MAJOR ARTICLE

Effect of Praziquantel Treatment during Pregnancy on Cytokine Responses to Schistosome Antigens: Results of a Randomized, Placebo-Controlled Trial

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Background. Praziquantel treatment of schistosomiasis boosts antischistosome responses, with type 2 helper T cell bias that may contribute to immunologically mediated killing and to protection against reinfection. Praziquantel treatment during pregnancy was recommended in 2002, but the immunological effects of the treatment had not been investigated.

Methods. A cohort of 387 *Schistosoma mansoni*–infected women were recruited from a larger trial of deworming during pregnancy. Women were randomized to receive either praziquantel or placebo during pregnancy. Six weeks after delivery, all women received praziquantel. Cytokine responses to *S. mansoni* worm and egg antigens were measured in whole blood culture before and 6 weeks after each treatment.

Results. Schistosome-specific cytokine responses were suppressed during pregnancy. Praziquantel treatment during pregnancy caused significant boosts in interferon-γ (IFN-γ), interleukin (IL)–2, IL-4, IL-5, IL-13, and IL-10 responses to schistosome worm antigen and in IFN-γ, IL-5, and IL-13 responses to schistosome egg antigen, but these boosts were not as substantial as those seen for women treated after delivery.

Conclusion. Pregnancy suppresses a potentially beneficial boost in cytokine responses associated with praziquantel treatment. Further studies are needed on the long-term effects that treatment of schistosomiasis during pregnancy have on morbidity and resistance to reinfection among treated women and their offspring.

Trial registration. International Standard Randomized Controlled Trial Number for the parent study: ISRCTN32849447.

Praziquantel, the drug of choice for treatment of schistosomiasis [1, 2], has shown excellent safety and therapeutic effect against schistosomiasis morbidity. Praziquantel became available in 1979 but had never been studied in pregnant or lactating women. Although its use during pregnancy was presumed to be safe on the basis of findings from studies involving animals, it was widely withheld from pregnant and lactating women during mass-treatment programs [3]. In 2002, an informal consultation by the World Health Organization (WHO) reviewed studies on the use of praziquantel during pregnancy. There was little evidence of adverse effects described in case reports or in pregnant women who inadvertently received praziquantel during mass-treatment programs, and it was recommended that pregnant and lactating women with schistosomiasis should be treated [4, 5]. However, the risks and benefits of treatment during pregnancy, particularly the immunological effects, had not been studied. Thus, in 2005, a WHO scientific working group called for randomized, placebo-controlled trials of treatment during pregnancy

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for all species of human schistosomes in both low- and high-transmission areas [6]. We here report immunological findings from such a trial.

Although the precise mode of action of praziquantel is still not clear, there is evidence of synergy between praziquantel and the immune system. First, it has been suggested that praziquantel-induced damage to the worm tegument may be supplemented by immunologically mediated killing of the exposed schistosome [7–12]. Second, praziquantel treatment has a marked effect on antischistosome immune responses [13, 14] and leads to a boost in schistosome antigen–specific cytokine responses [15], which may contribute to subsequent immunity to reinfection [16, 17].

Several factors, including age, sex, previous exposure, coinfections, and treatment, influence an individual's immune response to schistosomiasis [18, 19]. Of particular interest to this study is the influence of pregnancy. Pregnancy is characterized by a depression of cell-mediated immunity [20–22] with relative increases in Th2-associated responses and regulatory responses (i.e., increased production of IL-4 and IL-10) and decreased Th1 responses (i.e., low interferon-γ [IFN-γ] and IL-2 production) [23]. More specifically, it has been shown that schistosome-specific proliferative responses decrease with advancing pregnancy in women with schistosomiasis [24].

Thus, in a trial of deworming during pregnancy, we explored the effect of pregnancy on immune responses to *S. mansoni* infection by testing the hypothesis that the boost in immune responses during praziquantel treatment is reduced in pregnant women, compared with nonpregnant women. If this hypothesis is true, it might have important consequences for the efficacy of treatment (i.e., by reducing the synergy between drug-mediated and immunologic killing) and subsequent resistance to reinfection.

SUBJECTS, MATERIALS, AND METHODS

Study cohort, randomization to praziquantel treatment, and sample collection. A nested cohort of 387 pregnant women infected with *S. mansoni* was enrolled within the larger Entebbe Mother and Baby Study (EMABS) of the impact of helminths on the response to immunization and on the susceptibility to infectious diseases during childhood in Uganda (International Standard Randomized Controlled Trial Number: ISRCTN32849447) [25].

