Antibody Response Patterns against *Schistosoma mansoni* in a Recently Exposed Community in Senegal

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Acquired immune resistance is believed to be largely responsible for age-dependent infection and reinfection patterns in schistosomiasis. In a recently established but intense focus of *Schistosoma mansoni* in Senegal, the humoral immune response was studied in a random population sample of 289. Antibody levels of various isotypes to schistosome worm and egg antigens were determined by ELISA and related to egg counts (eggs per gram of feces [EPG]), age, and sex. Both IgG1 and IgG4 followed age-related patterns similar to egg counts and strongly correlated with EPG, even after allowing for age. Specific IgE levels increased slowly with age. The humoral immune response patterns in this recently infected population appeared to be largely similar to those in chronically infected communities. Thus far, the observations do not support the current hypothesis that age-related resistance to *Schistosoma* is determined by IgE-mediated protective immunity acquired during many years of exposure.

A number of studies have provided evidence for immune effector mechanisms that would explain why, in endemic areas, susceptibility to (re)infection with *Schistosoma* organisms decreases with age [1–4]. Seroepidemiologic studies demonstrated a positive correlation between the acquisition of resistance to reinfection after treatment and the level of parasite-specific IgE [2, 5], while levels of specific IgG4 were positively correlated with intensities of infection before treatment [4, 6] or with reinfection after treatment [2, 5]. Hagan et al. [2] combined these observations with additional evidence that IgE mediates in vitro antibody-dependent cytotoxicity against schistosomula involving mononuclear phagocytes, eosinophils, and platelets [7–9] and that IgG4, which is inefficient in complement activation and binding to cellular receptors, might compete with IgE for the same antigenic sites [10]. They provided seroepidemiologic evidence for the hypothesis that high levels of human IgE protect against reinfection with *Schistosoma haematobium* and that the late development of immunity reflects a slow maturation of the protective IgE response while early production of IgG4 against worm and egg antigens blocks the activity of anti-parasite IgE [2]. Subsequently, similar mechanisms for *Schistosoma mansoni* have been suggested by studies in Kenya [4] and Brazil [3, 11].

Apart from the role for parasite-specific IgE and IgG4, blocking antibodies of IgG2 and IgM isotypes directed against carbohydrate egg antigens and cross-reacting with schistosomulum surface antigens are supposed to be involved in age-related immunity [12–15]. It has been hypothesized that during early infections in young children, the major antigenic stimuli are the predominantly polysaccharide egg antigens, which elicit a T cell–independent antibody response consisting mainly of IgM and IgG2 isotypes [1, 13]. These levels of so-called blocking antibodies gradually decline with age, allowing the binding and action of effector antibodies such as IgG1, for example, in antibody-dependent cellular cytotoxicity, and hence the expression of immunity.

In both hypotheses, the expression of immunity against *Schistosoma* organisms would require a large time span to develop, which explains the typical decline of prevalences and intensities of infection in adults. Thus far, immunoepidemiologic studies have been done in endemic areas, where people are infected from a very young age. In these situations, the effect of history of exposure on the development of resistance to schistosomiasis cannot be separated from the effect of age.

Recently, an intense outbreak of schistosomiasis in a previously noninfected community in Richard-Toll, Senegal, has been described, which is probably due to human-made ecological changes [16]. This situation, although a troubling problem to the local community, provides an opportunity to analyze humoral immune responses to *Schistosoma* in a population that has been exposed recently [16–18] and where the history of

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Samples were collected in consultation and full cooperation with both the local authorities and the persons concerned. Persons showing *Schistosoma* egg counts were treated with praziquantel according to the protocol current at the moment of sample collection.

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exposure is not dependent on the age of the people. According to the above-described hypotheses, age-related patterns of (re)-infection and immune responses should be absent or less pronounced compared with those in "normal" endemic situations. We investigated the development of the immune response in the population according to a study design described by Gryseels et al. [18]. Four cross-sectional population samples (cohorts) were randomly selected from the population of Ndombo, Senegal, at 8-month intervals and surveyed, treated, and followed during reinfection. By assuming that each cohort is representative of the community, a longitudinal study is approximated as far as possible.

