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Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection*

Previous studies in school children have demonstrated the slow development with age of resistance to reinfection after chemotherapy of *Schistosoma mansoni* infections, and have indicated that inappropriate ("blocking") antibody responses prevent the expression of immunity in young children. The present study was designed to investigate further the nature of the protective responses, by serological studies on a group of 151 *S. mansoni*-infected individuals resident in an endemic area in Machakos District, Kenya. Antibody levels against various antigens in blood samples before treatment were related to intensity of previous infections; antibodies in blood samples taken 6 months after treatment were related to cumulative reinfection rates over the following 30 months.

IgE against an adult-worm antigen preparation correlated positively with age and negatively with reinfection. In contrast, IgE antibodies against other life-cycle stages showed either no relationship or the reverse correlation. Furthermore, antibodies of other isotypes against adult-worm antigens showed no correlations with reinfection.

The correlation with IgE could be demonstrated for different preparations of adult worms, including a periodate-treated preparation presumptively depleted of carbohydrate epitopes. For both the intact and the periodate-treated preparations, multiple regression analysis of the results for children ≤16 years old demonstrated an IgE effect after allowing for age, although this effect was not observed in a previously studied group of school children. Western blot analysis of the adult-worm preparation revealed a limited set of antigens recognized by IgE, among which an antigen of 22 kDa was prominent. The qualitative presence of IgE against this antigen could also be shown to be related to a lack of subsequent reinfection.

1 Introduction

During the past decade, studies of both *Schistosoma mansoni* and *S. haematobium* have shown that, following chemotherapeutic cure of a primary infection, older individuals show a lack of reinfection, in contrast to younger children, that is greater than can be accounted for by the relative lack of exposure alone [1–4]. Separate evidence has indicated that this age-dependent resistance to reinfection is also dependent on the previous experience of infection, and is, therefore, likely to represent a manifestation of an acquired immunity rather than an age-related physiological change [5]. A feature of this acquired resistance is the length of time required for its development. Children

usually start to acquire schistosome infections at about the age of 3–4 years: thereafter, worm burdens continue to rise until the second decade of life, and a resistance to reinfection after chemotherapy cannot be demonstrated until the age of 12 years, at earliest [2, 3, 6].

A simple explanation for this slow development of immunity might be that it takes many years for the host to acquire a sufficient antigenic load to mount an effective immune response. However, our previous studies on a group of 9–16-year-old children with *S. mansoni* infections indicated that this is not the case: all the children, including the younger ones who remained highly susceptible to reinfection after treatment, showed high levels of a wide range of immune responses against Ag from all stages of the parasite, and the young children in particular might be described as being immunologically hyperreactive to schistosome Ag [2]. Instead, our results were compatible with the hypothesis that young children's continued susceptibility to reinfection was because, in addition to any protective responses, they had also mounted inappropriate or "blocking" Ab responses that prevented the binding and expression of protective Ab [7–9]. These blocking Ab, which included both IgM and IgG2 isotypes, recognized carbohydrate determinants common to the eggs and schistosomula (the target of immune attack), and progressively declined with age.

Although these findings provided an explanation of why young children remained susceptible to reinfection, they

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Abbreviations: SEA: Soluble egg antigen Shed: Schistosomulum shed antigen SOM: Schistosomulum tegumental antigen

gave no indication about the nature of any protective responses that were subsequently expressed in older individuals. However, in a recent study of group of wider age range with *S. haematobium* infections, Hagan et al. [10] have demonstrated a significant relationship between IgE against adult-worm or egg Ag and resistance to reinfection. Although an association between IgE and immunity to infection has been clearly demonstrated in a rat model of schistosome infection [11–13], leading to the hypothesis that IgE-mediated reactions may have evolved primarily as defense mechanisms against large, tissue-invasive helminths, the results of Hagan et al. [10] for *S. haematobium* were the first to demonstrate a putative protective role of IgE in man. In this communication, we report a similar general finding for *S. mansoni* infections in man, with additional information on the nature of the Ag recognized by IgE in immune individuals.

2 Materials and methods

2.1 Study H: individuals with *S. mansoni* infection

The study was carried out within the framework of a comparative chemotherapy program, described in detail elsewhere [14]. This program was carried out in Kangundo Location in Machakos District, Kenya, approximately 90 km east of Nairobi. Study subjects were selected from Area H, in which treatment was offered to all individuals excreting >100 eggs/g feces (epg). Following one year of pretreatment observations, treatment was carried out in April 1985. Thereafter, community stool surveys were repeated at yearly intervals until 1989, when treatment was finally offered to all individuals who had been detectably infected in any of the previous surveys.

Area H comprised a community of 1500 individuals, living in a steep-sided valley with a river on its floor forming the only source of *S. mansoni* transmission. Initially, 115 individuals, aged 6 to 66 years, were selected from the community on the basis of (a) positive stool results in a preliminary survey, and (b) high frequencies of observed contact, during April to August 1984, with sites that had already been shown to contain infected snails. Six more stool samples were then collected from these individuals and Kato preparations [15] examined in duplicate, to provide an estimate of pretreatment intensity (see below). Blood samples (Bleed A) were collected immediately before treatment in April 1985. This treatment was also offered at the community level to all individuals excreting >100 epg at this time. Further blood samples were collected in October 1985 (Bleed B) and October 1986 (Bleed C), and post-treatment stool samples at 6-month intervals from October 1985 to October 1988.

Additional subjects were added to the Study H group in October 1986. Of these, 26 had already been treated in April 1985: they lacked Bleed A sera and pretreatment egg counts, but were included in the analyses of results for the Bleed B sera against reinfection (see below). Ten more subjects who had not previously been treated provided a further set of Bleed A sera for testing against pretreatment intensity of infection. These, however, were not included in subsequent analysis of reinfection. In addition to those who were treated late, all individuals from the first set whose

treatment failed, those who had zero egg counts in the definitive pretreatment stool surveys and those who consistently failed to provide post-treatment stool samples were excluded from the subsequent analysis of reinfection. Thus, results for the pretreatment blood sample (Bleed A) are analyzed only against pretreatment intensity of infection. Results for the post-treatment blood sample (Bleed B) are analyzed only against the intensity of reinfection. The groups of individuals studies in Bleeds A and B are overlapping but not identical.

2.2 Study H: stool examinations

Stool samples were examined quantitatively for *S. mansoni* eggs by a modification of the Kato thick smear technique [15]. For the pretreatment egg counts, results from duplicate 50-mg Kato preparations from each of six separate stool samples were summed, and results calculated and analyzed as the natural logarithm of the mean epg + 1 (to allow for zero counts). For the reinfection egg counts, an overall reinfection score was calculated, based on all six stool surveys carried out between March 1986 and November 1988, duplicate preparations of three stool samples being examined for each time point. Each individual's score for each time point was adjusted to remove any bias that might have arisen if missing data were non-randomly distributed. This was achieved by calculation for each time point of the mean score (m_t) for those individuals who had complete data for all time points, and then aligning all other time points with the mean for November 1987, $m_{\text{Nov } 87}$, as follows:

$$\text{Adjusted score} = \text{Unadjusted score} - m_t + m_{\text{Nov } 87}$$

Although this adjustment is additive, it will be multiplicative for egg counts, since the scores are on a logarithmic scale.

