

FACTORS AFFECTING HIGH AND LOW HUMAN IgE RESPONSES TO SCHISTOSOME WORM ANTIGENS IN AN AREA OF BRAZIL ENDEMIC FOR *SCHISTOSOMA MANSONI* AND HOOKWORM

MICHELLE WEBSTER, RODRIGO CORREA-OLIVEIRA, GIOVANNI GAZZINELLI, IRAMAYA R. C. VIANA, LUCIA ALVES DE OLIVEIRA FRAGA, ALDA MARIA SOARES SILVEIRA, AND DAVID W. DUNNE

Department of Pathology, University of Cambridge, Cambridge, United Kingdom; Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, Minas Gerais, Brazil; Cenbios-Univale-Universidade Vale do Rio Doce, Governador Valadares, Minas Gerais, Brazil

Abstract. We have previously examined the antibody isotype responses to schistosome worm and egg antigens in human populations living in areas of Kenya and the Philippines endemic for *Schistosoma mansoni* and *S. japonicum*, respectively. Here, we have analyzed antibody isotype responses to *S. mansoni* adult worm (AW) antigen and soluble egg antigen (SEA) in more than 500 Brazilian individuals, and found similar relationships with age and sex as in the Kenyan and Filipino populations. Isotype responses to AW antigen broadly increased with age whereas isotype responses to SEA decreased, and a higher proportion of males than females had detectable IgE against AW antigen. Most isotype responses to AW antigen and SEA correlated positively with intensity of infection with *S. mansoni* except AW antigen-specific IgG₂, which correlated negatively. The overall prevalence of infection with *S. mansoni* in this area was relatively low at only 39.5%; hookworm prevalence was higher at 57.4%. The majority of those infected with *S. mansoni* were also infected with hookworm (76%). Those individuals with high IgE responses to AW antigen were matched for sex, age, and total IgG to AW antigen as closely as possible with individuals with low levels of AW antigen-specific IgE. The two groups were compared for factors potentially influential in IgE production. No difference was found between the high and low IgE responders for 1) intensity or prevalence of infection with *S. mansoni*, 2) relative exposure to *S. mansoni*, 3) number of previous treatments for schistosomiasis, or 4) prevalence of infection with hookworm, but differences were found in other isotype responses to *S. mansoni*. The high IgE responders had higher IgA and IgG₄ against both AW antigen and SEA but lower IgG₃ responses to AW antigen than the low IgE responders. The IgE responses to three *S. mansoni* antigens (paramyosin, Sm22.6, and a 12-kD AW antigen band) were detected in individuals with IgE against AW antigen only.

Studies of human immunity to *Schistosoma* spp. in endemic populations have indicated that parasite-specific antibody isotypes vary in their association with either susceptibility or resistance to reinfection. Immunoglobulin E and IgA, which can mediate antibody-dependent cellular cytotoxicity of schistosomula in vitro,^{1–5} are associated with subsequent resistance to reinfection following treatment,^{6–11} whereas IgM, IgG₂, and IgG₄ are associated with susceptibility to reinfection.^{6, 12–16} In both scenarios the levels of specific antibodies vary with age. We have previously examined antibody isotype responses to schistosome adult worm (AW) antigen and soluble egg antigen (SEA) in sera from human populations living in areas of Kenya and the Philippines endemic for *S. mansoni* and *S. japonicum*, respectively.¹⁷ Despite differences between these two populations in terms of host genetics, exposure to the parasite, socioeconomics, and infection with different species of schistosomes, the parasite-specific immune responses were strikingly similar. For example, IgE against AW antigen broadly increased with age whereas most isotype responses to SEA decreased with age.¹⁷ In addition, levels of IgG₁, IgG₄, and IgE against AW antigen were higher in the males compared with the females.¹⁷ The same methods used in the Kenyan and Filipino studies¹⁷ have been used here to examine the pattern of isotype responses to AW antigen and SEA in a population from a *S. mansoni*-endemic area of Brazil, comprising more than 500 individuals, with particular reference to the relationships between isotype responses and age and sex. The association between *S. mansoni*-specific IgE levels and resistance to reinfection has been observed with post-treatment responses to schistosomal and adult worm stages only,^{7, 8} and we have

found that parasite-specific IgE responses do not correlate with total serum IgE levels (unpublished data). This indicates that the association between parasite-specific IgE and resistance to reinfection is not due simply to a general increase in IgE.

Production of IgE follows sequential isotype switching from IgM to IgE via IgG₄ and there is increasing evidence for the regulation of such antibody production by cytokines. The production of IgE is stimulated by interleukin-13 (IL-13) and IL-4, and modulated by IL-12 and interferon-gamma.^{18–21} The action of IgE may be blocked specifically by IgG₄.^{6, 15, 16} Production of IgG₄ is also stimulated by IL-4,¹⁹ but it is unclear as to whether these two isotypes are coregulated or independently regulated. Some studies have found in vivo that parasite-specific IgG₄ levels correlate with parasite-specific IgE levels,^{17, 22} suggesting that they may be coregulated, whereas other studies found no correlation between serum levels of these two isotypes, indicating that they may be independently regulated.^{23–25} Interferon-gamma, IL-8, IL-9, IL-10, IL-13, tumor necrosis factor-alpha, and prostaglandin E₂ may also be involved in the regulation of IgE production.

In light of the variability in isotype levels with age and sex,¹⁷ investigation of the control of parasite-specific IgE production would need to allow for such variation. In addition to examining whether or not parasite specific antibody isotype responses in this Brazilian population conformed to the age and sex patterns we have observed in Kenya and the Philippines, we have also initiated studies on the control of parasite-specific IgE production by identifying individuals with high and low IgE responses to AW antigen. The indi-

viduals were matched for age, sex, and total IgG responses to AW antigen as closely as possible in the absence of any reference to intensity of infection. The two groups were then compared for factors that may be influential in IgE production.