Details on recruitment, baseline findings, interventions, and randomization and allocation procedures for the main study have been described elsewhere [25]. In brief, the study was a randomized, double-blind, placebo-controlled trial that compared praziquantel with placebo and albendazole with placebo during pregnancy, using a 2×2 factorial design. Participants were recruited between April 2003 and November 2005. Women in the second or third trimester of pregnancy were eligible for inclusion if they were residents of the Entebbe peninsula of Lake Victoria, planned to deliver their baby in Entebbe Hospital, and were willing to participate. They were excluded if the pregnancy was not normal, they had any history of adverse reactions to anthelmintics or evidence of helminth-induced disease requiring immediate treatment, or they had participated in the study during an earlier pregnancy. Demographic and socioeconomic details and clinical history were obtained at enrollment. Women were asked to provide stool samples for examination for helminth ova and blood for diagnostic and immunological analysis. They were then randomized to receive single-dose praziquantel (40 mg/kg) or placebo and single-dose albendazole (400 mg) or placebo, taken under observation. Six weeks after delivery, all mothers received praziquantel and albendazole with additional anthelmintic treatment, if indicated by results of stool analyses.

The nested cohort study of immune responses to schistosomiasis began in November 2003, seven months after enrollment in the larger cohort had started, and was confined to women for whom stool specimens contained *S. mansoni* ova. Within this nested study, follow-up samples for immunological assays were obtained 6 weeks after enrollment during pregnancy (if the woman was still pregnant), 6 weeks after delivery but before receipt of postdelivery treatment, and 12 weeks after delivery (i.e., 6 weeks after receipt of postdelivery treatment). Follow-up stool samples were obtained 6 weeks after enrollment during pregnancy, 6 weeks after delivery, and 12 weeks after delivery.

At first, assays for responses to *S. mansoni* antigens were performed for all enrolled women. Later, to conserve limited stocks of schistosome antigens, single-step rapid test kit strips (BV European Veterinary Laboratory) for circulating cathodic antigen (CCA) in urine were performed to screen for *S. mansoni* infection, allowing assays to be selectively done on enrollment samples likely to be from schistosome-infected participants. Of 121 women enrolled when we used CCA screening, 78 were CCA positive and had assay results available, whereas 43 were CCA negative and did not provide specimens for testing. We compared the *S. mansoni* infection intensities between women who were CCA negative and Kato-Katz positive and women who were CCA and Kato-Katz positive and found no significant difference (median concentration in stool, 59.9 and 48.0 eggs/g, respectively; $P = .84$), thereby confirming no selection bias.

Ethical clearance was obtained from the Science and Ethics Committee of the Uganda Virus Research Institute, the Uganda National Council for Science and Technology and London School of Hygiene and Tropical Medicine.

Parasitological diagnosis. A single stool sample per individual, collected at each time point, was examined by duplicate Kato-Katz thick smears [26]. Slides were read $\leq$30 min after collection for hookworm ova and the next day for *S. mansoni* ova. *S. mansoni* infection intensities were categorized as light (median concentration in stool, 1-99 eggs/g), moderate (100-399 eggs/g), or heavy ($\geq$400 eggs/g) [3].

Schistosome antigens. Soluble *S. mansoni* adult worm (SWA) and egg (SEA) antigens were prepared as previously described [27]. The endotoxin level in both antigen preparations was below levels that could stimulate detectable production of the cytokines assayed.

Whole blood culture and cytokine assay. Cytokine responses were examined by whole blood culture assays as previously described [28, 29]. In brief, heparinized blood was diluted to a final concentration of 1 in 4 with serum-free medium (RPMI supplemented with glutamine, penicillin, and streptomycin), and 200 μL/well was added to 96-well, round-bottom plates (TC Microwell [Nunc]). The blood was stimulated with SWA, SEA, or phytohemagglutinin (PHA [Sigma]) at final concentrations of 10 μg/mL or left unstimulated. Cultures were incubated at 37°C with 5% CO_2, and supernatants were harvested after 6 days. Supernatants were virally inactivated with 0.03% tributyl phosphate and 1% Tween 80 (Sigma) at room temperature for 1 h and stored at −80°C. Supernatants were analyzed for IFN-γ, IL-2, IL-4, IL-5, and IL-10 by use of OptEIA ELISA Kits (BD Pharmingen) and for IL-13 by use of antibody pairs (BD PharMingen) with standards obtained from the UK National Institute for Biological Standards and Controls. Supernatants from all time points for each participant were assayed in duplicate on the same ELISA plate, and a positive control was used for all plates to monitor for interassay variations. The lower limit of detection for each assay, and the cut-off for positive response, was the lowest standard concentration (i.e., 7.8 pg/mL; 8.6 pg/mL was used for IFN-γ). The cytokine concentration in unstimulated wells was subtracted from concentrations in antigen-stimulated wells to obtain the antigen-specific response.

Statistical considerations and analysis. The sample size for the nested study was determined by the proportion of women in the main study who had schistosomiasis. We expected to enroll 250 schistosome-infected pregnant women (125 each in the praziquantel and placebo arms). Initial analyses were made using qualitative variables. However, quantitative analyses were found to be more informative, and results of these tests are presented in this article. No interim analyses were performed, and there were no stopping rules, but serious adverse events were reported to the data-monitoring committee to allow stopping if an excess number of events occurred in any group.