2.3 Study H: sera

Venous blood samples were taken before treatment (Bleed A), 6 months after treatment, but before reinfection had occurred (Bleed B), and 18 months after treatment (Bleed C: results not presented). No transmission occurred between treatment in April 1985 and the Bleed B sample in October 1985. Results from these samples were, therefore, an indication of post-treatment but pre-reinfection immune responses, and were taken to be predictive of subsequent reinfection. Blood was defibrinated and stored at room temperature during transport to Nairobi, before fractionation by centrifugation over Lymphoprep (Nyegaard, Oslo). The serum was recovered and stored initially in aliquots of 1.5 ml at -20°C (the lymphocytes also being recovered for studies of cell-mediated immune responses, to be described elsewhere). After shipment on dry ice to Cambridge, each serum sample was thawed once and re-frozen in aliquots at -70°C . Such aliquots were used only once in the assays described below.

2.4 Study of a group of 129 children

In addition to the new study on the Study H group described in Sect. 2.1, IgE Ab were also assayed and the

results related to reinfection in a group of 129 children, aged 9 to 16 years. This group has been described in detail previously [1, 2]. In brief, pretreatment stool and blood samples were collected from 129 children, who were treated with oxamniquine and observed for reinfection at 5 weeks and 3, 6, 9, 12, 15, 18 and 24 months after infection. In this case, in contrast to Study H, transmission in the study area remained high, and reinfection occurred very rapidly during the first 6 months after treatment. Therefore, results from the pretreatment and 5-week post-treatment blood samples were taken as predictive of reinfection, rather than the 6-month post-treatment sample that was more useful for Study H.

2.5 Parasite Ag

2.5.1 General remarks

All stages of the parasite life cycle used to obtain antigenic material were obtained from a Puerto Rican isolate of *S. mansoni* which was maintained in albino *Biomphalaria glabrata* and BALB/c mice. *S. mansoni* eggs were obtained from the livers and intestines of outbred TO mice (Tuck Ltd., Battedbridge, GB) as reported previously [8, 16]. Adult worms were recovered by perfusion of the hepatic portal system of TO mice 6–8 weeks after infection [17]. Cercariae were transformed into schistosomula by mechanical dissociation of tails from parasite bodies followed by separation by repeated sedimentation [18]. Separated bodies were incubated for 18 h in serum-free medium, NCTC (Flow Laboratories, Rickmansworth, GB), containing Hepes buffer, penicillin, gentamycin and streptomycin, before being used to prepare Ag.

2.5.2 Soluble egg Ag (SEA) and schistosomular “membrane” Ag

SEA was the supernatant fluid obtained after *S. mansoni* eggs were homogenized in PBS as described previously [16]. Schistosomular membrane was a detergent-solubilized extract of intact *S. mansoni* schistosomula. Schistosomula were mixed in PBS containing 1 % NP40 (Sigma, St. Louis, MO), 1 mM PMSF, 50 mg/ml N-p-tosyl-L-phenyl-alanine chloromethylketone (TPCK) and 25 mg/ml N-p-tosyl-L-lysine chloromethylketone (Sigma), at 4°C for 20 min (300 000 schistosomula/ml). The intact schistosomula were separated from the soluble material by gentle centrifugation. The resultant supernatant was stored at -20°C in aliquots until required, and the subsequently thawed material was centrifuged at $10\,000 \times g$ for 10 min before use.

2.5.3 Schistosomular ‘shed’ Ag

Shed Ag was recovered from the supernatant fluid collected from an 18-h culture of mechanically transformed schistosomula (containing approximately 10 000 parasites/ml). The supernatant fluid was then pressure dialyzed against PBS on a YM10 membrane (10-kDa pore size; Amicon, Lexington, MA) and reduced to approximately 10 % of the starting volume. This material was aliquoted and stored at -20°C until required.

2.5.4 Adult-worm Ag (detergent extract) and adult worm tegument

The basic worm Ag preparation was a 0.1 % NP40 extract of whole homogenized *S. mansoni* worms [8]. Procedures used to prepare tegumental Ag preparations were based on those described by Simpson et al. [19]. Freshly isolated worms were washed in serum-free medium (NCTC), sedimented and resuspended in PBS containing 2.4 mM CaCl_2 and 2 mM MgCl_2 . After 10-min incubation at 37°C, the supernatant was removed and the worms were gently vortexed twice in a minimum volume of the same buffer, the supernatant fluid being removed each time. The supernatant fluids were pooled and centrifuged at $100\,000 \times g$ for 1 h at 4°C. The resultant pellet was resuspended in PBS, designated ‘worm tegument’ and stored at -70°C.

2.5.5 Denuded worm Ag

Freshly isolated adult worms were gently mixed for 30 min in PBS containing 1 mM TPCK, 1 mM PMSF and 1 % NP40, to ensure the removal of the outer tegument. The worms were then removed, washed and finely ground in a mortar and pestle in liquid nitrogen. They were then resuspended in PBS containing 0.1 % NP40 and centrifuged at $10\,000 \times g$ for 1 h at 4°C. The resultant supernatant was stored at -70°C.

2.5.6 Con A-binding adult-worm Ag

A glycoprotein-enriched fraction of adult worms was obtained by passage of a crude antigenic extract over a column containing Con A-Sepharose as previously described [20].

2.5.7 Periodate-treated Ag and PBS extract of adult worm

ELISA were carried out using adult-worm Ag which was treated with sodium periodate after the Ag had been coated onto microtiter plates. The details are given with the description of ELISA methods.

PBS extract of adult worm comprised the supernatant fluid obtained after adult worms were homogenized in PBS and the resultant homogenate was centrifuged at $10\,000 \times g$ for 20 min.

