

## Chemotherapy Accelerates the Development of Acquired Immune Responses to *Schistosoma haematobium* Infection

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Treatment of 41 *Schistosoma haematobium*-infected children, 5–16 years old, with the drug praziquantel induced a switch from a predominantly IgA-specific antibody response to a predominantly IgG1 response within 12 weeks. A cross-sectional survey suggests that the same switch occurs naturally, but over several years, as children age ( $n = 251$ ). The switch may be driven by alterations in cytokine levels in response to the release of antigens by dead or damaged parasites. Adults are more resistant to schistosome infection than children, and the switch to an “adult” response suggests that praziquantel treatment may have an immunizing effect, with benefits extending beyond a transient reduction in levels of infection.

Praziquantel (a pyrazinoisoquinoline derivative) is widely used to treat human schistosome infections [1]. Praziquantel has two main effects on schistosomes (paralysis and tegument damage) and is highly effective, giving cure rates typically in the range 75%–100% [1]. Recently, it has been demonstrated that treatment of schistosome infection with praziquantel induces changes in specific antibody responses, especially in children [2, 3]. This result has a number of important implications: that antigens released by dead or damaged schistosomes alter the immune response, that a natural protective response to infection may not develop until some schistosomes die, and that praziquantel treatment of infected people may itself have an immunizing effect by accelerating the acquisition of immune responses which confer increased resistance to infection (i.e., those found in adults rather than children). However, there has not been a detailed comparison of changes in immune responses with age to those following chemotherapy.

The explanation of why levels of schistosome infection are typically higher in children than in adults remains controversial [4]; adults may have reduced exposure, lower innate susceptibility, or enhanced protective immunity compared with children. Acquired immunity has been demonstrated in animal

models [5], and a broad spectrum of immune responses to schistosomes has been described in naturally infected people. For example, specific IgE responses are associated with low levels of infection or reinfection following treatment for both *Schistosoma haematobium* and *Schistosoma mansoni* [6–8]. It is clear from these studies that immune responses develop slowly, over many years, even in areas with high levels of transmission.

Here we report the results of a field study of *S. haematobium* infections and associated immune responses in an endemically infected community in eastern Zimbabwe. We compared changes in antibody profiles, especially IgA and IgG1 responses, following treatment with praziquantel with changes that occur naturally with age.

### Methods

**Study site.** The study was carried out among the residents of two farm compounds in the Burma Valley district of eastern Zimbabwe, where *S. haematobium* is endemic [9].

**Sample collection.** A cross-sectional parasitologic survey was conducted during August to November 1994 in the two local schools and among persons who attended special clinics held in the farm compounds. Only permanent residents were included in the study, giving a total of 320 individuals between 1 and 67 years old who provided 2 or 3 urine specimens and 1 stool specimen. Specimens were screened for *S. haematobium*, *S. mansoni*, and geohelminth eggs using standard methods [9]. The 27 persons (9%) who excreted *S. mansoni* or geohelminth eggs were excluded from further study. No individuals reported prior treatment with anthelmintics. At the same time, a single blood specimen was collected from each individual. A cohort of schoolchildren 5–16 years old was treated with praziquantel at the recommended dose of 40 mg/kg during August to November 1994, and parasitologic and serologic surveys were conducted, as described, at 6-week intervals for 36 weeks; here we focus on data collected 18 weeks

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The study was approved by the Medical Research Council of Zimbabwe, and informed consent was obtained from the study population. Treatment was offered to all of the participants after initial screening and to all members of the follow-up group at the end of the study.

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after treatment. There were 173 treated children, of which 101 were egg-positive before treatment. One was still egg-positive after 6 weeks and was excluded as a failed treatment. Of the remainder, 143 children participated in the parasitologic follow-up surveys, and sera were collected from 71 of these (chosen at random), 41 of whom were egg-positive and 30 who were egg-negative before treatment.