MATERIALS AND METHODS

The same methods as used previously when studying Kenyan and Filipino populations were used to enable comparison of the results.^{8, 17}

Antigen preparation. The life cycle of a Puerto Rican strain of *S. mansoni* was maintained in outbred TO mice (Tuck Ltd., Battlebridge, United Kingdom) and the snail *Biomphalaria glabrata*. Adult worms were retrieved by perfusion of the hepatic portal system.²⁶ Soluble *S. mansoni* AW antigen was the supernatant obtained after adult worms were homogenized in liquid nitrogen,¹⁷ and tegument-stripped *S. mansoni* adult worm was a phosphate-buffered saline (PBS) extract of adult worms prepared as described previously.⁸ Soluble *S. mansoni* SEA was the supernatant obtained after eggs were homogenized in PBS.²⁷

Recombinant Sm22.6 (rSm22.6) was prepared by electroelution as described previously.⁹ Native paramyosin and a 12-kD adult worm band were also prepared by electroelution; paramyosin from sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) on a 6% gel²⁸ of tegument-stripped AW antigen and the 12-kD band from SDS-PAGE on a 14% gel²⁸ of AW antigen. The bands at 97 kD and 12 kD, respectively, were cut out of the appropriate gels and electro-eluted at 8–10 mA for 3–5 hr. The purity of rSm22.6 and the 12-kD adult worm band were checked by Western blot analysis with monospecific polyclonal rabbit sera raised against each antigen. Similarly, the purity of paramyosin was checked by Western blot analysis with an anti-paramyosin monoclonal antibody (kindly provided by Dr. Edward Pearce, Cornell University, Ithaca, NY).

Enzyme-linked immunosorbent assay. Anti-human isotype antibodies were used to quantify the AW antigen and SEA-reactive IgA, IgE, IgG, IgG₁, IgG₂, IgG₃, IgG₄, and IgM present in individual human infection sera by ELISA, using reagents described in detail elsewhere.^{8, 9, 17} The AW antigen was used at a concentration of 8.4 µg/ml for the detection of reactive IgE and at 4.2 µg/ml for all other assays. Recombinant Sm22.6, paramyosin, and 12-kD band-reactive IgE and IgG were quantified as described previously for rSm22.6.⁹ In all assays, sera were used at a dilution of 1:200 for the detection of IgG, IgG₁, IgG₄, IgA and IgM, at 1:100 for the detection of IgG₂ and IgG₃, and at 1:20 for the detection of IgE. Each assay was performed in triplicate and the results were expressed as absorbance units. The optical density (OD) values were adjusted for plate-to-plate variation by means of an internal standard.

Study population. The procedures for serum and stool collection followed the guidelines laid down by the Helsinki Convention and were approved by the FIOCRUZ ethics committee. All information was given to the patients and their written informed consent was obtained (from parents on behalf of children where necessary) prior to the study. Approximately 8 ml of whole blood was collected from 534 randomly selected inhabitants (ranging in age from 5 to 84

years) of Corrego do Bernardo (Governador Valadares County), an area that is endemic for *S. mansoni* and which is situated in Minas Gerais, Brazil. Blood was collected from as many patients as possible within Corrego do Bernardo, including two schools. A special effort was made, however, to recruit males between 25 and 35 years of age. Normal, uninfected human serum was obtained from residents in the United Kingdom with no history of schistosomiasis. Corresponding intensities of infection with *S. mansoni* for each individual were determined from duplicate slides of a single stool examination using the Kato-Katz technique. Prevalence of infection with *S. mansoni* was defined as a score of ≥ 1 eggs/gram of feces. The same stool samples were also used to assess qualitatively the presence of infection with hookworm (*Necator americanus*), *Ascaris lumbricoides*, *Trichuris trichiura*, and/or *Taenia solium*. Prevalence of infection with hookworm was defined as the detection of one or more eggs in the stool sample. All *S. mansoni*-infected individuals were treated with oxamniquine (Pfizer, Guarulhos, Sao Paulo, Brazil) according to the standard Brazilian dose of 15 mg/kg for adults and 20 mg/kg for children. Some individuals had been treated previously for schistosomiasis mansoni in 1990–1991. All individuals with hookworm, *Ascaris*, or *Trichuris* infections were treated with 600 mg of mebendazole (Central de Medicamentos, Rio de Janeiro, Brazil) (one week after treatment with oxamniquine where necessary). Treatment, clinical evaluation, and medical attention was offered to all individuals in the area, regardless of whether or not they participated in this project. Relative exposure to the parasite was estimated from the length of residence in Corrego do Bernardo and the frequency of water contact, both of which were determined during an interview with each patient.

Selection of the high and low IgE responders. From the population of 534 individuals, the 48 individuals with IgE levels against AW antigen of OD_{490 nm} values > 0.035 in the ELISA (an arbitrary cut-off value; Figure 1) were classified as high AW antigen-specific IgE responders and used to form a group of high IgE responders. They were matched first for sex, and then for age and IgG responses to AW antigen, as closely as possible, with individuals from the same population who had low levels of IgE against AW antigen. The pairs were classified as a group of low IgE responders. Following this selection procedure, two individuals left the study area. They were removed from all analysis, together with their respective pair, leaving 46 patients per group. All individuals in the high and low IgE responder groups were clinically examined for hepatosplenomegaly.

Statistical analysis. In analysis of the whole population, relationships between immune responses to AW antigen or SEA and age or intensity of infection with *S. mansoni* were examined by Spearman's rank correlation coefficient. Coinfection with *S. mansoni* and hookworm was analyzed by Pearson's chi-square test. Significant differences between the high and low IgE responders in mean intensity of infection and mean isotype responses to AW antigen and SEA were determined using the nonparametric Mann-Whitney test for unpaired data not normally distributed. Differences in prevalence of infection with *S. mansoni* and hookworm between the high and low IgE responders were analyzed by Pearson's chi-square test.