2.6 ELISA

ELISA were carried out using the following parasite Ag preparations: SEA; schistosomular tegument; schistosomular ‘Shed’; adult worm (detergent extract); adult-worm tegument; denuded adult worm; periodate-treated adult worm; and PBS extract of adult worm. For each Ag preparation, anti-human isotype Ab were used to quantify the specific IgG; IgG1; IgG2; IgG3; IgG4; IgM; IgA; and IgE present in individual human infection sera. The methods used, with some modifications, have been described previously [8, 21]. Optimum Ag concentrations were determined by “checkerboard” titration using pooled

positive and negative sera. Individual sera used to establish positive human serum pools for controlling IgG2, IgG3 and IgE assays had to be specially selected high responders for each isotype. The mouse and rabbit anti-human isotype Ab used were as follows: anti-human IgG (horseradish peroxidase) (HRP) conjugate, 1/400 (Dako Ltd., High Wycombe, GB); anti-human IgM HRP 1/400 (BRL, Life Technologies Inc., Gaithersburg, MD); mouse mAb anti-human IgG₁ (clone number NL16) 1/2000; mouse mAb anti-human IgG₂ (GOM) 1/2000; mouse mAb anti-human IgG₃ (ZG4) 1/1000; mouse mAb anti-IgG₄ (RJ4) 1/10000 (Oxoid Ltd., Basingstoke, GB); rabbit anti-human IgA, 1/10000 (Dako); mouse monoclonal anti-human IgE (HP6029) 1/2000 (Novabiochem Ltd., Nottingham, GB). Antisera used to detect non-conjugated anti-isotype sera were biotinylated donkey anti-mouse Ig (Amersham International PLC, Aylesbury, GB) or biotinylated donkey anti-rabbit Ig (Amersham). Each was subsequently treated with Streptavidin/biotin HRP (Amersham). All assays were developed using o-phenylenediamine (OPD, Sigma). Human infection sera were tested at a single dilution of 1/200 in total IgG, IgG1, IgG4, IgM and IgA assays, 1/100 for IgG2 and IgG3 assays, and at 1/20 in IgE assays. Incubation periods for human infection sera and anti-isotype sera were 3 h followed by three washes for the IgG, IgG1, IgG4, IgM and IgA assays. The corresponding incubation times for IgG2, IgG3, and IgE assays was 22 h followed by nine washes. Color development was measured spectrophotometrically at 490 nm on a Dynatech MR700 microplate reader (Dynatech Laboratories Ltd., Billingshurst, GB) linked to a Macintosh SE computer (Apple Corp., Cupertino, CA). The results were automatically standardized and checked for plate-plate variability and results are given as mean values of absorbance readings of triplicate assays on separate plates.

ELISA to detect human Ab that recognized periodate-treated adult-worm Ag employed adult-worm Ag (NP40 extract) coated on microtiter plates with subsequent treatment with sodium periodate. Thus, microtiter plates pre-coated with worm Ag were incubated overnight in the dark at 4°C with 0.01 M sodium periodate in 0.1 M sodium acetate. The microtiter plates were then washed and the assays completed as described for the standard ELISA. The extent sodium of periodate oxidation of terminal sugar residues on each plate was determined by comparing color development in periodate-treated and untreated microtiter-plate wells after incubation with biotinylated lectins (Sigma), biotin/streptavidin HRP and OPD. This indicated that periodate treatment of worm Ag resulted in a 90%–95% reduction in the binding of soybean agglutinin and wheat germ agglutinin, but only a 10% reduction in Con A binding.

2.7 Western blots

Individual patients were screened to identify reactivity with a 22-kDa worm Ag by 'mini'-Western blotting of worm Ag and probing with specific anti-IgE and -IgG Ab. Tests for each Ab isotype were carried out in duplicate. Worm Ag were separated by SDS-PAGE on 14% PAG using Mini-Protean II electrophoresis apparatus (Bio-Rad Laboratories Ltd., Hemel Hempstead, GB) and transferred to nitrocellulose paper (Bio-Trace, Gelman Sciences, Ann

Arbor, MI) using a Protean II Trans-Blot cell (Bio-Rad). Nitrocellulose sheets were then blocked with 1% BSA in washing solution of 1% Tween-20 (polyoxyethylene sorbitan monolaurate, Sigma) for 15 min, washed five times and transferred to Mini-Protean II Multi-Screening Apparatus. The worm Ag-coated nitrocellulose sheets were incubated with 600 µl of 1:20 (IgE assay) or 1:200 (IgG assay) dilutions of individual human sera for 23 h at room temperature. They were then washed five times in 1% Tween-20 and incubated with 1:2000 dilution of mouse mAb anti-human IgE or 1:400 dilution of rabbit anti-human IgG, in 1% Tween-20 plus 1% BSA, overnight at room temperature. After a further five washes, the bound human Ab was detected with biotinylated anti-mouse IgG followed by HRP-streptavidin/biotin and developed for one or two incubation periods with 0.05% diaminobenzoic acid. The sera were then scored as positive or negative for IgG and IgE reactivity with a 22-kDa worm Ag.

Western blots were also carried out to illustrate the range of Ag recognized by IgG and IgE in human infection sera. These were carried out as above, except that the worm Ag was separated using 15 × 15 cm, 8%–20% gradient polyacrylamide gels and nitrocellulose was cut into individual strips before incubation with 4 ml of the same dilutions of human sera.

2.8 Solid-phase RIA

Specific IgE Ab against the Con A-binding adult-worm Ag were measured by solid-phase RIA as previously described [22]. The increase in bound radioactivity in the presence of test serum (B_x) compared with a negative control serum (B_0) was calculated using the following formula:

$$\% = [(B_x - B_0)/B_0] \times 100$$

2.9 Statistical analysis

Relationships between the various immune responses, and between each response and age and pretreatment or post-treatment intensity of infection were initially examined by Pearson's correlation and Spearman's rank correlation coefficients: relationships with age were also tested by one-way analysis of variance. In general, results for the pretreatment sample (Bleed A) were tested against pretreatment intensity of infection: results for the sample (Bleed B) taken 6 months after treatment, but before significant reinfection had occurred, were tested against the cumulative reinfection score. Results of interest were further examined by multiple regression analysis, in which the effect of a given response was tested after allowing for the effect of age.

3 Results

3.1 Intensities of infection before and after treatment

Pretreatment intensities of infection are shown in Figs. 1A and 2 for individual patients and for age groups. The distribution of intensity of infection by age did not reflect the distribution in the community as a whole, which was

more convex [14]; this was because the subjects in the study group were pre-selected on the basis of a preliminary stool survey and observed water contact. Five individuals had no detectable infections at the definitive pretreatment stool surveys, in spite of yielding positive results in the preliminary survey, these individuals were excluded from subsequent analysis of reinfection.

Intensities of reinfection after treatment are shown in Figs. 1B and 2 as an adjusted mean score for the six surveys carried out after treatment (see Sect. 2.2). As with pretreatment intensities, reinfection intensity is shown on a logarithmic scale. Young children up to 8 years of age became reinfected to pretreatment levels. Older children

and adults showed a relative lack of reinfection, in comparison with pretreatment levels. This was particularly marked in individuals over 15 years of age. This strong age dependence of reinfection is in agreement with many previous observations on other study groups and communities.

