Increased Human IgE Induced by Killing Schistosoma mansoni In Vivo Is Associated with Pretreatment Th2 Cytokine Responsiveness to Worm Antigens


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In schistosomiasis endemic areas, children are very susceptible to postchemotherapy reinfection, whereas adults are relatively resistant. Different studies have reported that schistosome-specific IL-4 and IL-5 responses, or posttreatment worm-IgE levels, correlate with subsequent low reinfection. Chemotherapy kills i.v. worms providing an in vivo Ag challenge. We measured anti-worm (soluble worm Ag (SWA) and recombinant tegumental Ag (rSm22.6)) and anti-egg (soluble egg Ag) Ab levels in 177 Ugandans (aged 7–50) in a high Schistosoma mansoni transmission area, both before and 7 wk posttreatment, and analyzed these data in relation to whole blood in vitro cytokine responses at the same time points. Soluble egg Ag-IgG levels were unaffected by treatment but worm-IgG1 and -IgG4 increased, whereas worm-IgG increased in many but not all individuals. An increase in worm-IgE was mainly seen in >15-year-olds and, unlike in children, was inversely correlated to pretreatment infection intensities, suggesting this response was associated both with resistance to pretreatment infection, as well as posttreatment reinfection. The increases in SWA-IgE and rSm22.6-IgE positively correlated with pretreatment Th2 cytokines, but not IFN-γ, induced by SWA. These relationships remained significant after allowing for the confounding effects of pretreatment infection intensity, age, and pretreatment IgE levels, indicating a link between SWA-specific Th2 cytokine responsiveness and subsequent increases in worm-IgE. An exceptionally strong relationship between IL-5 and posttreatment worm-IgE levels in <15-year-olds suggested that the failure of younger children to respond to in vivo Ag stimulation with increased levels of IgE, is related to their lack of pretreatment SWA Th2 cytokine responsiveness. The Journal of Immunology, 2006, 177: 5490–5498.

Schistosomiasis is a major public health problem caused by infection with parasitic trematodes of the genus Schistosoma. It is contracted by exposure to fresh water containing cercariae that develop into blood-dwelling adult worms after they have penetrated intact human skin. An estimated 600 million people in 74 countries are at risk of infection, with some 200 million chronically infected. Although a drug, praziquantel (PZQ), is effective for treatment of individual cases, drug-based control programs are hampered by the continued susceptibility of treated individuals, particularly children, to reinfection. In schistosomiasis endemic areas, infection intensities increase with age in young children, peaking around the age of 12 years, before falling to lower adulthood levels (1–3). This age-dependent infection pattern is probably due to the natural death of older worms, which are replaced at a lower rate than they were originally acquired in childhood. This is most clearly demonstrated when chemotherapy is used to remove existing infections and reinfection is monitored over subsequent years. Young children are more susceptible to reinfection than older children or adults, with a striking decrease in susceptibility occurring in the mid-teenage years (4, 5). This reinfection age pattern is present even in fishing communities in which adults have more exposure to infection than children (3).

By examining immune responses pre- and posttreatment, and comparing these with subsequent reinfection intensities, a number of human antischistosome responses have been correlated with reinfection resistance. Parasite-specific IgE is associated with low reinfection for all three major schistosome species that infect man, Schistosoma mansoni (6), S. hematobium (7), and Schistosoma japonicum (8), even after allowing for host age by multiple regression analysis. In areas of Kenya with seasonal S. mansoni transmission, negative correlations between anti-worm IgE (worm-IgE) and subsequent reinfection were found using blood samples taken 3 mo posttreatment but before the occurrence of reinfection. In this study, no other Ab response against worm,
and no Ab response against egg, correlated with posttreatment immunity (6). Worm-IgE responses associated with reinfection immunity include those against the worm tegument and a 22-kDa tegument component. IgE recognition of this 22-kDa Ag (10), or its recombinant form, rSm22.6 (11), were significantly associated with low subsequent reinfection. The 22.6-kDa tegumental molecules, with a high degree of sequence homology to Sm22.6, are also major human IgE-binding Ags in S. japonicum (12, 13) and S. hematobium (14).

Other immunological correlates of human reinfection immunity include peripheral blood eosinophilia (15) and PBMC production of IL-5 and IL-4 in response to parasite Ag in vitro (16–18). Thus, these and other studies (6–9, 19–22) associate human Th2 type cytokine responses, and parasite-specific IgE, with resistance to reinfection. In a human population in which worm-IgE correlated with resistance to reinfection, worm-IgE levels were found to increase by 5 wk posttreatment, whereas anti-egg responses were unchanged (23). IL-4 induced by stimulation with S. hematobium worm Ag also increases after treatment (24), and we have reported worm-specific increases in IL-4, IL-5, and IL-13 after PZQ treatment of S. mansoni-infected Ugandans (25). As PZQ acts rapidly to disrupt the parasite tegument, it produces an i.v. release of multiple parasite Ags, which may contribute to protective immunity in older people in endemic areas, but not their children.