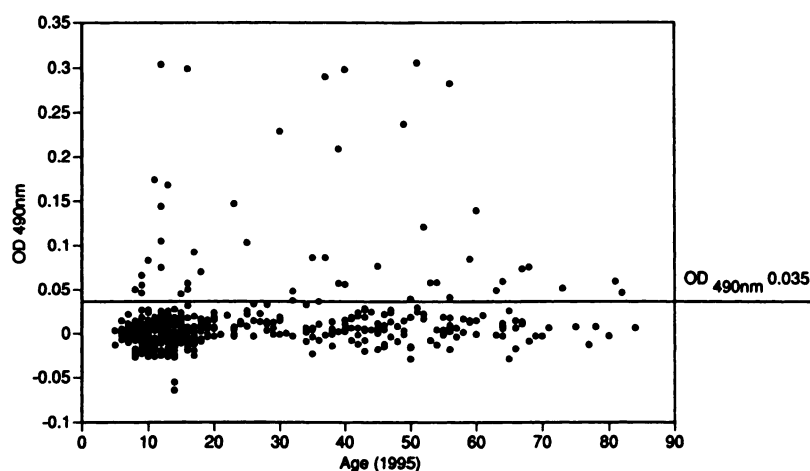


FIGURE 1. Individual IgE responses to *Schistosoma mansoni* adult worm antigen by age (in years). Results are expressed as mean absorbance values ($A_{490\text{ nm}}$) of triplicate assays. The line at an optical density of 490 nm ($OD_{490\text{ nm}}$) 0.035 indicates the arbitrary cut-off value for high IgE responders.

RESULTS

The overall prevalence of infection was 39.5% with *S. mansoni* and 57.4% with *N. americanus*; *A. lumbricoides*, *T. trichiura*, and *Taenia solium* were found to be of negligible prevalence (< 1%). Spearman's rank correlations suggested the presence of a negative association between age and intensity of infection with *S. mansoni* ($r = -0.136$, $n = 415$, $P < 0.05$). The majority of people infected with *S. mansoni* were also infected with hookworm (125 of 164; 76%). Pearson's chi-square test indicated that those infected with hookworm had a higher probability of also being infected with *S. mansoni* ($\chi^2 = 39.474$, $P < 0.001$). Of those who were infected with hookworm, 52.5% (125 of 238) were also infected with *S. mansoni*, compared with 22% (39 of 177) of those who were not infected with hookworm.

Antibody isotype responses to *S. mansoni*. Table 1 shows the Spearman's rank correlations between different antibody isotype responses to *S. mansoni* AW antigen or SEA and age or intensity of infection with *S. mansoni*. With the exception of IgG₂, all isotype responses to AW antigen correlated positively with intensity of infection and age. Most isotype responses to SEA correlated positively with intensity of infection

and negatively with age. Figure 1 shows the individual IgE responses to AW antigen by age and highlights how relatively few individuals in this population had high levels of IgE against AW antigen. A high AW antigen-specific IgE responder was arbitrarily defined as one with IgE against AW antigen of greater than $OD_{490\text{ nm}}$ 0.035 in the ELISA. The relationship between this arbitrary cut-off value and the level of individual responses is illustrated in Figure 1. The response by normal, uninfected human serum approached zero. Using this definition, only 48 (approximately 9%) of the 534 individuals tested could be classified as AW antigen-specific IgE responders, who varied in age from eight to 82 years. Although there were slightly more female than male AW antigen-specific IgE responders (28 females versus 20 males), there were proportionally more males. Figure 2 shows the proportion of male and female AW antigen-specific IgE responders in four age groups (i.e., 5–10 years, 11–14 years, 15–34 years, and 35–84 years) of approximately equal size. As well as showing that there were proportionally more male than female AW antigen-specific IgE responders,

TABLE 1
Correlations between age or intensity of infection with *Schistosoma mansoni* and different antibody isotype responses to *S. mansoni* adult worm and soluble egg antigens

	Adult worm antigens*		Soluble egg antigens*	
	<i>S. mansoni</i> intensity†	Age‡	<i>S. mansoni</i> intensity†	Age‡
IgA	0.198§	0.335§	0.085	0.008
IgE	0.141§	0.248§	-0.022	-0.023
IgG ₁	0.417§	0.097§	0.257§	-0.186§
IgG ₂	-0.151§	-0.073	0.119§	-0.077
IgG ₃	0.071	0.174§	0.063	0.170§
IgG ₄	0.349§	0.173§	0.590§	0.034
IgM	0.398§	0.098§	0.466§	-0.136§
IgG	0.067	0.163§	0.232§	-0.041

* Spearman's rank correlation coefficient.

† Minimum number of observations was 415.

‡ Minimum number of observations was 523.

§ $P < 0.05$.

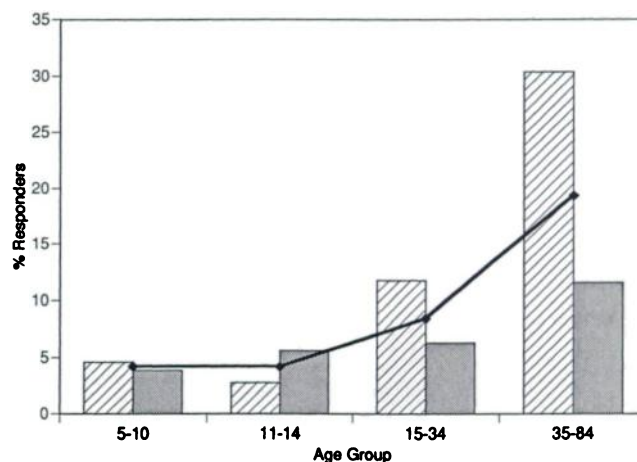


FIGURE 2. Percentage of individuals in the whole population (dark line) who were male (striped bars) and female (dotted bars), by age group (in years), who were classified as high IgE responders in the ELISA.