3.2 Relationship between intensities of infection or reinfection, age, and IgE to different life cycle stages

Sera from each blood sample were assayed for Ab of each isotype to preparations of crude Ag from each parasite stage, with or without periodate treatment to destroy carbohydrate epitopes. This generated a large amount of data, and the presentation in this manuscript is necessarily selective, concentrating on IgE and related responses. A full description of the entire data set will be presented elsewhere.

Table 1 shows the correlations of IgE responses to adult worm, SEA and schistosomulum (Shed and Som) Ag with age and intensity of infection. The corresponding data, grouped by age, are shown in Fig. 3. As described in Sect. 2.3, IgE in the pretreatment blood sample were tested against pretreatment intensities of infection: IgE in the blood sample taken 6 months after treatment (Bleed B) were tested against subsequent reinfection, as reflected by a cumulative score. Pretreatment IgE was correlated with pretreatment intensity, but significantly only in the case of the anti-SEA response, which was also significantly and negatively correlated with age. Post-treatment IgE against different Ag preparations behaved differently. IgE against egg and schistosomulum Ag were still negatively correlated with age (significantly so in the case of the schistosomulum Shed Ag) and positively with reinfection (significant for the Shed Ag and for SEA). In marked contrast, IgE against adult-worm Ag were correlated positively with age and negatively with reinfection. In other words, high IgE anti-adult-worm Ab are associated with *low* subsequent reinfection, and might, therefore, be involved in mediating protective immunity.

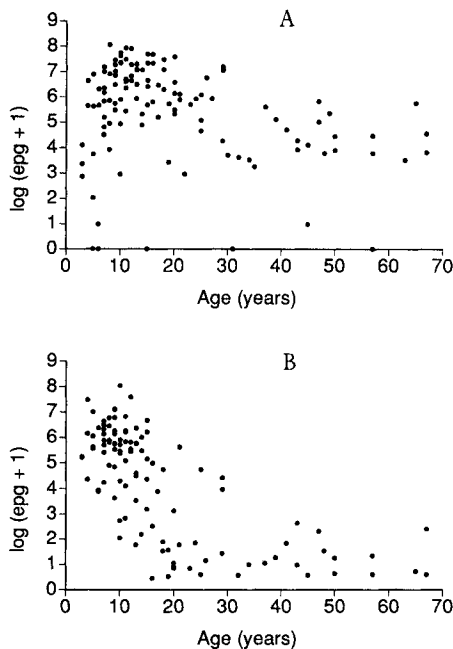


Figure 1. Intensities of infection by age: individual subjects in Study H. Values expressed as $\log_e (\text{epg} + 1)$. A: pretreatment intensity; B: cumulative reinfection intensity after treatment.

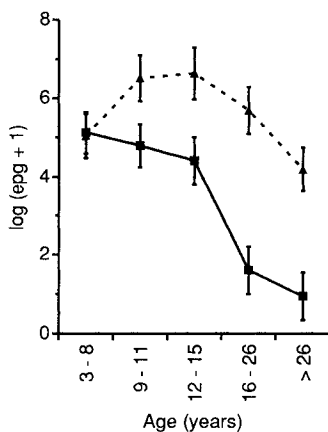


Figure 2. Intensities of infection by age: subjects in Study H, grouped by age. Values expressed as mean $\log_e (\text{epg} + 1) \pm 95\%$ confidence interval (▲) Pretreatment intensity; (■) cumulative score of reinfection intensity, as in Fig. 1.

3.3 Relationship between intensities of infection or reinfection, age, and Ab of different isotypes against adult worm Ag

The contrast between IgE responses to adult worm and those to other life-cycle stages was not simply a reflection of a generally different response to the adult worm, since it was not seen with other isotypes (Table 2 and Fig. 4). Most isotypes (except for IgM) showed a positive correlation with pretreatment intensity of infection and a negative correlation with age, the correlations being significant for total IgG, IgG2 and IgG4. In relation to the post-treatment blood sample, however, significant positive correlations with age were found only for IgA and IgE, while a significant negative correlation with reinfection was observed only for IgE. The correlation for IgA approached significance, and the IgA response to other Ag is discussed elsewhere [22] and by Capron et al. (in preparation).

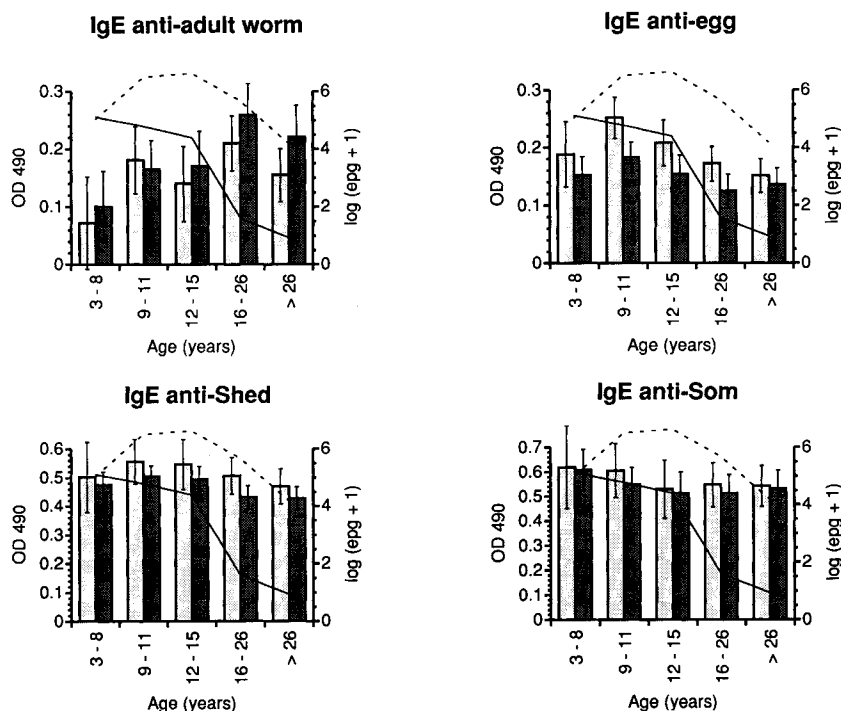


Figure 3. IgE against: adult worm; soluble egg Ag (EGG); schistosomulum shed Ag (Shed); schistosomulum tegument Ag (Som). Results expressed as mean A_{490} values, $\pm 95\%$ confidence interval, for the age groups shown, for pretreatment and 6 month post-treatment blood samples. Pretreatment and reinfection intensities of infection are shown in the dotted and solid lines respectively as mean $\log_e$ (epg + 1) for the same age groups.