**Serology.** Soluble egg antigen (SEA) and whole worm homogenate (WWH) were provided freeze-dried by the Theodore Bilharz Research Institute in Cairo and were reconstituted with PBS. Antibody levels were determined by a standard indirect ELISA. The ELISA plates were coated with 100  $\mu$ L of antigen in carbonate bicarbonate buffer (pH 9.6) at 10  $\mu$ g/mL and left overnight at 4°C. All plates were then emptied and blocked with 200  $\mu$ L of 5% skimmed milk for 1 h at room temperature. Following this, the plates were washed three times using PBS (pH 7.4) and 100  $\mu$ L of sera was plated out in duplicate at 1:100 for IgA and 1:10 for IgG1. All plates were incubated for 2 h at room temperature and washed three times with PBS; then 100  $\mu$ L of monoclonal antibody was added at 1:2000 dilution (using horseradish peroxidase-conjugated mouse anti-human IgG1 (MCA514P; Serotec, Oxford, UK) and goat anti-human IgA (A-7032; Sigma, St. Louis). The plates were incubated for 1 h at room temperature and washed six times with PBS; then 100  $\mu$ L of *o*-phenyldiamine in phosphate-citrate buffer (pH 5) was added. The enzyme reaction was allowed to take place for 15 min for IgA and for 5 min for IgG1, after which the reaction was stopped with 25  $\mu$ L of 10% H<sub>2</sub>SO<sub>4</sub> and the absorbance read at 492 nm. Sera and monoclonal antibodies were diluted in PBS (pH 7.4).

Three standards were run in duplicate on each plate: a positive control, made from a pool of 10 sera from people presenting the highest egg counts across the age range from both areas; a negative control, made from a pool of 10 Norwegian sera also across the age range; and a blank control, containing no sera. The background absorbance of reagents independent of sera was subtracted from the results. Not all sera were successfully assayed for all isotypes.

The two monoclonal antibodies (anti-human IgA and anti-human IgG1) have different specificities and do not cross-react. When significant responses occurred, those to SEA and WWH were strongly correlated: for IgA in the cross-sectional study for children up to 16 years old (Pearson's  $r = +0.28$ ,  $n = 134$ ,  $P < .001$ ) and for IgG1 in the cross-sectional study for adults (Pearson's  $r = +0.51$ ,  $n = 85$ ,  $P < .001$ ) and in the schoolchildren cohort 18 weeks after treatment (Pearson's  $r = +0.66$ ,  $n = 56$ ,  $P < .001$ ). Only results for SEA are reported here; WWH gave similar patterns throughout. Cross-reactivity between these responses is expected, given that adult female schistosomes contain developing eggs.

**Statistical analysis.** All statistical analyses were done using the SPSS statistical package (SPSS, Chicago) and, as appropriate, included sex and age group (figure 1) as categorical, infection as categorical (positive or negative, i.e., zero, egg count), and absorbances (square root-transformed) or changes in absorbances (untransformed) as continuous variables.