The analysis had 4 objectives. First, we assessed the effects of pregnancy on antischistosome cytokine responses by comparing responses during pregnancy (at enrollment and 6 weeks after enrollment) with responses after delivery in the placebo group, using the Wilcoxon signed rank test. Second, we investigated the effect of praziquantel treatment during pregnancy. To accomplish this, responses before and after treatment were compared in the praziquantel group, using the Wilcoxon signed rank test. Then, responses 6 weeks after enrollment were compared between the praziquantel group and the placebo group, using the Wilcoxon rank sum test (also known as the Mann-Whitney *U* test). Third, we examined the influence of pregnancy on the praziquantel-induced boost in cytokine responses 6 weeks after treatment. The boost during pregnancy was compared with the boost after delivery, using linear regression based on the change in $\log_{10}$ cytokine concentration + 1. The term "boost" is used to refer to the change in cytokine responses following praziquantel treatment, although in some instances decreased responses could occur. Fourth, we examined the influence of pregnancy on cure following praziquantel treatment by comparing posttreatment infection intensities between the group first treated during pregnancy and the group first treated after delivery, using the Pearson χ^2 test.

RESULTS

During the recruitment period for the nested cohort, 2208 women were enrolled in the main study, of whom 387 had stool samples positive for *S. mansoni.* Cytokine response data at enrollment were available for 103 women who received praziquantel and 105 women who received placebo. For logistical reasons (i.e., missed appointments, omission of schistosome antigens in assays, or insufficient samples), the number of specimens analyzed varied across time points: for the praziquantel and placebo groups, results for 80 and 72 specimens, respectively, obtained 6 weeks after enrollment (i.e., during pregnancy) were available, as were results for 91 and 95, respectively, obtained 6 weeks after delivery and results for 68 and 77, respectively, obtained 6 weeks after receipt of postdelivery treatment. At baseline, the praziquantel and placebo groups showed similar demographic characteristics, intensities of schistosome infection, prevalences of hookworm and HIV, and profiles of cytokine responses to schistosome antigens (table 1). At baseline, type 2 IL-4 ($\rho = 0.13$; $P = .061$), IL-5 ($\rho = 0.14$; $P = .036$), and IL-13 ($\rho = 0.16$; $P = .021$) responses to SWA showed positive associations with schistosome infection intensity. Baseline responses to SEA showed negative associations with infection intensity for IFN-γ ($\rho = -0.17$; $P = .017$) and IL-13 ($\rho = -0.14$; $P = .041$) and a positive association for IL-4 ($\rho = 0.14$; $P = .05$). Cytokine responses to SWA and SEA showed no association with maternal age, estimated gestational age, or other coinfections, such as hookworm.

Cytokine responses to SWA and SEA are suppressed during pregnancy. Results from the placebo group were examined to assess the effects of pregnancy on antischistosome cytokine responses in the absence of treatment (figures 1*B* and 2*B*). Six weeks after enrollment, IFN-γ, IL-5, and IL-13 responses to SWA and SEA were lower than those at enrollment ($P = .008$ for IFN-γ and IL-5 and $P = .006$ for IL-13 responses to SWA; $P < .001$ for IFN-γ, $P = .002$ for IL-5, and $P = .001$ for IL-13 responses to SEA). Six weeks after delivery, IFN-γ, IL-5, and IL-13 responses to SWA had increased and were higher than those at enrollment ($P = .02$ for IFN-γ, $P = .007$ for IL-5, and

Table 1. Characteristics at enrollment before treatment initiation among pregnant women with *Schistosoma mansoni* infection who received praziquantel or placebo.