3.4 Relationships between intensities of infection or reinfection, age, and IgE Ab against different worm Ag preparations

The negative correlation observed between post-treatment IgE and subsequent reinfection was not limited to the particular adult-worm Ag preparation that was tested. Table 3 shows that similar negative correlations with reinfection, and positive correlations with age, were found with Ag prepared either from the tegument or denuded body, as well as in a PBS extract of worms. In addition, a significant correlation was found with a different assay method (RIA) against a fraction of adult worms eluted from a Con A column, indicating that the relevant IgE Ab can be directed against carbohydrate containing Ag. However, this was also seen for a worm preparation whose carbohydrate epitopes had been destroyed by periodate treatment and, in this

case, it may be noted that the significant positive correlation with age was lost (Fig. 5).

In this context, it was considered possible that the association of high IgE anti-adult-worm Ab with lack of reinfection after treatment was fortuitous rather than causal, being simply attributable to the fact that both properties are strongly correlated with age (Fig. 3). Initial attempts to control for the effects of age in multiple regression or logistic regression analysis were unsuccessful, since the age effects were too strong for residual effects to be detected. However, adults are heterogeneous not only in their immune responses but also in their exposure, and it is possible that many adults, being unexposed, neither become reinfected nor show strong immune responses (Fig. 6A and B). Therefore, separate analyses were carried out of IgE responses to adult-worm Ag (Fig. 6A) and of

Table 1. Correlations between IgE responses against Ag from different life cycle stages, intensities of infection and age

Blood sample: Tested against:	Pretreatment (Bleed A)		Post-treatment (Bleed B)	
	Pretreatment intensity	Age	Post-treatment intensity	Age
IgE Ab against ^{a)} :				
Adult	+0.171 ^{b)} (79, NS)	-0.015 (81, NS)	-0.307 (96, $p < 0.01$)	+0.261 (96, $p < 0.01$)
SEA	+0.260 (77, $p < 0.05$)	-0.340 (79, $p < 0.01$)	+0.295 (95, $p < 0.01$)	-0.160 (96, NS)
Shed	+0.050 (77, NS)	-0.140 (79, NS)	+0.300 (95, $p < 0.01$)	-0.273 (96, $p < 0.01$)
Som	+0.102 (75, NS)	-0.099 (77, NS)	+0.155 (94, NS)	-0.115 (95, NS)

a) Ag tested: Adult, adult worm Ag; SEA, soluble egg Ag; Shed, schistosomulum shed Ag; Som, schistosomulum tegumental Ag.

b) Spearman's correlation coefficient: number of observations and level of significance in parentheses. NS: Not significant.

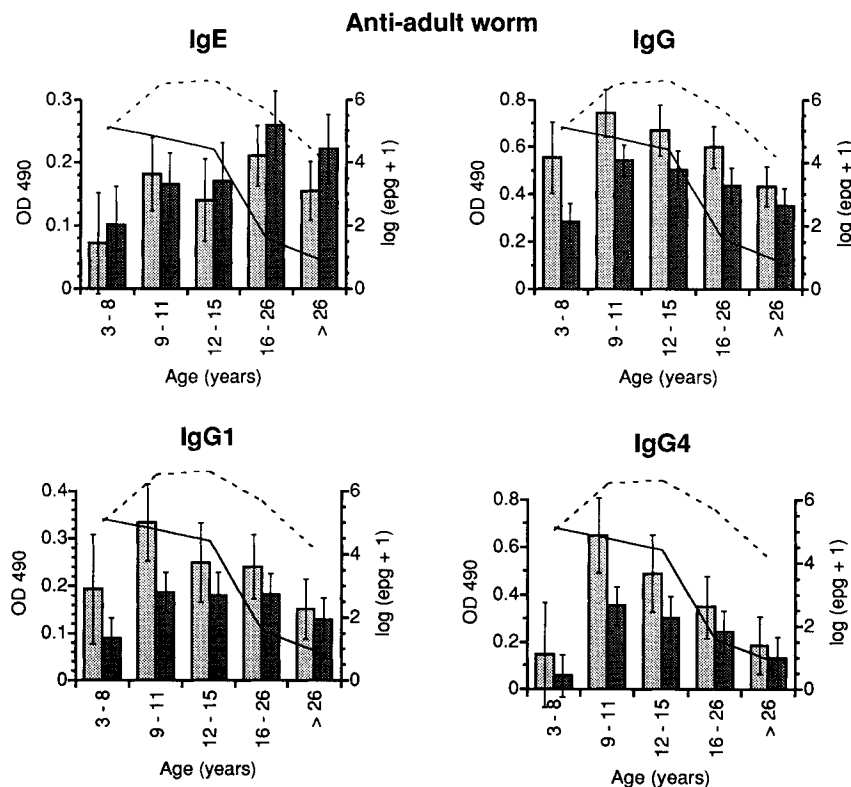


Figure 4. Different Ab isotypes against adult-worm Ag. Results expressed as in Fig. 3.

periodate-treated adult-worm Ag (Fig. 6B) only in children aged 16 years or less. The correlation coefficients were significant and were greater than those found with the whole study group. In the case of responses to the intact Ag, multiple regression analysis now showed a significant effect of IgE *after* allowing for the effect of age ($F = 4.96$

with 1,56 *df*; $p < 0.05$). This significant effect of IgE, after allowing for age, was even more marked for the response against the periodate-treated Ag ($F = 15.01$ with 1,56 *df*; $p < 0.001$). In this case, it will be recalled that the correlation with age was not in fact significant (Table 3 and Fig. 5).

Table 2. Correlations between different isotypes of Ab responses against adult-worm Ag, intensities of infection and age

Blood sample: Tested against:	Pretreatment (Bleed A)		Post-treatment (Bleed B)	
	Pretreatment intensity	Age	Post-treatment intensity	Age
Anti-adult worm:				
Total IgG	+0.391 ^{a)} (78, $p < 0.001$)	−0.416 (89, $p < 0.001$)	+0.134 (97, NS)	−0.106 (98, NS)
IgG1	+0.185 (86, NS)	−0.308 (88, $p < 0.01$)	−0.005 (101, NS)	+0.034 (102, NS)
IgG2	+0.265 (76, $p < 0.05$)	−0.275 (77, $p < 0.05$)	−0.041 (96, NS)	+0.054 (97, NS)
IgG3	−0.034 (86, NS)	−0.025 (88, NS)	−0.075 (97, NS)	+0.159 (98, NS)
IgG4	+0.289 (86, $p < 0.01$)	−0.324 (88, $p < 0.001$)	+0.073 (101, NS)	−0.085 (102, NS)
IgM	−0.211 (86, $p < 0.05$)	+0.254 (88, $p < 0.05$)	+0.041 (104, NS)	+0.108 (105, NS)
IgA	+0.082 (85, NS)	+0.130 (87, NS)	−0.185 (96, NS)	+0.307 (97, $p < 0.01$)
IgE	+0.171 (79, NS)	−0.015 (81, NS)	−0.307 (95, $p < 0.01$)	+0.261 (96, $p < 0.01$)

a) Spearman's correlation coefficient: number of observations and level of significance in parentheses. NS = Not significant.