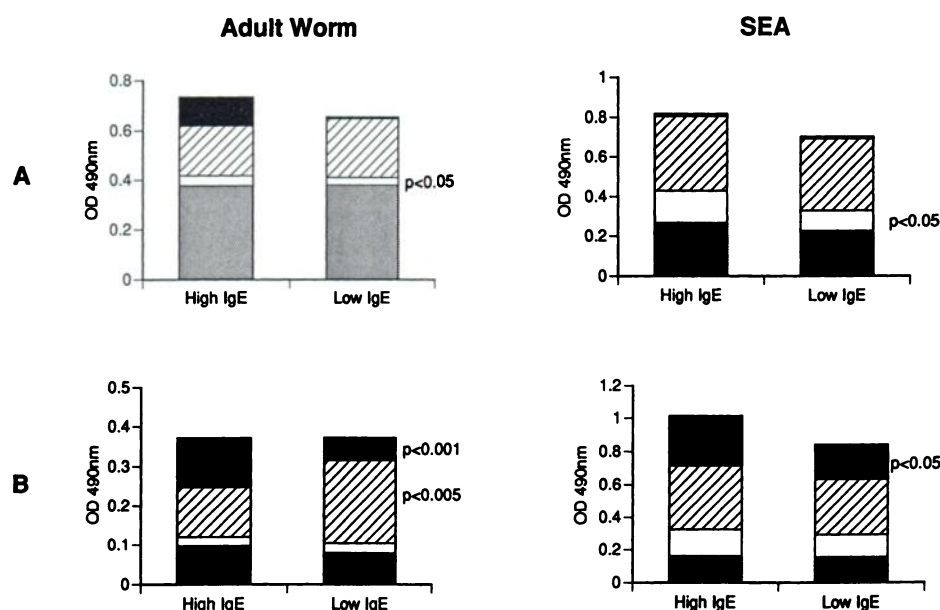


FIGURE 3. Antibody isotype responses to *Schistosoma mansoni* adult worm and soluble egg antigen (SEA) detected by ELISA. A, IgG (■), IgA (□), IgM (▨), and IgE (▩). B, IgG₁ (■), IgG₂ (□), IgG₃ (▨), and IgG₄ (▩). Results are expressed as the overall mean absorbance values ($A_{490\text{ nm}}$) for the high and low IgE responders. A level of significance is shown when a difference between the two groups was observed. OD = optical density.

Figure 2 illustrates the increase in proportion of AW antigen-specific IgE responders with age.

Comparison of the high and low IgE responders. The 46 high IgE responders were matched with individuals from the same study population with low levels of IgE against AW antigen for sex, age, and IgG responses to AW antigen as closely as possible. The IgG levels were not matched exactly but were balanced, and the mean $OD_{490\text{ nm}}$ values were not significantly different (high IgE responders: 0.3779; low IgE responders: 0.380). The two groups were compared in terms of 1) other isotype responses to AW antigen and SEA, 2) prevalence and intensity of infection with *S. mansoni*, 3) prevalence of infection with hookworm, 4) relative exposure to *S. mansoni*, 5) the number of previous treatments for schistosomiasis, and 6) IgE and IgG reactivity with a selection of *S. mansoni* antigens. Figure 3 shows the mean $OD_{490\text{ nm}}$ values for IgA, IgM, IgG₁, IgG₂, IgG₃, and IgG₄ isotype responses to AW antigen and SEA for the high and low IgE responders, together with the level of significance of differences between the two groups.

The high IgE responders had significantly higher IgA and IgG₄ against both AW antigen and SEA but lower IgG₃ against AW antigen than the low IgE responders. As shown in Table 2, no significant difference in either prevalence or intensity of infection with *S. mansoni* was observed between the high and low IgE responders. Similarly, no significant difference was found in prevalence of infection with hookworm. No significant difference was observed in either 1) the length of residence in Corrego do Bernardo, 2) the estimated frequency of water contact, 3) the number of previous treatments for schistosomiasis, or 4) the degree of morbidity between the two groups. Figure 4 shows the IgE and total IgG levels against three *S. mansoni* antigens (rSm22.6, a 12-kD AW antigen band, and paramyosin) for the individuals in the high and low IgE responder groups. The IgG responses were observed to these antigens by some individuals from both groups, whereas IgE specifically reactive with rSm22.6, the 12-kD AW antigen band, or paramyosin was detected in sera from individuals in the high IgE responder group only.

TABLE 2

Percentage prevalence of infection with *Schistosoma mansoni* and/or hookworm, and mean intensities of infection with *S. mansoni* in the high and low IgE responder groups

		High IgE	Low IgE	
<i>S. mansoni</i>	Infected*	18	19	$\chi^2 = 0.006$
	Not infected	22	24	
	% infected	45	44.2	
	Intensity of infection†	133.5 ± 63.1	127.3 ± 53.2	
Hookworm	Infected*	24	24	$\chi^2 = 0.255$
	Not infected	16	20	
	% infected	60	54.6	

* Number individuals infected with either *S. mansoni* (=eggs per gram [epg] of feces count ≥1) or hookworm (=egg count ≥1), and percentage prevalence of infection.

† Mean intensity of infection with *S. mansoni* expressed as mean ± SE epg.