Characteristic	Praziquantel group	Placebo group
Albendazole treatment	57/103 (55.3)	49/105 (46.7)
Age, median (IQR), years	22 (20–26)	22 (18–26)
Gravidity		
Primigravida	31/103 (30.1)	22/105 (21.0)
Multigravida	72/103 (69.9)	83/105 (79.0)
Gestational age at time of treatment, median (IQR), weeks	27 (23–31)	26 (22–30)
S. mansoni infection intensity		
Light	64/103 (62.1)	71/105 (67.6)
Moderate	21/103 (20.4)	22/105 (21.0)
Heavy	18/103 (17.5)	12/105 (11.4)
Hookworm infection	47/103 (45.6)	38/105 (36.2)
HIV infection	15/103 (14.5)	13/105 (12.4)
Response to SWA antigen, by cytokine		
IFN-γ		
Overall	58/103 (56.3)	59/103 (57.2)
Median (IQR), pg/mL	32.0 (14–128.5)	34 (10–85.3)
IL-2		
Overall	51/97 (52.5)	47/98 (52.5)
Median (IQR), pg/mL	15.0 (7–54.2)	20 (5.2–41.0)
IL-4		
Overall	20/97 (20.6)	24/97 (24.7)
Median (IQR), pg/mL	18.6 (4.4–23.8)	7.8 (3.2–16.3)
IL-5		
Overall	49/103 (47.5)	49/103 (47.5)
Median (IQR), pg/mL	14.2 (7.6–60.8)	17.0 (4.2–85.5)
IL-13		
Overall	55/103 (53.4)	48/103 (46.6)
Median (IQR), pg/mL	18.1 (6.2–42.2)	32.1 (5.9–73.6)
IL-10		
Overall	79/103 (76.7)	77/103 (74.7)
Median (IQR), pg/mL	30.0 (13.2–59.0)	39.2 (11.1–72.6)
Response to SEA antigen, by cytokine		
IFN-γ		
Overall	34/103 (33.0)	56/103 (54.3)
Median (IQR), pg/mL	36.7 (6.2–165.5)	34.6 (9.4–76.8)
IL-2		
Overall	40/97 (41.2)	35/98 (35.7)
Median (IQR), pg/mL	14.3 (4.4–47.7)	13.0 (5.8–32.2)
IL-4		
Overall	20/97 (20.6)	21/97 (21.6)
Median (IQR), pg/mL	16.7 (4.4–29.7)	8.4 (5.1–22.4)
IL-5		
Overall	22/103 (21.3)	27/103 (26.2)
Median (IQR), pg/mL	22.5 (4.0–50.0)	11.0 (5.2–45.6)
IL-13		
Overall	26/103 (25.2)	32/103 (31.0)
Median (IQR), pg/mL	8.6 (2.0–57.4)	11.6 (2.2–48.9)
IL-10		
Overall	74/103 (71.8)	69/103 (67.0)
Median (IQR), pg/mL	23.1 (10.1–61.8)	31.0 (14.5–69.1)

NOTE. Data are no. of patients with the characteristic/no. evaluated (%), unless otherwise indicated. IFN, interferon; IQR, interquartile range; SEA, *S. mansoni* adult egg; SWA, *S. mansoni* adult worm.