In this study, we focus on changes in IgE and other Ab responses to S. mansoni soluble egg Ag (SEA), soluble worm Ag (SWA), and rSm22.6, induced by treatment in a Ugandan fishing community in an area highly endemic for S. mansoni. We analyze changes in Ab levels before and 7 wk after treatment in relation to host age and pretreatment infection intensity, which are parameters that can also serve as proxies, respectively, for length of exposure to infection and the in vivo Ag dose released by treatment. In addition, we examine the relationships between the changing levels of different Ab isotypes and pre- and posttreatment in vitro cytokine responses to SEA or SWA.

Materials and Methods

Study area and study cohort

This study took place in Booma village on Lake Albert, Uganda, where occupational activities, such as fishing, cause adults to have greater exposure to schistosomiasis mansoni than children (3, 26). A cohort of volunteers were selected as follows: a stratified random sample balanced for sex and age, living close to the lake shore, resident for at least 10 years or since birth, having provided three stool samples for pretreatment parasitological examination, willing to donate blood samples before and after treatment, and having no history of schistosomiasis treatment. The cohort age distribution was deliberately weighted toward the teenage years when the largest changes in parasite burden and immunological response are observed. Pregnant women were excluded. S. mansoni egg counts (egg per gram of stool, epg) were determined on two Kato-Katz thick smears (27) per person sample. The cohort was also examined for gut helminth infections with low subsequent reinfection. In a human population in which worm-IgE correlated with resistance to reinfection, worm-IgE levels were found to increase by 5 wk posttreatment, whereas anti-egg responses were unchanged (23). IL-4 induced by stimulation with S. hematobium worm Ag also increases after treatment (24), and we have reported worm-specific increases in IL-4, IL-5, and IL-13 after PZQ treatment of S. mansoni-infected Ugandans (25). As PZQ acts rapidly to disrupt the parasite tegument, it produces an i.v. release of multiple parasite Ags, which may contribute to protective immunity in older people in endemic areas, but not their children.

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Parasite Ags

The Puerto Rican strain of S. mansoni was maintained in outbred mice and Biomphalaria glabrata. Adult worms were recovered from mice by portal system perfusion 6 wk after infection, washed three times in medium, with care being taken not to damage the outer tegument (28), and snap frozen in liquid nitrogen. SWA was prepared from frozen worms in the presence of protease inhibitors, 1 mM tosylamide-2-phenylethyl chloromethyl ketone, and 1 mM PMSF (Sigma-Aldrich), and parasite eggs were isolated from liver tissue and washed, and saline SEA was prepared from snap-frozen eggs, all as described previously (6, 11). The recombinant tegument worm Ag rSm22.6 was prepared as described previously (11).

Assays

Plasma was prepared from venous blood samples, aliquoted, transported, frozen, and stored at −80°C until required. Human IgE and IgG1–4, IgA, and IgM responses to S. mansoni SWA, SEA; and IgE, IgG1, and IgG4 responses to rSm22.6 were measured using biotin-labeled isotype specific mAb as described previously (29). Plasma samples were assayed at dilutions of 1/200 for IgG1, IgG4, IgM, and IgA; 1/100 for IgG2 and IgG3; and 1/20 for IgE. For these assays, all of the human test plasma samples from the two time points were randomly distributed on 96-well microtiter plates; each plate was tested in triplicate, with each of the triplicate samples being on a different microtiter plate. OD readings for each sample were automatically adjusted and taken into account for adjusting any plate-to-plate variation in readings. Merging of data was done automatically using in-house computer programs. Ab isotype (e.g., IgE) data assayed using SEA or SEA-IgE or SEA-IgG and so on; where worm data from other studies were originally given designations of SEA, E. coli-IgE, and E. coli-IgG, it is referred to here as worm-IgE, and so on. In vitro whole-blood cytokine responses to SEA and SWA were taken from the databases resulting from previous reports based on this same study cohort (25, 30).