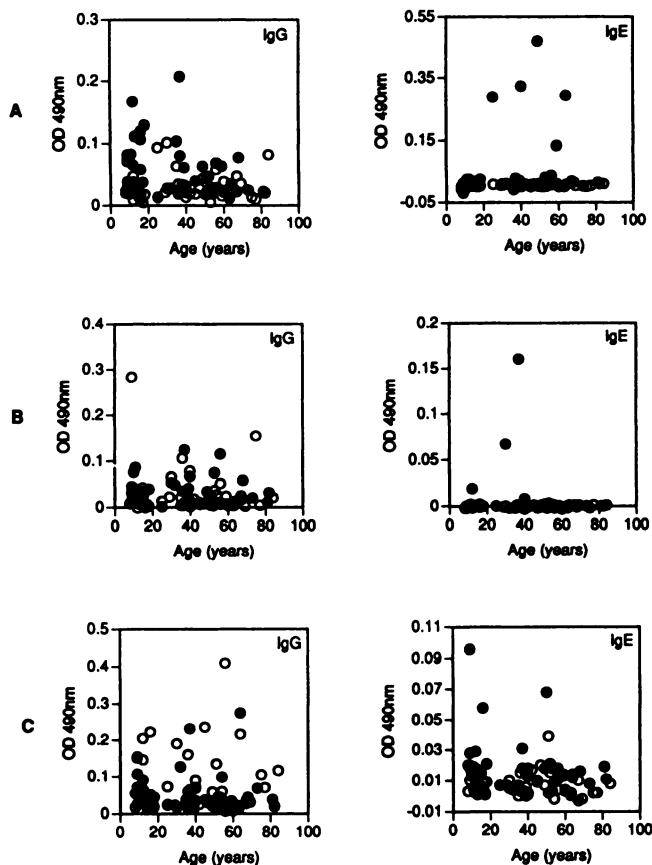


FIGURE 4. Individual IgE and IgG responses to A, the 12-kD band, B, recombinant Sm22.6, and C, paramyosin in the high (●) and low (○) IgE responder groups. Results are expressed as the mean absorbance values ($A_{490\text{ nm}}$) of triplicate assays. OD = optical density.

DISCUSSION

The 534 Brazilian individuals who participated in this work comprised a study population described here for the first time. This population, which was essentially unselected, will form the basis of many future investigations, both cellular and humoral, primarily directed towards elucidating the control of IgE production. In this paper, we have described pretreatment isotype responses to *S. mansoni* adult worm and soluble egg antigens at the population level, which is important for defining the underlying immune responses in this new population. As with previous studies on both Kenyan and Filipino individuals,¹⁷ we found that isotype responses to AW antigen generally increased with age whereas similar responses to SEA tended to decrease with age. In addition, with the exception of IgG₂ against AW antigen, most isotype responses against AW antigen and SEA correlated positively with the intensity of infection with *S. mansoni*. Despite using the same detection methods, we found fewer high AW antigen-specific IgE responders in the Brazilian population in comparison with previous population-based studies in both Kenya and the Philippines.^{8,17} This may be due to differences in the intensity of transmission of the parasite, host genetics, or socioeconomic factors, for example. However, albeit using different techniques, Rihet and others⁷ and De-meure and others¹⁶ detected numerous high AW antigen-specific IgE responders in Brazil.

Interleukin-5-dependent eosinophilia is well documented as a characteristic of helminth infection,²⁹ which, together with in vitro evidence for IgE-dependent cell-mediated cytotoxicity of schistosomula principally by eosinophils¹⁻³ and negative correlations between IgE responses to AW antigen and low levels of both pretreatment³⁰ and posttreatment⁶⁻⁹ intensities of infection with *Schistosoma* spp., indicates that IgE may either be directly involved in mediating resistance to reinfection or be an indirect marker for a resistant phenotype. However, as in previous studies on *S. mansoni*,^{8,17} we did not find a negative correlation between IgE levels against AW antigen and pretreatment intensity of infection. Similarly, we found no significant difference in the prevalence or mean intensity of infection with *S. mansoni* between the age- and sex-matched high and low IgE responders. Antibody-dependent, eosinophil-mediated reactions occur against other helminths, including *Trichinella spiralis*,³¹ *Onchocerca volvulus*,³² *Toxocara canis*,³³ *Brugia malayi*,³⁴ and *N. americanus*³⁵ (but not *Fasciola hepatica*³⁶), and Pritchard and others³⁷ observed negative correlations between IgE or eosinophil levels and hookworm (*N. americanus*) weight or fecundity. Infection with other parasites, therefore, may have had an influence on the levels of total IgE and/or AW antigen-specific IgE. We tested for the presence of *A. lumbricoides*, *T. trichiura*, *Taenia solium*, and hookworm infections, and found that although the prevalence of infection with *A. lumbricoides*, *T. trichiura*, and *Taenia solium* was negligible, the overall prevalence of hookworm (41%) was higher than that with *S. mansoni*. *Necator americanus* is immunologically cross-reactive with *A. lumbricoides*³⁸ and *Toxocara canis*,³⁹ but the degree of IgE cross-reactivity between *N. americanus* and *A. lumbricoides* is less than with other antibody isotypes.⁴⁰ *Schistosoma mansoni* is known to be immunologically cross-reactive with *F. hepatica*⁴¹ but the degree of IgE cross-reactivity between *S. mansoni* and hookworm remains to be elucidated. Although coinfection with *S. mansoni* and hookworm was observed in only 30% (125 of 415) of the whole population, the majority of those infected with *S. mansoni* were also infected with hookworm. It remains to be determined if this is a reflection of a general susceptibility of some individuals to infection with parasitic helminths, and although it is not clear how immunologic interactions between coinfecting helminths might influence the distribution of helminth species in human populations, this may be of significance in terms of an immune response to one parasite influencing the response to a second parasite. In an experimental murine model, it has been shown that *S. mansoni* infection can induce cytokine mediated changes to immune responses directed against coinfecting *Trichuris muris*.⁴²