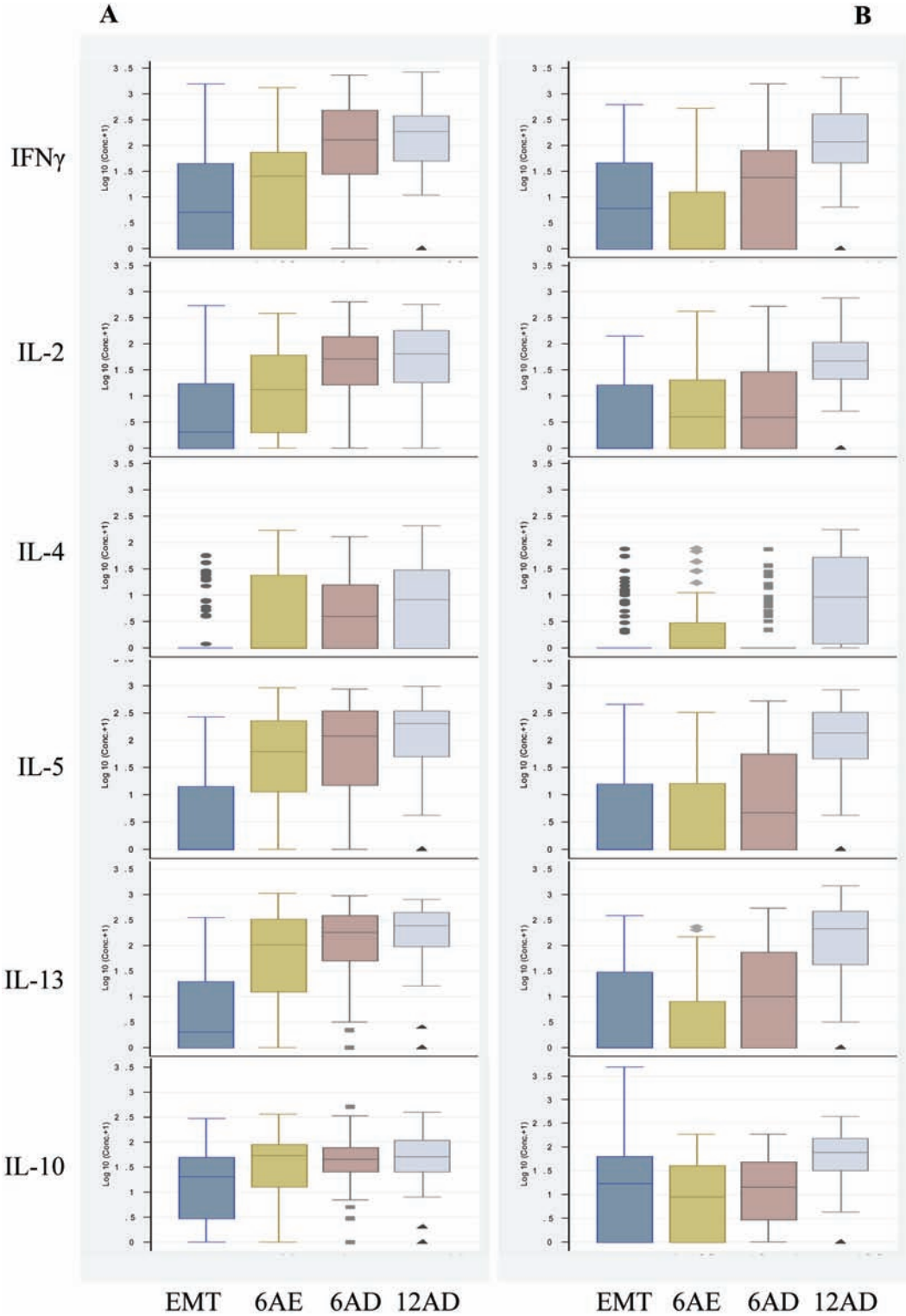

Figure 1. Findings of whole blood culture to determine cytokine responses to adult *Schistosoma mansoni* adult worm antigen and the effects of praziquantel treatment during pregnancy and after delivery. *A,* Participants who received an initial dose of praziquantel at enrollment (EMT) during pregnancy and a second dose 6 weeks after delivery (6AD). IFN-γ, IL-5, IL-13, and IL-10 responses were measured at EMT for 103 eligible participants, 6 weeks after enrollment (6AE) for 80, 6AD for 91, and 12 weeks after delivery (12AD) for 68, whereas IL-2 and IL-4 responses were measured at EMT for 97 of 103, 6AE for 79 of 80, 6AD for 85 of 91, and 12AD for 64 of 69. *B,* Participants who received an initial dose of praziquantel 6AD. IFN-γ, IL-5, IL-13, and IL-10 responses were measured at EMT for 105 eligible participants, 6AE for 72, 6AD for 95, and 12AD for 77, whereas IL-2 and IL-4 responses were measured at EMT for 98 of 105, 6AE for 67 of 72, 6AD for 89 of 95, and 12AD for 75 of 77.