Statistical analysis

Egg counts from all of an individual’s stools were totaled and converted to epg. When entered as a covariate in multiple regression models, epg was transformed to the logarithm, log(epg + 1). OD from ELISA samples were analyzed as the logarithm with 0.02 added to all readings to avoid logarithms of nonpositive numbers. The change in Ab responses before and after treatment was calculated as the difference between the logarithms of post- and pretreatment ODs. Three levels of analysis were conducted regarding changes in Ab response: 1) to test the significance of the mean change in response induced by treatment; a simple paired t test; 2) to detect whether the change in response differed between adults (>15 years) and children (<15 years); a one-way ANOVA of the difference on age group; and 3) to detect an association between the change in response and pretreatment egg count (epg), and how this might differ between adults and children: multiple regression of the difference in response on age group, sex, pretreatment epg, and the interaction between age and epg.

Archived data from the Booma study cohort (30) was used to analyze the relationships between cytokines produced by whole-blood cultures stimulated with SEA or SWA, and Ab levels. Cytokine OD readings were transformed to concentration levels using adapted calibration curves (30) and then log transformed (log(cytokine + 1)). To allow an assessment of the effect of specific Ag-induced cytokine production on Ab levels, cytokine production in medium alone was added to all models as a covariate. The relationship between cytokine production and Ab levels was analyzed in three stages. First, estimates for partial correlation coefficients between the effect of specific Ag-induced cytokine production on Ab levels, cytokine production in medium alone, and infection intensity, and pretreatment Ab levels. Second, multiple regressions were used to investigate the effect of pretreatment cytokine production on posttreatment Ab levels after allowing for cytokine production in medium alone, age, infection intensity, and pretreatment Ab levels. Third, the interaction of age with cytokine production with Ag stimulation was analyzed in a separate multiple regression model after adjusting for cytokine production in medium alone.

The influence of gut helminth infections on anti-schistosome Ab and cytokine responses was investigated by using Wilcoxon’s rank sum tests, and resulting p values were corrected for multiple testing. In addition, covariates representing these helminth infections were also entered in all models. The statistical computing language R was used to carry out all the analyses (The R Foundation for Statistical Computing, version 2.1.1 (2005-06-20)).
**Results**

Mean pre- and posttreatment *S. mansoni* infection intensity and prevalence in the cohort are tabulated by age (Table I). Those who donated two blood samples (177 of 275) did not differ significantly from the rest of the cohort in age (23.2 vs 24.5 years), or pretreatment infection intensity (geometric mean of fecal egg count, 269 vs 284 epg). Although the double PZQ treatment was effective in reducing egg counts by 99.4%, initial intensities were high and at least one egg was detected per three posttreatment stools in 19% of individuals. As is commonly observed, infection prevalence posttreatment was higher among children than adults. The pretreatment age patterns of mean Ab isotype to SEA and SWA (Fig. 1) were broadly similar to those we had observed in the neighboring community of Piida (29). Generally, the mean responses against SEA declined, particularly IgG2, or were unchanged with age, whereas most SWA Ab isotype responses, particularly SWA-IgG1, -IgG2, -IgG4, and -IgE, increased (Fig. 1).

The effect of treatment was examined by directly comparing the mean responses of the same individuals before and 7 wk after treatment. Little or no change in the mean level of any SEA response was apparent (Fig. 1) except for SEA-IgE, which modestly increased in the 13- to 16-year-olds. In contrast, most SWA responses clearly increased posttreatment (Fig. 1), with only the SWA-IgM and SWA-IgG3 showing no change from pretreatment levels. SWA-IgG1 and SWA-IgG2 mean OD values increased less in the younger age groups than in older children and adults, whereas SWA-IgA, -IgG4, and -IgE increases were restricted to those older than 15 years of age. There were dramatic posttreatment increases in rSm22.6-IgG1 and -IgG4, peaking in the 13- to 16-year-old group, but with little increase in younger children (Fig. 2). The increase in rSm22.6-IgE was clearly restricted to those older than 13 years of age.

Changes in individual responses, rather than age-group means, to SEA, SWA, and rSm22.6 were examined for IgG1, IgG4, and IgE, the responses that showed the clearest posttreatment increases (Fig. 3). Few individuals had posttreatment changes in SEA-IgG1, -IgG4, and -IgE, and any changes were evenly divided between increases and decreases (Fig. 3a). In contrast, several posttreatment SWA responses (Fig. 3b) increased in many individuals. The majority of individuals SWA-IgG1 and -IgG4 increased, irrespective of pretreatment level, with only a few individuals showing decreases. However, although a number of individuals with low pretreatment SWA-IgE showed dramatically increased levels posttreatment, a significant number remained low. Thus, a broad spectrum of posttreatment SWA responses were seen, particularly for SWA-IgG1 and -IgG4, with most responses being additionally boosted by treatment to give a general upward shift in response across the whole cohort (Fig. 3b). In contrast, only a minority of individuals had detectable pretreatment Ab against rSm22.6, but there was a marked posttreatment increase in these responses, plus the recruitment of additional responders, giving a distinctive L-shaped pattern to the scatter plot, most noticeable for rSm22.6-IgG1 and -IgG4 (Fig. 3c). These observations were quantified and tested statistically (using one-way ANOVA) in Table II.