The ability to mount an IgE response against defined antigens may be dependent on the level of total IgE which, in turn, may be genetically determined. In both Kenya and the Philippines, we found that only those with relatively high IgE responses to either *S. mansoni* or *S. japonicum* AW antigen had detectable IgE responses against a 22-kD SDS-PAGE AW antigen band containing the tegumental antigen Sm22.6.¹⁷ This is supported by our results; IgG responses to three antigens associated with low intensities of reinfection following treatment (i.e., rSm22.6,⁹ paramyosin,⁴³ and a 12-kD AW antigen band [Dunne DW and others, unpub-

lished data)] were observed in some individuals from both groups, whereas specifically reactive IgE was detected in sera from a few individuals in the high IgE responder group only. The low number of individuals with high IgE responses against these antigens may be due to the low number of high AW antigen-specific IgE responders. We are currently investigating the possibility that the ability to mount a high IgE response is genetically determined by screening high and low IgE responders for genetic mutations that may be associated with atopy. These include a mutation in the β -subunit of the high affinity receptor for IgE (Fc ϵ RI) that has been linked to atopic responses in some asthmatic families,⁴⁴ and possible mutations in the area of the genome encoding the inflammatory cytokines (i.e., IL-3, IL-4, and IL-5).^{45, 46} It would be interesting to measure total (nonspecific) IgE in the high and low IgE responders to see if the ability to mount a high IgE response against specific antigens is dependent on the level of total IgE and T helper cell 2 (Th2) cell activation. Individuals infected with *S. mansoni* have higher parasite-specific IgE than those who are uninfected,⁴⁷ and total IgE levels correlate negatively with hookworm weight and fecundity.³⁷ However, we have examined pretreatment total IgE levels in a Kenyan community⁸ and found no relationship between total nonspecific IgE and *S. mansoni*-specific IgE (unpublished data). Similarly, Pritchard and others³⁷ and Hagel and others⁴⁸ found no correlation between total and parasite-specific IgE.

The high IgE responders had higher IgG₄ and IgA to both AW antigen and SEA but lower IgG₃ to AW antigen than the low IgE responders. The difference in IgG₄ would not be unexpected if IgE and IgG₄ are indeed coregulated. Likewise, since IgG₃ production may be regulated by IL-10,^{49, 50} and not affected by either IL-4 or IL-8,⁵¹ the difference in IgG₃ was not surprising. The difference in IgA, however, was harder to explain. Although very little is currently known about the regulation of IgA production in humans, class switching to this isotype may be associated with transforming growth factor- β .⁵² Class switching to IgG₁ may also be induced by IL-10,^{49, 50} but we found no significant difference in IgG₁ against either AW antigen or SEA between the high and low IgE responders. Subsequent cellular studies on age-matched male and female pairs from the high and low IgE responder groups described here have suggested that the high IgE responders were exhibiting a more Th2-type response than the low IgE responders.

To summarize, we have found that the pattern of antibody isotype responses to *S. mansoni* AW antigen and SEA in a Brazilian population were similar to those observed in populations from Kenya and the Philippines; that individuals infected with *S. mansoni* had a higher probability of also being infected with hookworm; and that subjects with high levels of AW antigen-specific IgE had higher IgA and IgG₄ but lower IgG₃ than those with low levels of AW antigen-specific IgE. This study population is extremely valuable in that it gives us the opportunity to investigate immune responses in a population coinfecting with two helminths, as well as to examine antibody isotype responses to hookworm antigens, to assess the degree of cross-reactivity between *S. mansoni* and hookworm, and to investigate the control of IgE production further by more direct means.

Acknowledgments: We thank Dr. Marcia Cordeiro, Susan Arnold, Marluce Rodrigues Lima, Maria de Fatima Silva, Lilian Cardoso Moreira, Raimunda Magalhaes da Silva, and Philip Smith for technical assistance. We are indebted to the inhabitants of Corrego do Bernardo for participation in this study.

Financial support: This work was supported by grants from the European Commission, the Medical Research Council, and the UNDP/CNPq World Bank/WHO Special Program for Research and Training in Tropical Diseases. David W. Dunne was in receipt of a CNPq Visiting Scientist Fellowship.

Authors' addresses: Michelle Webster and David W. Dunne, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, United Kingdom. Rodrigo Correa-Oliveira, Giovanni Gazzinelli, and Iramaya R. C. Viana, Ministerio da Saude, Fundacao Oswaldo Cruz, Centro de Pesquisas Rene Rachou, Av. Augusto de Lima, 1715, CP 1743, CEP 30190-002, Belo Horizonte, Minas Gerais, Brazil. Lucia Alves de Oliveira Fraga and Alda Maria Soares Silveira, Cenbios-Univale-Universidade Vale do Rio Doce, Rua Moreira Sales, 850, Vila Bretas, CEP 35030-390, Governador Valadares, Minas Gerais, Brazil.

Reprint requests: David W. Dunne, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, United Kingdom.

REFERENCES

1. Butterworth AE, Sturrock RF, Houba V, Mahmoud AAF, Sher A, Rees PH, 1975. Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature* 256: 727-729.
2. Butterworth AE, David JR, Franks D, Mahmoud AAF, David PH, Sturrock RF, Houba V, 1977. Antibody-dependent eosinophil-mediated damage to ⁵¹Cr-labelled schistosomula of *Schistosoma mansoni*: damage by purified eosinophils. *J Exp Med* 145: 136-150.
3. Capron M, Rousseaux J, Mazingue C, Bazin H, Capron A, 1978. Rat mast cell-eosinophil interaction in antibody-dependent eosinophil cytotoxicity to *Schistosoma mansoni* schistosomula. *J Immunol* 121: 2518-2525.
4. Grezel D, Capron M, Grzych J-M, Fontaine J, Lecocq J-P, Capron A, 1993. Protective immunity induced in rat schistosomiasis by a single dose of the Sm28GST recombinant antigen: effector mechanisms involving IgE and IgA antibodies. *Eur J Immunol* 23: 454-460.
5. Dunne DW, Richardson BA, Jones FM, Clark M, Thorne KJI, Butterworth AE, 1993. The use of mouse/human chimeric antibodies to investigate the roles of different antibody isotypes, including IgA2, in the killing of *Schistosoma mansoni* schistosomula by eosinophils. *Parasite Immunol* 15: 181-185.
6. Hagan P, Blumenthal UR, Dunn D, Simpson AJG, Wilkins HA, 1991. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* 349: 243-245.
7. Rihet P, Demeure CE, Bourgois A, Prata A, Dessein AJ, 1991. Evidence for an association between human resistance to *Schistosoma mansoni* and high anti-larval IgE levels. *Eur J Immunol* 21: 2679-2686.
8. Dunne DW, Butterworth AE, Fulford AJC, Kariuki HC, Langley JG, Ouma JH, Capron A, Pierce RJ, Sturrock RF, 1992. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. *Eur J Immunol* 22: 1483-1494.
9. Webster M, Fulford AJC, Braun G, Ouma JH, Kariuki HC, Havercroft JC, Kimani G, Sturrock RF, Butterworth AE, Dunne DW, 1996. 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* 64: 4042-4046.
10. Aurialt C, Gras-Masse H, Pierce RJ, Butterworth AE, Wolowczuk I, Capron M, Ouma JH, Balloul J, Khalife J, Neyrinck JL, Tartar A, Koech D, Capron A, 1990. Antibody response of *Schistosoma mansoni*-infected subjects to the recombinant