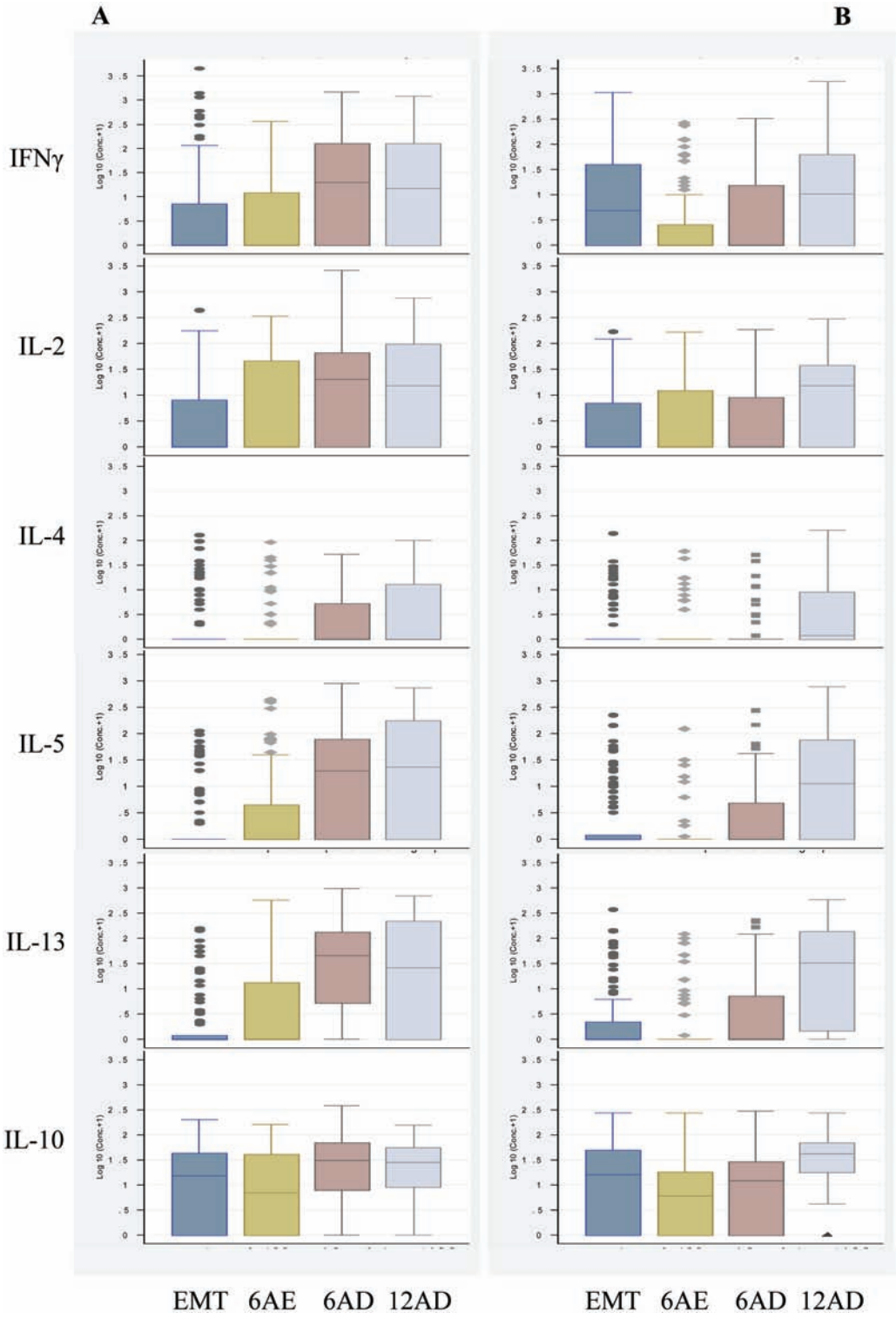

Figure 2. Findings of whole blood culture to determine cytokine responses to *Schistosoma mansoni* egg antigen and the effects of praziquantel treatment during pregnancy and after delivery. *A,* Participants who received an initial dose of praziquantel at enrollment (EMT) during pregnancy and a second dose 6 weeks after delivery (6AD). IFN-γ, IL-5, IL-13, and IL-10 responses were measured at EMT for 103 eligible participants, 6 weeks after enrollment (6AE) for 80, 6AD for 91, and 12 weeks after delivery (12AD) for 68, whereas IL-2 and IL-4 responses were measured at EMT for 97 of 103, 6AE for 79 of 80, 6AD for 85 of 91, and 12AD for 64 of 69. *B,* Participants who received an initial dose of praziquantel 6AD. IFN-γ, IL-5, IL-13, and IL-10 responses were measured at EMT for 105 eligible participants, 6AE for 72, 6AD for 95, and 12AD for 77, whereas IL-2 and IL-4 responses were measured at EMT for 98 of 105, 6AE for 67 of 72, 6AD for 89 of 95, and 12AD for 75 of 77.

Table 2. Comparison of the change in cytokine responses to *Schistosoma mansoni* adult worm (SWA) and egg (SEA) 6 weeks following praziquantel treatment during pregnancy or after delivery.

Antigen, cytokine	Change in cytokine responses, median (IQR), pg/mL		Change in $\log_{10}$ cytokine concentration +1, regression coefficient (95% CI)[a]		
	First treated during pregnancy	First treated after delivery	Crude analysis	Adjusted analysis[b]	*P*
SWA					
IFN-γ	11.7 (5.0–58.8)	56.7 (−3.0 to 319.5)	−0.32 (−0.74 to 0.08)	−0.57 (−0.89 to −0.23)	.001
IL-2	4.3 (−6.1 to 53.9)	29.3 (0.8–82.0)	−0.47 (−0.80 to −0.16)	−0.46 (−0.73 to −0.19)	.001
IL-4	0 (0–19.5)	7.2 (0–45.4)	−0.37 (−0.65 to −0.10)	−0.37 (−0.64 to −0.11)	.006
IL-5	58.2 (4.3–177.2)	82.8 (19.2–245.0)	0.05 (−0.32 to 0.42)	−0.20 (−0.51 to 0.10)	.18
IL-13	97.2 (12.2–280.4)	192.0 (12.0–405.6)	0.14 (−0.24 to 0.53)	−0.15 (−0.47 to 0.17)	.37
IL-10	20.0 (0–68.0)	46.0 (11.8–96.2)	−0.29 (−0.55 to −0.02)	−0.27 (−0.50 to −0.04)	.02
SEA					
IFN-γ	0 (0–4.0)	0 (0–39.0)	−0.30 (−0.63 to 0.03)	−0.33 (−0.63 to −0.03)	.03
IL-2	0 (0–20.1)	8.0 (0–33.05)	−0.20 (−0.52 to 0.11)	−0.17 (−0.45 to 0.12)	.25
IL-4	0 (0–0)	0.2 (0–9.4)	−0.48 (−0.69 to −0.26)	−0.40 (−0.60 to −0.20)	.001
IL-5	0 (0–4.2)	7.2 (0–61.7)	−0.42 (−0.77 to −0.07)	−0.47 (−0.79 to −0.16)	.004
IL-13	0 (0–11.0)	14.6 (0–103.2)	−0.46 (−0.79 to −0.12)	−0.55 (−0.87 to −0.23)	.001
IL-10	0 (−14.2 to 19.0)	18.2 (−1.0 to 55.8)	−0.51 (−0.81 to −0.22)	−0.50 (−0.76 to −0.25)	.001

NOTE. CI, confidence interval; IFN, interferon; IQR, interquartile range.

[a] Regression coefficients denote the mean boost in $\log_{10}$ cytokine concentration + 1 six weeks following treatment received during pregnancy minus the mean boost (in $\log_{10}$ cytokine concentration + 1 six weeks following treatment received after delivery.