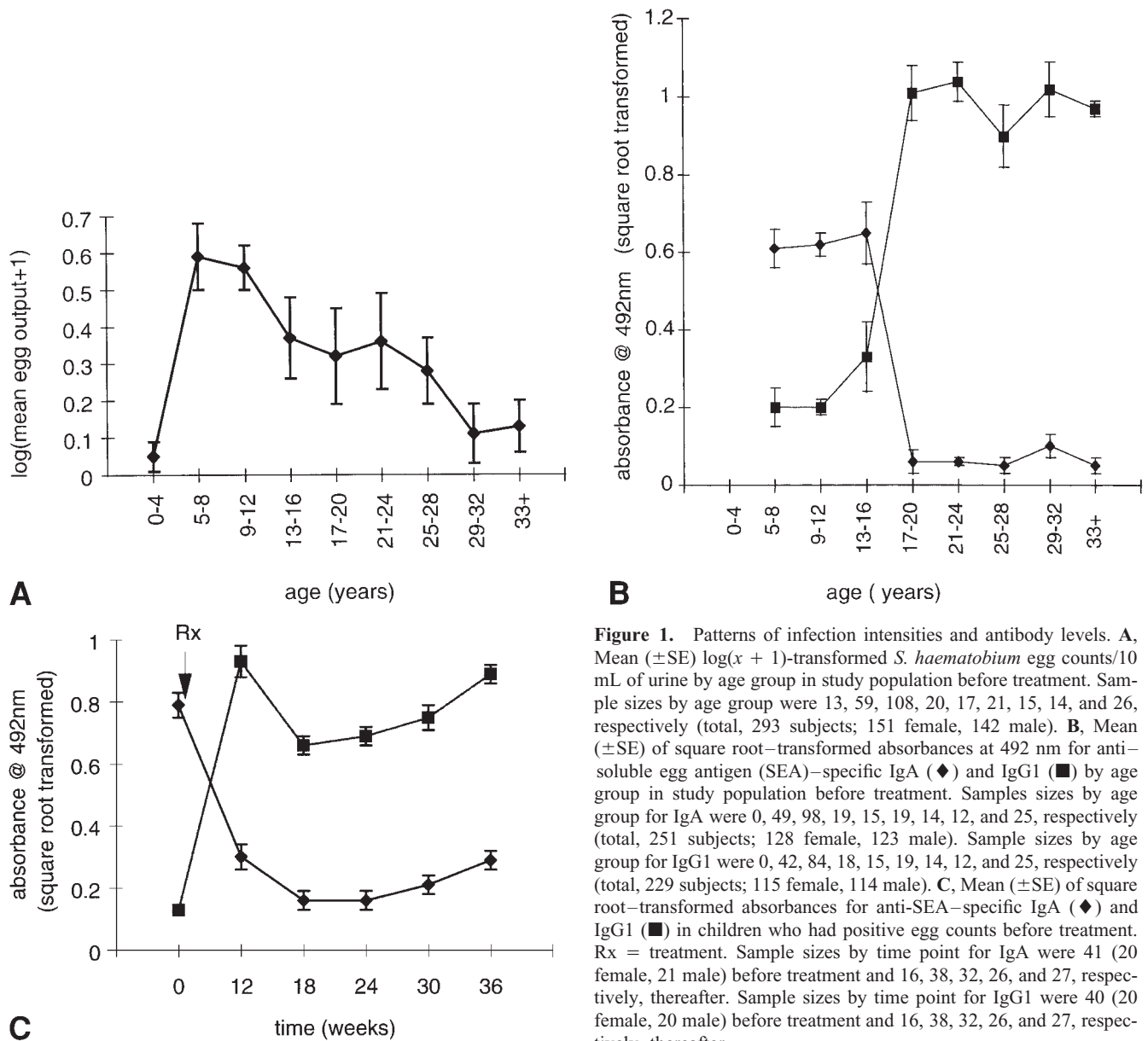
## Results

The prevalence of *S. haematobium* infection in the study population before treatment was 49% overall, 58% in subjects

5–16 years old, and 35% in those >16. The arithmetic mean infection intensity was 14.3 eggs/10 mL of urine, peaking between 5 and 12 years old (figure 1A). Levels of IgA were high in children up to 16 years old and were low in adults; levels of IgG1 showed the reverse pattern (figure 1B), suggesting a marked shift in antibody production in late childhood. General factorial model analysis showed that high IgA level was associated with male rather than female subjects ( $F$  statistic with 1 and 192  $df$  [ $F_{1,192}$ ] = 21.9,  $P < .001$ ), with children rather than adults ( $F_{7,192} = 71.8$ ,  $P < .001$ ), with positive egg counts ( $F_{1,192} = 23.5$ ,  $P < .001$ ), and with low levels of IgG1 ( $F_{1,192} = 57.5$ ,  $P < .001$ ), with an interaction between age and IgG1 ( $F_{7,192} = 6.1$ ,  $P < .001$ ). Similarly, high IgG1 level was associated with female rather than male subjects ( $F_{1,192} = 59.5$ ,  $P < .001$ ), with adults rather than children ( $F_{7,192} = 101.4$ ,  $P < .001$ ), and with a low level of IgA ( $F_{1,192} = 52.8$ ,  $P < .001$ ), but not with egg count, with an interaction between age and IgA ( $F_{7,192} = 3.45$ ,  $P < .01$ ). That is, high levels of IgA within age groups were associated with infection but high levels of IgG1 were not, although levels of the two antibodies were strongly negatively correlated.

The prevalence of infection in the cohort was 58% before treatment, but reinfection rates were low; only 2 individuals (2%) were reinfected after 18 weeks. Levels of IgA in infected children were high before treatment and low after treatment but levels of IgG1 showed the reverse pattern, suggesting a marked shift in antibody production after treatment (figure 1C). Levels of IgA in treated, infected children were slightly higher than those in untreated adults, and levels of IgG1 were slightly lower. Results were very similar throughout the follow-up period (figure 1C) and we focus on results at 18 weeks after treatment. The increase in IgG1 levels at 18 weeks was significantly greater for egg-positive than egg-negative children (analysis of variance,  $F_{1,59} = 9.8$ ,  $P < .01$ ) but was independent of age and sex. The decrease in IgA absorbance showed similar trends, but these were not statistically significant. However, there was a significant increase in IgG1 levels in egg-negative children, possibly due to the presence of undetected schistosomes (from immature, single-sex, or very light infections) in many of these children.