- P28 glutathione-S-transferase and to synthetic peptides. *J Clin Microbiol* 28: 1918–1924.
11. Grzych J-M, Grezel D, Bo Xu C, Neyrinck J-L, Capron M, Ouma JH, Butterworth AE, Capron A, 1993. IgA antibodies to a protective antigen in human schistosomiasis mansoni. *J Immunol* 150: 527–535.
 12. Butterworth AE, Bensted-Smith R, Capron A, Capron M, Dalton PR, Dunne DW, Grzych J-M, Kariuki HC, Khalife J, Koech D, Mugambi M, Ouma JH, Arap Siongok T, Sturrock RF, 1987. Immunity in human schistosomiasis mansoni: prevention by blocking antibodies of the expression of immunity in young children. *Parasitology* 94: 281–300.
 13. Butterworth A, Dunne D, Fulford A, Capron M, Khalife J, Capron A, Koech D, Ouma J, Sturrock R, 1988. Immunity in human schistosomiasis mansoni: cross reactive IgM and IgG2 anti-carbohydrate antibodies block the expression of immunity. *Biochimie* 70: 1053–1063.
 14. Khalife J, Dunne DW, Richardson BA, Mazza G, Thorne KJI, Capron A, Butterworth AE, 1989. Functional role of human IgG subclasses in eosinophil-mediated killing of schistosomula of *Schistosoma mansoni*. *J Immunol* 142: 4422–4427.
 15. Rihet P, Demeure CE, Dessein AJ, Bourgois A, 1992. Strong serum inhibition of specific IgE correlated to competing IgG4, revealed by a new methodology in subjects from a *S. mansoni* endemic area. *Eur J Immunol* 22: 2063–2070.
 16. Demeure CE, Rihet P, Abel L, Ouattara M, Bourgois A, Dessein AJ, 1993. Resistance to *Schistosoma mansoni* in humans: influence of the IgE/IgG4 balance and IgG2 in immunity to reinfection after chemotherapy. *J Infect Dis* 168: 1000–1008.
 17. Webster M, Libranda-Ramirez BDL, Aligui GD, Olveda RM, Ouma JH, Kariuki HC, Kimani G, Olds GR, Fulford AJC, Butterworth AE, Dunne DW, 1997. 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* 114: 383–393.
 18. Punnonen J, Aversa G, Cocks BG, McKenzie ANJ, Menon S, Zurawski G, de Waal Malefyt R, de Vries JE, 1993. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci USA* 90: 3730–3734.
 19. Spiegelberg HL, O'Connor RD, Falkoff RJM, Beck K, 1991. Interleukin-4 induced IgE and IgG4 secretion by B cells from atopic dermatitis patients. *Int Arch Allergy Appl Immunol* 94: 181–183.
 20. King CL, Hakimi J, Shata MT, Medhat A, 1995. IL-12 regulation of parasite antigen-driven IgE production in human helminth infections. *J Immunol* 155: 454–461.
 21. Thyphronitis G, Tsokos GC, June CH, Levine AD, Finkelman FD, 1989. IgE secretion by Epstein-Barr virus-infected purified human B lymphocytes is stimulated by interleukin 4 and suppressed by interferon gamma. *Proc Natl Acad Sci USA* 86: 5580–5584.
 22. Jeannin P, Delneste Y, Tellie-Leblond I, Wallaert B, Carlier A, Pestel J, Tonnel AB, 1994. Abnormal IgG4 antibody response in aeroallergens in allergic patients. *Int Arch Allergy Immunol* 104: 191–198.
 23. Merrett J, Burr ML, Merrett TG, 1984. A community survey of IgG4 antibody isotypes. *Clin Allergy* 13: 397–407.
 24. Falconer AE, Friedmann PS, Bird P, Calvert JE, 1992. Abnormal immunoglobulin G subclass production in response to keyhole limpet haemocyanin in atopic patients. *Clin Exp Immunol* 89: 495–499.
 25. Li Z, King CL, Ogundipe JO, Licate LS, Blanton RE, 1995. Preferential recognition by human IgE and IgG4 of a species-specific *Schistosoma haematobium* serine protease inhibitor. *J Infect Dis* 171: 416–422.
 26. Smithers SR, Terry RJ, 1965. The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of the adult worms. *Parasitology* 55: 695–700.
 27. Dunne DW, Lucas S, Bickle QD, Pearson S, Madgwick L, Bain J, Doenhoff MJ, 1981. Identification and partial purification of an antigen (ω 1) from *Schistosoma mansoni* eggs which is putatively hepatotoxic in T-cell deprived mice. *Trans R Soc Trop Med Hyg* 75: 54–71.
 28. Laemmli UK, 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685.
 29. Butterworth AE, 1984. Cell-mediated damage to helminths. *Adv Parasitol* 23: 143–235.
 30. Ndhlovu P, Cadman H, Vennervald BJ, Christensen NO, Chidimu M, Chandiwana SK, 1996. Age-related antibody profiles in *Schistosoma haematobium* infections in a rural community in Zimbabwe. *Parasite Immunol* 18: 181–191.
 31. Kazura JW, Aikawa M, 1980. Host defense mechanisms against *Trichinella spiralis* infection in the mouse: eosinophil-mediated destruction of newborn larvae in vitro. *J Immunol* 124: 355–361.
 32. Greene BM, Taylor HR, Aikawa M, 1981. Cellular killing of microfilariae of *Onchocerca volvulus*: eosinophil and neutrophil-mediated immune serum-dependent destruction. *J Immunol* 127: 1611–1618.
 33. Fattah DI, Maizels RM, McLaren DJ, Spry CJ, 1986. *Toxocara canis*: interaction of human blood eosinophils with the infective larvae. *Exp Parasitol* 61: 421–431.
 34. Sim BK, Kwa BH, Mak JW, 1982. Immune responses in human *Brugia malayi* infections: serum dependent cell-mediated destruction of infective larvae in vitro. *Trans R Soc Trop Med Hyg* 76: 362–370.
 35. Desakorn V, Suntharasamai P, Pukrittayakamee S, Migasena S, Bunngun D, 1987. Adherence of human eosinophils to infective filariform larvae of *Necator americanus* in vitro. *South-east Asian J Trop Med Public Health* 18: 66–72.
 36. Duffus WP, Franks D, 1980. In vitro effect of immune serum and bovine granulocytes on juvenile *Fasciola hepatica*. *Clin Exp Immunol* 41: 430–440.
 37. Pritchard DI, Quinnell RJ, Walsh EA, 1995. Immunity in humans to *Necator americanus*: IgE, parasite weight and fecundity. *Parasite Immunol* 17: 71–75.
 38. Pritchard DI, Quinnell RJ, McKean PG, Walsh L, Leggett KV, Slater AFG, Raiko A, Dale DDS, Keymer AE, 1991. Antigenic cross-reactivity between *Necator americanus* and *Ascaris lumbricoides* in a community in Papua New Guinea infected predominantly with hookworm. *Trans R Soc Trop Med Hyg* 85: 511–514.
 39. Ball PAJ, Voller A, Taffs LF, 1971. Hypersensitivity to some nematode antigens. *Br Med J* 1: 210–211.
 40. Turner KJ, Fisher EH, McWilliams AS, 1980. Homology between roundworm (*Ascaris*) and hookworm (*N. americanus*) antigens detected by human IgE antibodies. *Aust J Exp Biol Med* 58: 249–257.
 41. Hillyer GV, 1984. Immunity to schistosomes using heterologous trematode antigens - a review. *Vet Parasitol* 14: 263–283.
 42. Curry AJ, Else KJ, Jones F, Bancroft A, Grecis RK, Dunne DW, 1995. Evidence that cytokine mediated immune interactions induced by *Schistosoma mansoni* after disease outcome in mice concurrently infected with *Trichuris muris*. *J Exp Med* 181: 769–774.
 43. Correa-Oliveira R, Pearce EJ, Oliveira GC, Golgher DB, Katz N, Bahia LG, Carvalho OS, Gazzinelli G, Sher A, 1989. The human immune response to defined immunogens of *Schistosoma mansoni*: elevated antibody levels to paramyosin in stool negative individuals from two endemic areas in Brazil. *Trans R Soc Trop Med Hyg* 83: 798–804.
 44. Young RP, Sharp PA, Lynch JR, Faux JA, Lathrop GM, Cookson WO, Hopkin JM, 1992. Confirmation of genetic linkage between atopic IgE responses and chromosome 11q13. *J Med Genet* 29: 236–238.
 45. Warrington JA, Bailey SK, Armstrong E, Aprelikova O, Alitalo K, Dolganov GM, Wilcox AS, Sikela JM, Wolfe F, Lovett M, Wasmuth JJ, 1992. A radiation hybrid map of 18 growth factor, growth factor receptor, hormone receptor, or neurotransmitter receptor genes on the distal region of the long arm of chromosome 5. *Genomics* 13: 803–808.
 46. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH, 1994. Linkage analysis of IL-4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 264: 1152–1156.