From Fig. 1, it appeared that, for some SWA-Abs, IgE in particular, the boost in posttreatment response was stronger in adults than children. This was confirmed when the sizes of the boost (i.e., log{ODpost-trt} / log{ODpret-treatment}) in children (≤15 years) and adults (>15 years) were compared by a simple one-way ANOVA. Of the 14 responses illustrated in Fig. 1, a greater adult boost was the most pronounced for SWA-IgE (among adults: mean boost, 36%; 95% confidence interval (CI95%), 19–54%; p < 0.001; among children: mean boost, 1.7%; CI95%, −12 to 17%; p = NS; p value for equality of means, 0.004). The boosting of adult SWA-IgG4 and anti-rSm22.6 IgE was also significantly greater than that for children (p = 0.014, p = 0.041, respectively), whereas SEA-IgA decreased more in children than adults, but not significantly (Figs. 1 and 2).

Further analysis controlled the effects of age on each Ag-specific Ab boost for sex and log pretreatment epg. SWA-IgE and SWA-IgG4 effects remained significant (p = 0.025, p = 0.017), the rSm22.6-IgE effect weakened (p = 0.075), whereas that for rSm22.6-IgG1 became significant (p = 0.023). SWA-IgE had also a strong negative relationship with pretreatment infection level (β = −0.071, p < 0.001), whereas in the case of rSm22.6-IgG1, this regression coefficient was positive (β = 0.163, p < 0.001). When these models were rerun with the addition of a term for the interaction between age group (≤15 or >15 years) and pretreatment infection intensity, the interaction term was found to be significant for both SWA-IgE (F1,112 = 10.7, df = 1 and 172, p = 0.001) and rSm22.6-IgE (F1,112 = 7.5, df = 1 and 172, p = 0.007). In both cases, the larger boosts in adults were associated with lower pretreatment infection intensities, whereas little or no association was seen for children (Fig. 4). Two of the other 15 Ab responses (illustrated in Figs. 1 and 2) also showed weakly significant epag-group interactions, (SEA-IgG3, p = 0.017; rSm22.6-IgG4, p = 0.054), but with no obvious pattern.

We have previously reported the whole blood in vitro pre- and posttreatment cytokine responses to SEA and SWA in this cohort (25). Using these data, we examined relationships between pre- and posttreatment cytokine responses and Ab levels, for all individuals for whom both datasets were available (n = 152).

There was no statistically significant correlation between any posttreatment SEA or SWA cytokine response and the level of any posttreatment Ab (data not shown). There was also no significant relationship between the level of any pretreatment SEA cytokine

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number Bled Twice</th>
<th>Mean EPG</th>
<th>(CI)</th>
<th>Mean Prevalence</th>
<th>7 wk after Treatment</th>
<th>Mean EPG</th>
<th>(CI)</th>
<th>Mean Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>7–9</td>
<td>24</td>
<td>167.5</td>
<td>(54.4, 511.2)</td>
<td>87.5%</td>
<td>3.0</td>
<td>(0.7, 8.1)</td>
<td>36.4</td>
<td></td>
</tr>
<tr>
<td>10–12</td>
<td>28</td>
<td>664.0</td>
<td>(326.4, 1349.6)</td>
<td>96.4%</td>
<td>4.6</td>
<td>(1.4, 11.7)</td>
<td>52.0</td>
<td></td>
</tr>
<tr>
<td>13–16</td>
<td>22</td>
<td>1116.0</td>
<td>(674.9, 1845.0)</td>
<td>100.0%</td>
<td>0.7</td>
<td>(0.2, 1.5)</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>17–23</td>
<td>22</td>
<td>374.3</td>
<td>(139.0, 1005.1)</td>
<td>95.5%</td>
<td>0.1</td>
<td>(−0.1, 0.2)</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>24–30</td>
<td>27</td>
<td>80.2</td>
<td>(32.6, 195.1)</td>
<td>88.9%</td>
<td>0.2</td>
<td>(−0.1, 0.7)</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>31–38</td>
<td>23</td>
<td>93.5</td>
<td>(32.1, 269.0)</td>
<td>87.0%</td>
<td>0.0</td>
<td>−</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>39–50</td>
<td>31</td>
<td>306.0</td>
<td>(153.5, 608.9)</td>
<td>96.8%</td>
<td>0.6</td>
<td>(0.0, 1.6)</td>
<td>17.9</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>177</td>
<td>268.7</td>
<td>(190.0, 379.8)</td>
<td>93.2%</td>
<td>0.9</td>
<td>(0.5, 1.4)</td>
<td>21.3</td>
<td></td>
</tr>
</tbody>
</table>
and any pre- or posttreatment Ab. However, some pretreatment SWA cytokine responses were significantly correlated with both pre- and posttreatment SWA and rSm22.6 Ab levels after adjusting for cytokine produced in medium alone (Table III). These included the pretreatment, Th2-associated SWA cytokine responses, IL-5 and IL-13. However, there were no significant partial correlations of pretreatment IL-4, IL-10, or IFN-γ responses with either concurrent or subsequent Ab levels after adjusting for cytokine production in medium alone. Both pre- and posttreatment SWA-IgE were strongly correlated to pretreatment SWA-IL-5 and -IL-13, the strongest correlation being between pretreatment SWA-IL-5 and posttreatment SWA-IgE ($p < 0.001$).