Table 3. Correlations between IgE responses against different adult-worm Ag preparations, intensities of infection and age

Blood sample: Tested against:	Pretreatment (Bleed A)		Post-treatment (Bleed B)	
	Pretreatment intensity	Age	Post-treatment intensity	Age
IgE Ab against ^{a)} :				
Adult worm	+0.171 ^{b)} (79, NS)	−0.015 (81, NS)	−0.307 (95, $p < 0.01$)	+0.261 (96, $p < 0.01$)
Adult worm (periodate-treated)	+0.259 (77, $p < 0.05$)	−0.162 (79, NS)	−0.239 (95, $p < 0.05$)	+0.053 (96, NS)
Adult worm (Con A eluate)	−0.046 (64, NS)	+0.160 (66, NS)	−0.243 (88, $p < 0.05$)	+0.278 (89, $p < 0.01$)
Tegument	+0.015 (70, NS)	+0.041 (72, NS)	−0.258 (93, $p < 0.05$)	+0.169 (94, NS)
Denuded body	−0.046 (64, NS)	+0.070 (66, NS)	−0.292 (93, $p < 0.01$)	+0.240 (94, $p < 0.05$)
PBS extract	+0.089 (71, NS)	−0.098 (73, NS)	−0.217 (93, $p < 0.05$)	+0.113 (94, NS)

a) See Sect. 2.5 for details of the adult-worm preparations tested.

b) Spearman's correlation coefficient: number of observations and level of significance in parentheses. NS = Not significant.

Thus, these results demonstrated that IgE against adult-worm Ag were associated with the development of an age-related resistance to reinfection and could be dissociated from the effect of age itself. However, this pattern was not invariably observed. When IgE against adult-worm

Ag were assayed in sera from a previously studied group of 129 children aged 9 to 16, no significant relationship with reinfection was detected for blood samples taken before treatment or at 5 weeks or 3 months after treatment ($r = 0.090$, -0.025 and -0.043 respectively, >120 df), even though positive correlations with age were again observed ($r = 0.210$, 0.266 and 0.340 , respectively, >120 df;

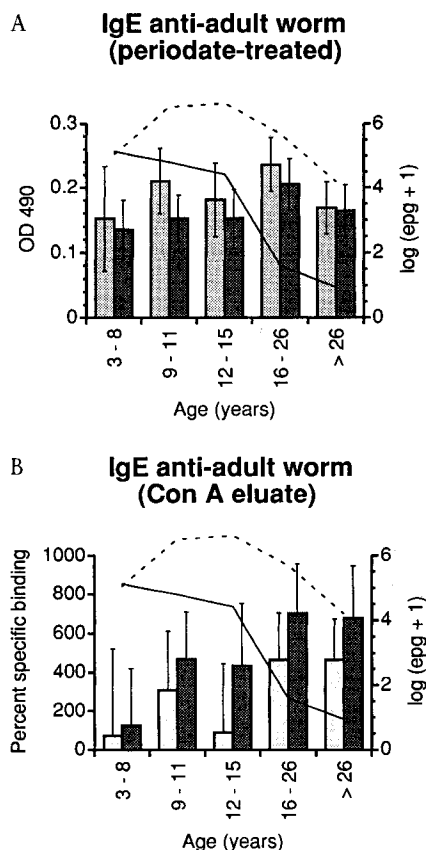


Figure 5. IgE against: A: sodium periodate-treated adult-worm Ag; B: Con A-binding fraction of adult worm. Results expressed as in Fig. 3, except that results for B represent % binding in an RIA.

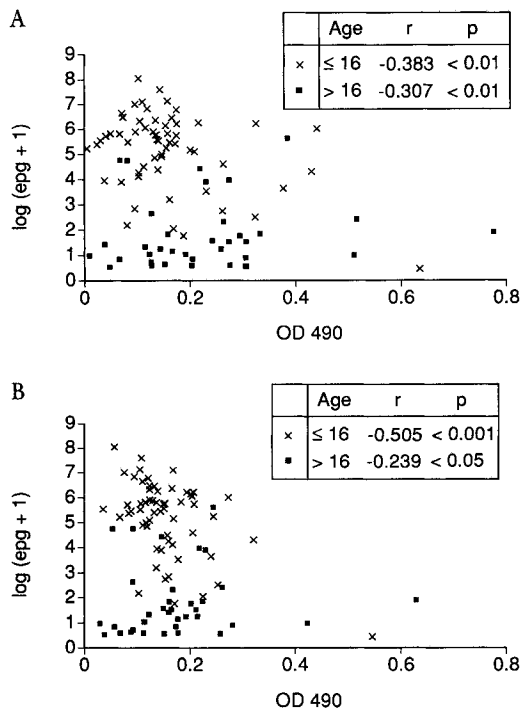
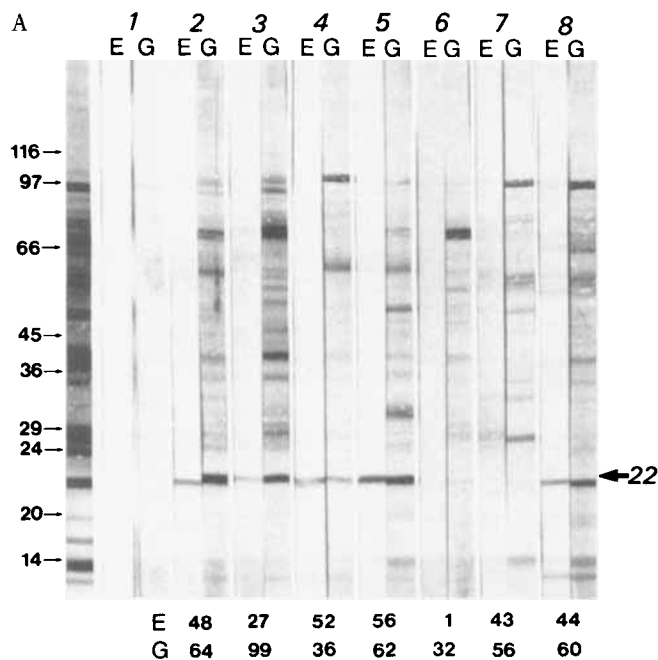


Figure 6. (A) IgE against adult-worm Ag for (X) individuals ≤ 16 years; (■) individuals > 16 years in Study H. (B) IgE against a sodium periodate-treated adult-worm Ag preparation for: (X) individuals ≤ 16 years; (■) individuals > 16 years. Results shown as individual values for the post-treatment blood sample (Bleed B), plotted against individual reinfection scores expressed as $\log_e$ (epg + 1).

$p < 0.01$, 0.01 and 0.001, respectively). Possible explanations for the differences between this study group and Study H are discussed below.