Inspection of antibody levels in untreated children up to 16 years old showed that most had high levels of IgA and low levels of IgG1, though a minority (including 1 child only 7 years old) had high levels of IgG1 and low levels of IgA (figure 2A). All untreated individuals >16 years old had low levels of IgA and most had high levels of IgG1 (figure 2B). The population as a whole showed a shift from a predominantly IgA response to a predominantly IgG1 response between 7 and 15 years of age, although for any single individual, the shift may be much more rapid. Before treatment, the egg-positive children up to 16 years old all showed a predominantly IgA response (figure 2A), but 18 weeks after treatment, all had low levels of IgA and most had high levels of IgG1 (figure 2C).



**Figure 1.** Patterns of infection intensities and antibody levels. **A**, Mean ( $\pm$ SE)  $\log(x + 1)$ -transformed *S. haematobium* egg counts/10 mL of urine by age group in study population before treatment. Sample sizes by age group were 13, 59, 108, 20, 17, 21, 15, 14, and 26, respectively (total, 293 subjects; 151 female, 142 male). **B**, Mean ( $\pm$ SE) of square root-transformed absorbances at 492 nm for anti-soluble egg antigen (SEA)-specific IgA (◆) and IgG1 (■) by age group in study population before treatment. Samples sizes by age group for IgA were 0, 49, 98, 19, 15, 19, 14, 12, and 25, respectively (total, 251 subjects; 128 female, 123 male). Sample sizes by age group for IgG1 were 0, 42, 84, 18, 15, 19, 14, 12, and 25, respectively (total, 229 subjects; 115 female, 114 male). **C**, Mean ( $\pm$ SE) of square root-transformed absorbances for anti-SEA-specific IgA (◆) and IgG1 (■) in children who had positive egg counts before treatment. Rx = treatment. Sample sizes by time point for IgA were 41 (20 female, 21 male) before treatment and 16, 38, 32, 26, and 27, respectively, thereafter. Sample sizes by time point for IgG1 were 40 (20 female, 20 male) before treatment and 16, 38, 32, 26, and 27, respectively, thereafter.

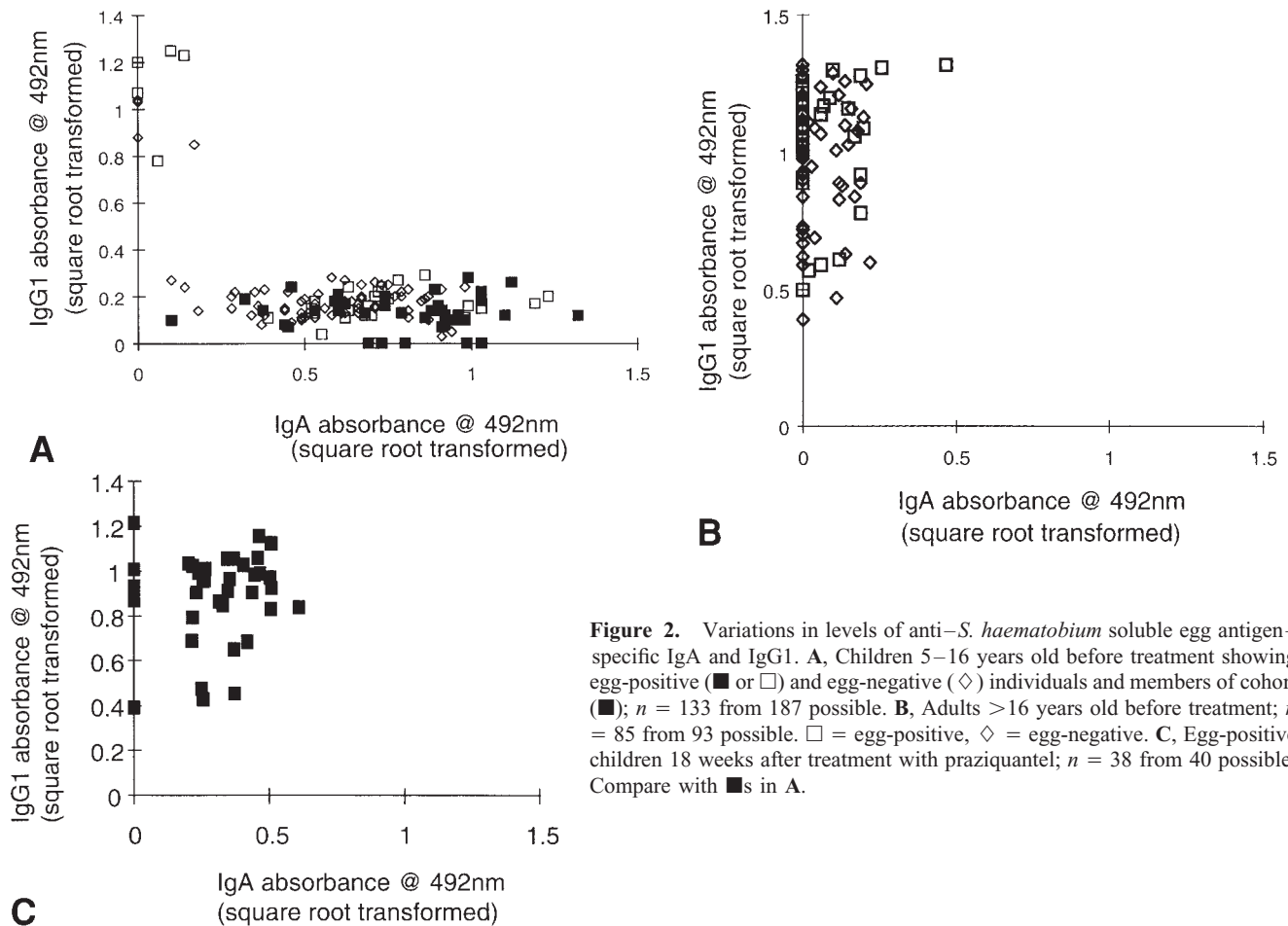
This “adult” antibody profile was seen in treated individuals as young as 6 years old.