**FIGURE 1.** Mean Ab isotype levels against SEA and SWA vs host. Number for each age group is given in Table I. Horizontal axis shows mean age of stratum in years. The dashed line represents pretreatment Ab levels, as indicated by ELISA OD values, and the full line represents posttreatment Ab levels. The error bars show the SEM Ab level in each age group.
Because the levels of SWA- and rSm22.6-IgG1, -IgG4, and -IgE were higher in adults compared with children, and the posttreatment boosts in these responses were also greater in adults, the relationships between pretreatment Th2 cytokine responsiveness and Ab levels for adults and children were compared using multiple regression models allowing for cytokine production against medium and the interaction term of age group (≤15 or >15 years) with cytokine production against SWA. Those models showed that the positive relationships between Th2 cytokines and posttreatment Ab in the whole cohort (Table III, posttreatment) were due to very strong relationships between these parameters in children, but not adults. The age effect was strong in the SWA-IL-5 responses against all Abs, i.e., SWA- and rSm22.6-IgG1, -IgG4, and -IgE (p ≤ 0.02), with the strongest age effect being found for SWA-IL-5 against rSm22.6-IgG1 (p = 0.001). This is illustrated in Fig. 5, which shows that only children whose whole blood had a relatively high pretreatment IL-5 response to in vitro SWA had the capacity to produce high levels of circulating SWA-IgE 7 wk after treatment. The difference between age groups was less strong in the SWA-IL-13 responses against rSm22.6-IgG1 and SWA- and rSm22.6-IgG4 and -IgE (p ≤ 0.05).

Because pretreatment and posttreatment levels of individual Ab isotypes were also strongly correlated to each other, even for those isotypes that greatly increased posttreatment (p ≪ 0.0001), and because age and infection intensity influenced the boost in Ab levels, we tested whether the strong correlations between pretreatment cytokines and posttreatment Ab levels remained after allowing for pretreatment Ab levels, as well as for age and infection intensity, in a multiple regression model. We found pretreatment SWA-IL-5 and -IL-13 were both still correlated to posttreatment SWA-IgG and rSm22.6-IgG1, -IgG4, and -IgE. The strongest effects were produced by SWA-IL-5 on SWA-IgE and rSm22.6-IgG1 (both p ≤ 0.0007), followed by SWA-IL-13 on SWA-IgE and rSm22.6-IgG1 (both p < 0.003), and SWA-IL-5 and SWA-IL-13 both on rSm22.6-IgG4 and -IgE (p < 0.04). One other positive significant effect was produced by SWA-IL-10 on posttreatment SWA-IgE (β = 0.074, p = 0.003). Pretreatment IFN-γ

FIGURE 2. Mean Ab isotypes against rSm22.6 vs host age. Number for each age group is given in Table I. The horizontal axis shows the mean age of stratum in years. The dashed line represents pretreatment Ab levels, as indicated by ELISA OD values, and the full line represents posttreatment Ab levels. The error bars show the SEM Ab level in each age group.

Because the levels of SWA- and rSm22.6-IgG1, -IgG4, and -IgE were higher in adults compared with children, and the posttreatment boosts in these responses were also greater in adults, the

FIGURE 3. Scattergrams of Ab levels. Ab levels are represented by ELISA OD values at 7 wk posttreatment vs pretreatment for SEA, SWA, and rSm22.6, and IgG1, IgG4, and IgE. Line y = x represents equal pre- and post-treatment level.
and IL-4 responses were not significantly associated with posttreatment Ab levels after adjusting for pretreatment Ab levels. Thus, in addition to the influence of age, pretreatment infection intensity, and pretreatment Ab levels on posttreatment Ab levels, there was an independent positive effect of the pretreatment S. {mansoni} cytokine responses on 7-wk posttreatment S. {mansoni}-IgE induced by PZQ.