3.5 Adult-worm Ag recognized by human IgE Ab

The extent of diversity among the individual adult-worm Ag recognized by human IgE, in comparison with IgG, was



examined in Western blots. Fig. 7A shows the pattern of Ag recognized by IgE and IgG in human sera. Both for these and other sera (not shown), there was no evidence of the selective recognition by IgE of Ag not recognized by IgG. However, whereas IgG recognized a large number of Ag, the pattern of Ag recognized by IgE was much more restricted. In particular, a band of 22-kDa was strongly recognized by IgE in many, although not all, sera that were selected on the basis of high IgE ELISA responses against the adult-worm Ag (Fig. 7B).

Quantitative estimates of the recognition by IgE of the 22-kDa or other Ag are not yet available. However, the relationship could be examined qualitatively on the basis of the presence or absence of a band in IgE Western blots. Table 4 demonstrates that incidence and intensities of reinfection were significantly lower among those who had detectable IgE against the 22-kDa Ag than among those who did not.

4 Discussion

Two distinctive features of human schistosomiasis are the markedly convex shape of the typical age-prevalence or age-intensity curve (implying a lack of new infections in older individuals) and the relative lack of reinfection after chemotherapeutic cure that is observed in older children and adults. Although a reduction in exposure may provide part of the reason for this lack of superinfection or of reinfection after treatment [23, 24], it is now generally accepted that, in addition, there is also a marked effect of an age-dependent acquired resistance to infection or

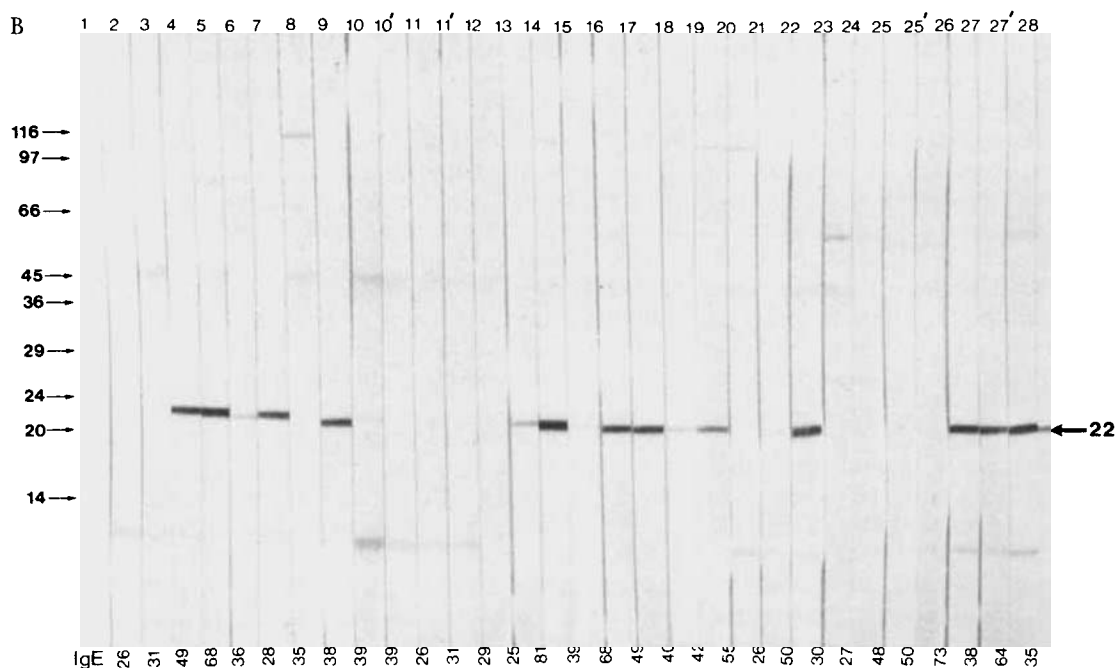


Figure 7. Western blots of adult-worm Ag with selected individual infection sera and developed for the presence of IgE or IgG. The predominant 22-kDa Ag is arrowed. (A) Normal human serum (1) and sera from *S. mansoni*-infected individuals (2–8), each showing the presence of anti-worm IgE (E) and IgG (G) Ab. The results at the bottom of blot show the corresponding IgE (E) and IgG (G) values (A_{490}) obtained for each serum in the anti-adult-worm ELISA. (B) Western blot of adult-worm Ag showing IgE responses in normal human serum (lane 1) and 24 *S. mansoni*-infected individuals (lanes 2 to 28) all selected as high anti-worm IgE responders in ELISA; corresponding ELISA results are shown at the bottom of each lane. 10 and 10', 11 and 11', 25 and 25', 27 and 27' are pairs of sera taken from the same individuals at two time points at least 6 months apart.

Table 4. Relationship between the recognition by IgE Ab of a 22-kDa adult-worm Ag and prevalence and intensity of reinfection after treatment

		IgE anti-22 kDa ^{a)}			
		+	–		
Number ^{b)}	Not reinfected	12	21	$\chi^2 = 9.22$ $p < 0.01$	a) IgE Ab against a 22-kDa Ag detected in Western blots. b) Number of individuals becoming 'reinfected' = epg score of $\log(\text{epg} + 1) > 2.5$ or not becoming 'reinfected' = $\log(\text{epg} + 1) < 2.5$. c) Mean intensity of reinfection, expressed as geometric mean epg (95 % confidence interval in parentheses).
	Reinfected	6	53		
	% reinfected	33	72		
Intensity of reinfection ^{c)}		10.9 (3.3, 31.6)	65.9 (39.7, 109.1)	$F = 9.04; 1, 90 \text{ df}$ $p < 0.01$	

reinfection [3, 4, 25, 26]. This finding, together with the extensive demonstration, in experimental animals, of the induction of protective immunity by a variety of procedures, suggests that it may eventually be possible to induce such a resistance by vaccination. However, successful vaccination will require not only an appropriate protective Ag, but also the induction of appropriate protective immune responses. One approach to determining what responses might be appropriately elicited by a vaccine is to identify those responses that are associated with the expression of immunity under conditions of natural infection and exposure.

In this context, a striking feature of *S. mansoni* infection is that, although young children manifest a wide range of immune responses to a variety of schistosome Ag from the age of 6 or 7 years at the latest [2, 8], they remain susceptible to reinfection after treatment; immunity to reinfection cannot be demonstrated until the children reach about the age of 12 years [3]. Thus, the responses that develop in young children are insufficient, *in toto*, to mediate protection, and we have previously presented evidence that in such children the predominant responses are "blocking" Ab that prevent the expression of protective responses. Our hypothesis has been that these blocking Ab, of the IgM and IgG2 isotypes, are elicited in response to carbohydrate epitopes expressed in Ag released from the egg (the major immunogenic stimulus in young children), cross-react with epitopes expressed at the surface of the migrating larva (*schistosomulum*) that is a potential target for immune attack, and prevent the binding and action of potentially protective Ab. However, these early studies gave no clear indication as to the nature of any protective responses.