[b] Adjusted for pretreatment cytokine responses and *S. mansoni* infection intensity.

$P < .001$ for IL-13) and 6 weeks after enrollment ($P < .001$ for IFN-γ, $P = .003$ for IL-5, and $P = .001$ for IL-13). Similarly, IFN-γ and IL-13 responses to SEA were significantly higher than those 6 weeks after enrollment ($P = .009$ for IFN-γ and $P = .002$ for IL-13). IL-2, IL-4, and IL-10 responses showed no statistically significant changes.

Thus, IFN-γ, IL-5, and IL-13 responses to SWA and SEA were suppressed during pregnancy: they decreased with progression of pregnancy, and they were higher after delivery than at either time point during pregnancy.

Praziquantel treatment during pregnancy causes significant boosts in cytokine responses to SWA and SEA. Samples obtained during pregnancy from the praziquantel group and the placebo group were compared to assess the effect of praziquantel treatment during pregnancy. Among women who received praziquantel at enrollment (figure 1*A*), cytokine production in response to SWA 6 weeks after enrollment was significantly higher than production at enrollment ($P = .01$ for IFN-γ and IL-2 and $P < .001$ for IL-4, IL-5, IL-13, and IL-10). Six weeks after enrollment, cytokine responses to SWA were significantly higher among women in the praziquantel group than among those in the placebo group ($P < .001$ for IFN-γ, IL-5, IL-13, and IL-10 and $P = .003$ for IL-2 and IL-4).

The effects of praziquantel treatment during pregnancy on responses to SEA were more limited (figure 2*A*). Among women who received praziquantel at enrollment, production of IL-2, IL-5, and IL-13 in response to SEA had increased 6 weeks after enrollment ($P = .02$ for IL-2, $P = .01$ for IL-5, and $P = .002$ for IL-13). At this time point, IL-5 ($P = .003$) and IL-13 ($P < .001$) responses to SEA were significantly higher among the women in the praziquantel group than among those in the placebo group.

Pregnancy is associated with reduced boosts in cytokine responses to SWA and SEA. The magnitude of the praziquantel-induced boost in cytokine responses (between treatment and 6 weeks after treatment) was compared between the praziquantel group (first treated at enrollment during pregnancy) and the placebo group (first treated 6 weeks after delivery) to assess the influence of pregnancy on the boost in responses. Initial comparisons showed that the boost was lower for treatment during pregnancy than for treatment after delivery for all responses except the IL-5 and IL-13 responses to SWA (table 2). Several possible sources of bias were considered in relation to this analysis because, at the time of treatment, the group first treated after delivery (originally the placebo group) differed in several important ways from the group first treated during pregnancy (originally the praziquantel group). The pretreatment prevalence of hookworm was lower in the group first treated after delivery, because 47% had already received albendazole (table 1); all women in the group first treated after delivery received albendazole concurrently with their first praziquantel treatment, compared with 55% of those first treated during pregnancy. However, analyses during pregnancy showed no effect of hookworm coinfection or concurrent receipt of albendazole on the boost in antischistosome responses following praziquantel treatment (data not shown). Therefore, these differences were con-

sidered unlikely to bias the comparison. Pretreatment cytokine responses were higher in the group first treated after delivery. Although no major differences in schistosome infection intensities at enrollment were noted between the 2 groups, enrollment schistosome intensity showed positive correlations with posttreatment SWA-specific IL-4 ($\rho = 0.38$; $P < .001$), IL-5 ($\rho = 0.21$; $P = .05$), and IL-13 ($\rho = 0.23$; $P = .039$) responses. Posttreatment responses to SEA showed a tendency to negatively correlate with enrollment schistosome intensity, but the association did not attain statistical significance. However, after adjustment for pretreatment cytokine responses and enrollment infection intensity, the boost in responses remained markedly higher for women treated after delivery than for those treated during pregnancy for all responses except the IL-5 and IL-13 responses to SWA and the IL-2 response to SEA (table 2).

In keeping with the suppression of responses during pregnancy and the reduced boost in response following treatment during pregnancy, all responses 6 weeks after treatment were lower following treatment during pregnancy than following treatment after delivery. However, among women first treated during pregnancy, most of the responses 6 weeks after delivery had increased and were not significantly different from the responses 6 weeks after treatment among women first treated after delivery; IL-4 and IL-10 responses to SWA were still lower among women first treated during pregnancy than among women first treated after delivery ($P = .002$ for IL-4 and $P = .008$ for IL-10).

Pregnancy is not associated with a reduced cure rate. To address the hypothesis that suppression of the boost in immune response during pregnancy is associated with a reduced cure rate, the infection intensities 6 weeks after treatment were compared between the group first treated during pregnancy and the group first treated after delivery. Of the 80 women in the group first treated during pregnancy, 68 (85.0%) did not have egg detected in their stool, 8 (10.0%) had light infection, 3 (3.8%) had moderate infection, and 1 (1.2%) still had heavy infection 6 weeks after treatment. Of the 77 women in the group first treated after delivery, eggs were not detected in 68 (89.5%) of 76, and 8 (10.5%) of 76 still had light infection. One of the women did not submit a stool sample 6 weeks after treatment. The difference in posttreatment infection intensities were not statistically significant ($P = .27$).