Discussion

Before treatment, the age infection intensity profile was typical of schistosomiasis endemic areas (Table I). Infection intensities, estimated by {S. mansoni} egg excretion, increased with age in young children, peaking in the 13- to 16-year-old age group, and then declined. {S. mansoni} transmission was very high, with mean infection intensity in the 13- to 16-year-old group of >1000 epg. Even the less heavily infected adult age groups had mean fecal egg counts of 80–300 epg. Treatment did not reduce infection intensities in proportion to pretreatment epg, because the age group with highest pretreatment epg, 13- to 16-year-olds, had lower epg than the younger age groups after treatment; thus treatment efficacy was lower in the youngest children. We also noted this in the neighboring community of Piida (26). Because PZQ killing of mature worms is partly dependent on the host’s anti-worm Ab response (31, 32), it is possible that reduced treatment efficacy in the youngest children may be related to their lower Ab levels.

In schistosomiasis endemic areas, children are particularly susceptible to posttreatment reinfection, whereas adults are relatively resistant. Schistosome-specific IgE correlates with resistance to reinfection, as does eosinophilia, and Th2 cytokine responses. Human parasite-specific IgE combined with a variety of FcεR-bearing cell types can mediate killing of schistosome larvae in vitro, although it is not known whether these mechanisms are effective in vivo. Baboons, like man natural hosts for {S. mansoni}, when exposed to infection and cured with PZQ, are up to 80% protected against subsequent infection. An immunological correlate of this immunity is the posttreatment level of worm-IgE (33). In populations living in schistosomiasis endemic areas, anti-parasite Ab levels are strongly age dependent. As in this study, most Ab isotype responses against worm Ag, such as S. {mansoni}, increase with age, whereas SEA responses often decline, or are unchanged. We observed similar relationships between pretreatment S. {mansoni} Ab levels with age in a neighboring Ugandan fishing community (29), in S. {mansoni} endemic areas of Kenya (6, 23) and Brazil (34), and in S. {japonicum} endemic areas of the Philippines (13). Generally similar, but not identical, Ab-age patterns, including an age-dependent increase in worm-IgE, have been reported by others (8, 35, 36).

In this study, chemotherapy was followed by an increase in all S. {mansoni} Abs, except IgM and IgG3 and, with the exception of a modest SEA-IgE increase, largely unchanged levels of SEA-Abs. We have reported increased posttreatment worm-IgE in a Kenyan population, in which this response correlated with subsequent resistance to reinfection, in the absence of change in SEA-Ab (23). Similarly, in Brazilians classified as resistant to {S. mansoni} reinfection, worm-IgE increased posttreatment, suggesting that worm-IgE boosted by treatment is associated with reinfection immunity (20). Schistosome worms live for many years in the blood, and although some Ags are released continuously (37), others are concealed from the host’s immune system. PZQ disrupts the parasite’s outer tegument, exposing underlying Ags (38), which may boost S. {mansoni} Ab levels without boosting in this study were IgG3 and IgM. Anti-schistosome IgG3 responses, in S. {mansoni} endemic areas of Kenya (6, 23) and Brazil (34), and in S. {japonicum} endemic areas of the Philippines (13). Generally similar, but not identical, Ab-age patterns, including an age-dependent increase in worm-IgE, have been reported by others (8, 35, 36).
been reported. A Gambian associations between SEA-IgE and reinfection immunity have increases in younger compared with older children (41). Some infected Zimbabwean children is reported to show greater in-

Children (40), and increased posttreatment SEA-IgE in 15 years) are shown and emphasized using /H11349

Relationship between pretreatment SWA-IL-5 responses to posttreatment SWA-IgE levels in children (≤15 years) are shown and emphasized using a linear regression line.

FIGURE 5. Correlation between SWA-IL-5 and SWA-IgE in children. Relationship between pretreatment SWA-IL-5 responses to posttreatment SWA-IgE levels in children (≤15 years) are shown and emphasized using a linear regression line.