Subsequently, Wilkins and colleagues [4] carried out similar treatment and reinfection studies on patients with *S. haematobium* infection. They found clear evidence for an age-dependent resistance to reinfection after chemotherapy, distinguishable from a lack of reinfection attributable to a lack of exposure. Hagan et al. [10] also demonstrated that certain immune responses correlated with the expression of such resistance. After allowing for age, sex and exposure in a logistic regression analysis, they were able to show that individuals with the highest levels of IgE Ab against either adult-worm or egg Ag were ten times less likely to become reinfected after treatment than those with the lowest levels. In contrast, those with the highest IgG4 levels against the same Ag were ten times more likely to become reinfected than those with the lowest levels (although this could

simply have reflected the levels of infection before treatment).

Stimulated by these results, we carried out a similar but more extensive analysis of IgE responses in *S. mansoni* infections, as part of a wider serological investigation of the Study H group of patients, which will be published elsewhere. The basic finding was that, as in the study of Hagan et al. on *S. haematobium*, IgE Ab to adult-worm Ag in a blood sample taken after treatment, but before any reinfection had occurred, were negatively correlated with intensity of subsequent reinfection, indicating that such Ab are predictive of an associated with resistance to reinfection.

Although care must be taken in assigning causality to this observed association, various other findings suggest that this might indeed be the case. First, although both reinfection and anti-worm IgE were strongly correlated with age, in a multiple regression analysis of the results for children ≤ 16 years old, the negative correlation of IgE with reinfection was still significant even after allowing for age. In fact, most of the information in the study group as a whole was in the younger ages, older individuals showed little reinfection, irrespective of their IgE levels. This may be attributable to exposure being heterogeneous in older adults; and individual may fail to become reinfected either because he has high IgE levels in spite of high exposure, or because he has low levels of exposure. In this context, it is worth noting that the data in Figs. 6A and 6B are asymmetrical. Although there are many individuals with low levels of reinfection and low levels of IgE, there are no individuals with high levels or reinfection and high levels of IgE.

Second, the observed negative correlations between IgE anti-adult-worm Ab and reinfection was not simply a manifestation of a high degree of immune responsiveness in general, or of IgE responses in particular. Thus, there were no negative correlations with reinfection for Ab of other isotypes against adult-worm Ag. More importantly (and in contrast to the results of Hagan et al. [10], the negative correlations for IgE were restricted to IgE Ab against adult-worm Ag, some other IgE responses in fact being positively correlated with reinfection.

Third, negative correlations of IgE with reinfection were consistently observed for several preparations of adult-worm Ag. Such preparations included a PBS extract of adult worms that had been denuded of the tegumental membrane, as well as the tegumental membranes them-

selves, implying that the relevant Ag can be distributed throughout the worm. Although correlations were observed with a fraction of adult-worm Ag that bound to Con A, implying that Ab against carbohydrate-containing Ag can be involved, the presence of carbohydrate epitopes is not essential, since negative correlations were also observed (and were in fact the strongest) for a worm preparation that had been treated with sodium periodate, a procedure that destroys many carbohydrate epitopes.

In other studies, various workers have concentrated on investigating correlations for individual Ag, such as paramyosin [27] or the 37-kDa glyceraldehyde-3-phosphate dehydrogenase described by Dessein and colleagues [26]. Similarly, in separate studies to be reported elsewhere, we have also specifically examined IgE and other Ab against one candidate vaccine Ag, the 28-kDa glutathione-S-transferase (Capron et al., in preparation). In the present study, however, we deliberately chose to start with a crude and heterogeneous mixture of Ag from each life cycle stage to avoid any preconception as to which Ag might be important. This approach could have been unhelpful, since the average of the responses to all Ag in a preparation, (relevant and irrelevant), might well have yielded uninformative results. The fact that significant correlations were obtained allowed us to concentrate on analyzing in more detail the responses of interest, namely IgE to the adult-worm preparations. As an initial step, IgE-specific Western blots of adult-worm Ag were prepared. These demonstrated that the IgE response was restricted in specificity, in comparison with the IgG response, with one 22-kDa Ag in particular being commonly recognized. Subsequently, it could be shown that the presence of IgE against this Ag was itself associated with resistance to reinfection, although this does not exclude the possibility that IgE responses to other Ag present in the adult-worm preparations might also be important. The nature of the 22-kDa Ag and its relation to Ag of similar size described by other workers remain unknown. A monospecific rabbit serum raised against the eluted material has revealed positive clones in a λ gt11 expression library (Havercroft et al., unpublished observations).

Not surprisingly, a variety of questions remain unanswered by this study. In particular, it is not clear why the negative correlations of IgE with reinfection should have been observed only in Study H and not in Study 129 [1, 2]. One possible explanation is that reinfection occurred much more rapidly in group Study 129 than in Study H. Thus, those protective immune responses that were present immediately after treatment would have been more important. These may have included Ab against schistosomulum surface Ag that mediate antibody-dependent cellular cytotoxicity (ADCC) reactions, these Ab being blocked by IgM and IgG2. In contrast, in Study H the time before re-exposure was sufficiently long that such anti-schistosomal protective Ab may have declined, leaving a later stage of the parasite as the target for protective immunity mediated by IgE. In this context, it should also be pointed out that the exact target of an IgE-mediated protective response remains uncertain. At present, it is reasonable to assume that adult worms are not susceptible to immune attack in their human hosts, and that egg output accurately reflects the adult-worm burden [28]. The assumption, therefore, must be that the target is some stage of the parasite found

during its migration from the skin to the hepatic portal system. Finally, it should also be pointed out that the mechanism of an IgE-mediated protective effect is equally uncertain. Although ADCC reactions involving IgE and eosinophils, macrophages or platelets have been clearly demonstrated for skin-stage schistosomula in both the rat [29] and human [30], there is no evidence yet that such reactions can act on older organisms; other IgE-dependent reactions may also be important.

Although various issues remain unresolved, the key finding presented here is the confirmation for *S. mansoni* of an association, which may be causal in nature, between IgE and immunity. Although a protective role of IgE in helminth infections has been postulated for many years, and clearly demonstrated in the rat model of schistosomiasis [11, 12, 29], recent studies in murine models of some helminth infections have been interpreted as showing that responses controlled by the T_H2 subset of T_H cells, including IgE production, are associated with pathology, while T_H1 responses are associated with protection [31]. We are now attempting to test this directly by measuring T_H1 and T_H2 cytokine production in response to adult-worm and other Ag in a new group of study patients. Our prediction is that humans are rat-like in their immune effector mechanisms against helminths, and that production of T_H2 cytokines (especially IL-4 and IL-5) will be associated with the expression of immunity.

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