DISCUSSION

The aim of this study was to elucidate the influence of pregnancy on the immune response to schistosome antigens in *S. mansoni*–infected women and, particularly, its influence on the effects of praziquantel treatment on cytokine responses, using the hypothesis that pregnancy causes a reduced boost in cytokine responses following treatment.

First, the study showed alterations in cytokine responses to schistosome antigens during pregnancy among women who did not receive praziquantel at enrollment. Schistosome-specific IFN-γ, IL-5, and IL-13 responses were depressed during pregnancy. Furthermore, although there was no association between the stage of gestation and the cytokine responses at enrollment (perhaps because of the variability of responses between individuals and the difficulty in estimating the stage of gestation accurately), there was a decrease in these 3 responses within individuals over a 6-week period during pregnancy. This evidence of a suppression of responses during pregnancy confirmed and extended the earlier findings by Novato-Silva et al. [24] and is consistent with effects reported for other antigens [20, 21]. From the onset of pregnancy, the immunological environment of the uterus is controlled to allow fetal allograft retention [30], and this is associated with a general depression of cellular responses [31, 32]. Schistosome-specific IL-2, IL-4, and IL-10 remained relatively unaffected during pregnancy. IL-2 and IL-4 responses were detected at low frequency, and the detection limits of the assay may have limited our ability to detect any changes. However, these results accord with reports that Th2-associated responses and regulatory activity are generally sustained during pregnancy [33–35], whereas Th1 responses are suppressed.

Second, despite the immunosuppressive effects of pregnancy, praziquantel treatment of *S. mansoni* during pregnancy caused a detectable increase in all measured cytokine responses to SWA, as well as an increase in all responses to SEA except the IL-4 response, which was perhaps below the level of detection of the assay in many cases; thus, praziquantel treatment reversed the otherwise decreasing cytokine profiles observed during pregnancy. The effects were particularly marked for the responses to SWA. Other studies involving nonpregnant individuals from *S. mansoni*–endemic areas in Uganda have reported that praziquantel treatment caused boosts in IL-4, IL-5, IL-13, and IL-10 responses to SWA but did not boost the IFN-γ response to SWA or any response to SEA [15, 36].

Responses to SWA and SEA were boosted to different extents following praziquantel treatment during pregnancy. This could have a bearing on the morbidity of schistosomiasis during pregnancy, an aspect that was not examined in this study. In both human and experimental murine studies, schistosomiasis morbidity has been closely linked to the host immune responses, with evidence that excess of either type 1 or type 2 responses can cause severe disease [18, 37–39]. Dominance of IFN-γ has been associated with acute and childhood-associated hepatosplenic disease [37, 40, 41], whereas IL-13 together with low IL-10 has been associated with severe hepatic fibrosis [42].

The significant boost in IFN-γ responses observed in the current study has not been reported by related studies involving nonpregnant individuals in areas of high *S. mansoni* transmission [15, 43]. The current study involved pregnant women, and the relatively low prevalence and intensities of infection indi-

cated low *S. mansoni* transmission. Furthermore, posttreatment follow-up times were different. The boost in IFN-γ responses observed in this study (following treatment either during pregnancy or after delivery) but not in high-transmission areas suggests that the praziquantel-induced boost profile depends on transmission and the intensity of infection. In fact, we noted a negative correlation between the IFN-γ boost and the pretreatment infection intensity in this study, although this association was not statistically significant (data not shown).

Studies elsewhere have reported that praziquantel treatment of schistosomiasis due to *S. mansoni* and *Schistosoma hematobium* not only resolves some of the schistosomiasis-associated morbidities, but can also delay resurgence of these morbidities after subsequent reinfection [44, 45]. Thus, praziquantel treatment can induce immunological changes that favor type 2 responses that are associated with resistance to reinfection [27, 46–48] and also prevent or delay onset of specific morbidities in reinfection [44, 45]. Our study has shown that the boost in cytokine responses to schistosome antigens was suppressed following treatment during pregnancy, compared with the boost observed following treatment after delivery. However, the responses among women treated during pregnancy increased substantially after delivery. Moreover, despite the suppressed boost, the cure rate during pregnancy was not significantly different from that after delivery. This suggests that the long-term benefits associated with treatment-induced alterations of the immune responses may not be affected by the observed suppression of the boost in cytokine responses following treatment during pregnancy.

This is first study to demonstrate effects of pregnancy on immune response following praziquantel treatment of schistosomiasis. Further studies are needed to examine the likely impact of the treatment-induced cytokine alterations during pregnancy on morbidity in women and in babies delivered by treated women, in order to assess the morbidity and immune responses in the children and the long-term effects of praziquantel treatment in pregnant women.

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