Table III. Estimates for partial correlations of pretreatment cytokine responses to SWA and pre- and posttreatment SWA and rSm22.6 Ab levels in the whole cohort (pretreatment, n = 210, and posttreatment, n = 173) after adjusting for cytokines against medium

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>SWA-IgG1</th>
<th>SWA-IgG4</th>
<th>SWA-IgE</th>
<th>Sm22.6-IgG1</th>
<th>Sm22.6-IgG4</th>
<th>Sm22.6-IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>0.19</td>
<td>0.12</td>
<td>0.16</td>
<td>0.17</td>
<td>0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>IL-5</td>
<td>0.36*</td>
<td>0.29*</td>
<td>0.36*</td>
<td>0.22*</td>
<td>0.23*</td>
<td>0.28*</td>
</tr>
<tr>
<td>IL-13</td>
<td>0.25*</td>
<td>0.20</td>
<td>0.25*</td>
<td>0.13</td>
<td>0.14</td>
<td>0.20</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.17</td>
<td>0.18</td>
<td>0.15</td>
<td>0.12</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>−0.05</td>
<td>−0.10</td>
<td>−0.04</td>
<td>0.00</td>
<td>−0.04</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Posttreatment

<table>
<thead>
<tr>
<th>SWA-IgG1</th>
<th>SWA-IgG4</th>
<th>SWA-IgE</th>
<th>Sm22.6-IgG1</th>
<th>Sm22.6-IgG4</th>
<th>Sm22.6-IgE</th>
</tr>
</thead>
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<tr>
<td>IL-4</td>
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<td>0.11</td>
<td>0.15</td>
<td>0.16</td>
<td>0.09</td>
</tr>
<tr>
<td>IL-5</td>
<td>0.30*</td>
<td>0.25*</td>
<td>0.42*</td>
<td>0.36*</td>
<td>0.29*</td>
</tr>
<tr>
<td>IL-13</td>
<td>0.22</td>
<td>0.19</td>
<td>0.34*</td>
<td>0.26*</td>
<td>0.23</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.16</td>
<td>0.15</td>
<td>0.21</td>
<td>0.17</td>
<td>0.14</td>
</tr>
<tr>
<td>IFN-γ</td>
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<td>−0.02</td>
<td>0.10</td>
<td>0.02</td>
<td>−0.01</td>
</tr>
</tbody>
</table>

*p < 0.0017 (equivalent to p < 0.05 when Bonferroni correction applied).

posttreatment was reported in S. mansoni-infected Ethiopian children (40), and increased posttreatment SEA-IgE in S. hematobium-infected Zimbabwean children is reported to show greater increases in younger compared with older children (41). Some associations between SEA-IgE and reinfection immunity have been reported. A Gambian S. hematobium study (8) found both SEA-IgE and worm-IgE associated with low reinfection, whereas a Chinese S. japonicum study of SEA-Abs (9) reported that an increase in posttreatment SEA-IgE was correlated with subsequent reinfection immunity.

In the present study, the boost in SWA-IgE posttreatment was significantly greater for adults (>15 years) than for children (≤15 years). This contrasts with a previous study of S. hematobium infection in which worm-IgG4, worm-IgE, and SEA-IgE increased 5 wk posttreatment in children but remained unchanged in adults (42). However, in a study of S. hematobium-infected schoolchildren, worm-IgE, -IgM, and -IgG subclasses, all increased by 4 wk posttreatment, particularly in the oldest age group (11–13 years) (36). The same tendency is discernible in our data at 7 wk posttreatment, with older children having greater increases in SWA-IgE than younger children (Fig. 1). However, caution should be exercised with “boosted” responses, because they are essentially interactions and therefore can be dependent on the scale used. Therefore, we repeated the SWA-IgE and SWA-IgG4 assays, using appropriate isotype myeloma proteins for standard curves to give direct estimates of Ab concentrations, and the SWA-IgE as-

says were also repeated using 1/10, rather than 1/20, test plasma dilutions (data not shown). Analysis of these repeated assays gave the same results as the data shown. Thus, the differences in posttreatment changes we observed for responses to SWA, and the differences between adults and children, were robust and may have significance for protection against infection.

We also found that pretreatment infection intensity significantly interacted with posttreatment increases in SWA-IgE and rSm22.6-IgE. When analyzed separately, the greater increases in adults were found to be associated with lower pretreatment infection intensities, whereas there was little or no such association in the children. Thus, whereas posttreatment SWA-IgE levels have been shown to correlate negatively with subsequent reinfection (6), the current study also showed that SWA-IgE induced by treatment was associated with lower pretreatment infection in adults, perhaps indicating a relationship with pretreatment immunity in older individuals. However, pretreatment egg counts are the product of worm burdens that have accumulated over a large and variable number of years, during which the immunological status of each individual may have varied greatly from that currently observed. It is therefore difficult to demonstrate associations between protective immune responses and pretreatment infection intensity. In addition, an individual’s potential response to Ag challenge is not necessarily reflected in current circulating levels of antiparasite effector molecules. The “boostibility” of an individual’s responses to treatment, in this study indicated by increased posttreatment SWA-IgE, may reflect the potential to mount a protective response when required.

