fine line between immunological control and immunopathology. *Adv Parasitol* **2002**; 52: 265–307.