**Discussion**

IgA and IgG1 responses to schistosome infection have distinct characteristics but both may be protective. The IgA response toward Sm28-GST (the 28-kDa enzyme glutathione-S-transferase from *S. mansoni*) has been associated with a significant impairment of the schistosome egg-laying and hatching of eggs into miracidia in *S. mansoni* [10]. In contrast, IgG1 responses are directed against a range of carbohydrate and

peptide epitopes [11] and play a major role in the killing of larval schistosomes [3, 12]. In the cross-sectional study reported here, the fall in egg counts (at 13–16 years old) precedes the switch from an IgA to an IgG1 response (at >16 years old) (figure 1). This is consistent with accumulated exposure to antigens from dead worms and the reduced number of eggs inducing the switch. The fall in egg counts itself may be due to nonimmunologic as well as immunologic factors [4].

The capacity of praziquantel to alter the immune response to schistosomes may stem from damage caused to the schistosome tegument, releasing subsurface antigens that would not otherwise be available to the host immune system [1] combined



**Figure 2.** Variations in levels of anti-*S. haematobium* soluble egg antigen-specific IgA and IgG1. **A**, Children 5–16 years old before treatment showing egg-positive (■ or □) and egg-negative (◇) individuals and members of cohort (■);  $n = 133$  from 187 possible. **B**, Adults >16 years old before treatment;  $n = 85$  from 93 possible. □ = egg-positive, ◇ = egg-negative. **C**, Egg-positive children 18 weeks after treatment with praziquantel;  $n = 38$  from 40 possible. Compare with ■s in **A**.

with a reduction in numbers of eggs. Praziquantel efficacy is enhanced by prior immunization with schistosome tegument antigens [13] and by passive transfer of immune sera [14]. However, the potential mechanisms controlling a switch from IgA to IgG1 production are not well understood. Interleukin (IL)-4, IL-5, and IL-10 are all associated with both IgA and IgG1 production [15, 16] and transforming growth factor- $\beta$  is also associated with IgA production [17]. The roles of these cytokines and growth factors warrant further investigation.

Taken together, our observations suggest the following hypothesis: The release of subsurface antigens from dead or damaged schistosomes and a reduction in numbers of eggs stimulates a shift to a predominantly IgG1 antibody response from a predominantly IgA response. This change occurs naturally as schistosomes die; their long life expectancy, at least 3 years, may help explain slow development of acquired immunity. The shift can be dramatically accelerated by praziquantel treatment, inducing “adult”-like responses even in young children. These changes may be associated with increased partial protection against schistosome infection, implying that chemotherapy may

have an “immunizing” effect and that the benefits of treatment may extend beyond a transient reduction in levels of infection.

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