The change in individual response patterns to SWA and rSm22.6 were qualitatively different in a way that cannot easily be described statistically (Fig. 3). The wide range of pretreatment SWA-IgG1, -IgG4, and -IgE levels increased proportionally posttreatment. Pretreatment rSm22.6 levels were low, but were boosted to high levels posttreatment, with some continuing nonresponders, giving a characteristic L-shaped pre- vs posttreatment scatter plot. SWA is a complex mixture of worm Ags, which will include Ags that are continuously released in vivo by live worms and others that are sequestered. As treatment exposes sequestered Ags, perhaps causing increased SWA-Ab levels, the striking changes in rSm22.6-Ab levels after treatment suggest that this tegumental worm component may be one such sequestered Ag. Those who continued to be rSm22.6 nonresponders, may be genetically non-responsive to this Ag, or may not have been sensitized by previous experience of in vivo dying worms. The latter circumstance would be more likely to be occurring in children than in adults. It is not
possible to distinguish between age per se and “history of infection” in schistosomiasis endemic areas, because residents are exposed to infection from an early age. However, studies have been conducted in new transmission foci where pretreatment infection intensity is a reasonable estimate of exposure. In the present study, rSm22.6-IgE increased with age. However, when rSm22.6-Abs were assessed in a comparatively new “epidemic” Senegalese focus, posttreatment increases in rSm22.6-IgG1, -IgG4, and -IgE correlated positively with pretreatment infection intensities, but not with age, suggesting that rSm22.6 levels are Ag exposure dependent rather than age dependent per se (43). S. japonicum-infected individuals living in a Chinese endemic area were compared with infected individuals from a new focus. In the endemic area, worm-IgE and -IgG4 increased by 8 wk posttreatment and were significantly correlated with age and number of previous treatments. Worm-Abs also increased in the new focus, but worm-IgE was lower at all time points (44). Both these studies suggest that multiple episodes of infection and worm death may be required to generate the elevated worm-IgE associated with reinfection resistance. A study by Karanja et al. (45) also reported that adults can develop increased resistance to S. mansoni reinfection after multiple PZQ treatments.

IgE and cytokine responses to SEA were low and, relative to worm-Abs, unaltered by PZQ treatment. The low number of SEA responders made any links between cytokine and Ig response difficult to identify. In vitro cytokine production induced by SWA in this cohort increased with treatment, with particularly dramatic increases in IL-4, IL-5, and IL-13 (25). Although there were a number of significant positive correlations between pretreatment Th2-associated cytokines and several pre- and posttreatment Ab isotypes, such relationships were not evident between cytokine responses and Ab levels measured at the same 7 wk posttreatment time point. Differences in the dynamics of Ab and cytokine responses to treatment may account for this disassociation of cytokine responsiveness and Ab levels at the posttreatment time point. There is no reason to expect synchronous posttreatment change in Ab and cytokine, and in the absence of multiple time point sampling, it is not known whether Ab levels or cytokine responses had reached a posttreatment metaequilibrium or a peak by the 7 wk posttreatment time point.

Th2 cytokine responsiveness to SWA predicted increased posttreatment in vivo SWA-IgE. IL-4 is pivotal to Th2 immune skewing and, with IL-13, Ig isotype switching. We did not find significant correlations between SWA-IL-4 responses and Ab levels in the whole cohort. This might have been because SWA-IL-4 responses were much lower than SWA-IL-5 and -IL-13 responses, despite positive correlations between them (30). IL-5 has a major role in the generation, activation, and survival of eosinophils. However, it is ploetrophic in its effects on different cell types, being involved in B cell development, proliferation, and differentiation (46) and enhancing T cell-dependent B cell Ig isotype switching (47). IL-5 responses have been found to correlate with human resistance to reinfection with schistosomes (17) and, more recently, hookworm (48). In relation to hookworm, it was suggested that this was probably due to its role in promoting eosinophil development and function (48, 49).

The biological effects of cytokines, reported from in vitro studies or in murine models, are often difficult to demonstrate immunologically at the human population level. However, the exceptionally high correlations between pretreatment IL-5 and subsequent increased antischistosome worm-IgE levels, which were still statistically significant after allowing for effects of age, infection intensity, and pretreatment IgE levels, strongly supports the idea that the association of reinfection resistance with both pretreatment IL-5 (17, 22) and posttreatment IgE (6) may be causally linked to form, together with eosinophils, an important and coordinated human anthelminth response. As hypothesized by Woolhouse and Hagan (50), these responses may be progressively enhanced with host age by the experience of i.v. worm death, repeated over many years, and this may well be the basis for the slow development of adult protective immunity in human populations living in schistosomiasis endemic areas.

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Disclosures

The authors have no financial conflict of interest.

References