47. Sauer H, Leykun J, Schubert S, 1992. Determination of parasite-specific antibodies during human schistosomiasis - diagnostic relevance. *Angelwandte Parasitol* 33: 151-159.
48. Hagel I, Lynch NR, Perez M, Di Prisco MC, Lopez R, Rojas E, 1993. Modulation of the allergic reactivity of slum children by helminthic infection. *Parasite Immunol* 15: 311-315.
49. Briere F, Servet-Delprat C, Bridon J-M, Saint-Remy J-M, Banchereau J, 1994. Human interleukin 10 induces naive surface immunoglobulin D⁺ (sIgD⁺) B cells to secrete IgG1 and IgG3. *J Exp Med* 179: 757-762.
50. Servet-Delprat C, Bridon J-M, Blanchard D, Banchereau J, Briere F, 1995. CD40-activated human naive surface IgD⁺ B cells produce IgG2 in response to activated T-cell supernatant. *Immunology* 85: 435-441.
51. Kimata H, Yoshida A, Ishioka C, Lindley I, Mikawa H, 1992. Interleukin 8 (IL-8) selectively inhibits immunoglobulin E production induced by IL-4 in human B cells. *J Exp Med* 176: 1227-1231.
52. Defrance T, Vanbervliet B, Briere F, Durand I, Rousset F, Banchereau J, 1992. Interleukin 10 and transforming growth factor B cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J Exp Med* 175: 671-682.