Here we provide baseline data from the first cohort. Antibody levels to schistosome worm and egg antigens were determined by ELISA and related to egg counts, age, and sex. The results were compared with published data of similar studies in populations in which schistosomiasis is endemic.

Materials and Methods

Population. The study took place in Ndombo, a village of ~4000 inhabitants, 4 km south of Richard-Toll, in northern Senegal. The populations of Richard-Toll and Ndombo largely depend on the same canal for their water supply. The epidemiologic design and results have been published elsewhere [19] and are only briefly summarized here.

Sera and egg counts. From a randomly drawn 10% population sample (422 persons), stools and venous or capillary blood samples were obtained. Sera were prepared in Senegal and stored frozen until they were transported on dry ice to Leiden, where they were aliquoted and stored at −70°C. For each person, 2 duplicate 25-mg Kato slides were prepared from 2 stool samples within an interval of 2 weeks. Individual results were calculated as arithmetic mean of the egg counts and converted to eggs per gram of feces (EPG). In the data analysis, the egg counts were expressed as $\log_{10}(\text{EPG} + 1)$ (to allow for zero counts), and means were calculated as the geometric mean of EPG + 1.

Parasite antigen preparations. Adult *S. mansoni* worms (Puerto Rico strain) were collected from golden hamsters by perfusion of the hepatic portal system with a balanced salt solution 7 weeks after infection with 1500 cercariae. Adult worm antigen (AWA) was prepared from supernatant obtained after the worms were homogenized in PBS (0.035 *M* PBS, 0.15 *M* NaCl, pH 7.6) and centrifuged at 25,000 *g* for 1 h at 4°C. This preparation was then dialyzed against deionized water and freeze-dried [20]. Soluble egg antigen (SEA) was prepared following the same procedure using *S. mansoni* eggs isolated from infected hamster livers by means of homogenization and trypsinization as described [21].

Immunoassays. ELISAs were done using the antigen preparations AWA and SEA as target antigen. To allow comparison of results, the methods used followed as closely as possible those described by others [6, 22, 23]. Antigen concentrations and serum and conjugate dilutions were optimized by checkerboard titration using pooled positive and negative sera. For both antigens, anti-human isotype antibodies were used to quantify the specific IgA, IgE, IgG, IgG1, IgG2, IgG3, IgG4, and IgM present in individual human sera.

Specifications of the optimized procedures are summarized in table 1. In all assays, Maxisorp plates (Nunc, Roskilde, Denmark) were used, and incubation volumes were 100 μL for coating, 120 μL for postcoating and substrate, and 80 μL for samples and conjugates. As coating buffer, PBS was used, and as assay buffers, 0.1% Tween 20 in PBS was used for all isotypes, except for IgE, for which 0.1 or 0.05 *M* TRIS-HCl, pH 7.5, and 0.05% Tween 20 was used for the sample dilutions, with addition of 10% fetal calf serum for the conjugates. The ELISA plates were coated overnight at 4°C with AWA or SEA (5 μg/mL) or with 0.3% bovine serum albumin (BSA) as a control antigen, followed by postcoating with 0.3% BSA for 1 h at 37°C in PBS. This was done for all assays except for the IgE assay, in which no BSA control or postcoating was found to be necessary.

Enzyme conjugates labeled with horseradish peroxidase (HRP) were used, and the assays were developed with 3,3′,5,5′-tetramethylbenzidine (TMB) [24]. The antibody levels of individual sera covered a long range of reactivities and therefore the absorbance at 630 nm was measured kinetically at two time points within 10 min (the time span in which TMB-HRP color development occurs linearly [25]). The calculated A_{630} slopes (in milli–optical density units per minute, ΔA_{630}) were used for further data analysis, resulting in a more accurate quantitation than with a single absorbance measurement after a longer period of time [25].

The cutoff levels for the various ELISAs were determined as the arithmetic mean + 3 SD of the antibody activities of 20 sera from healthy Dutch blood donors.

Expression and quality control of ELISA data. To correct for inter- and intraassay variation, for each of the different ELISA systems (combination of isotype and antigen) a different serum was selected to serve as a standard, which covered as much as possible the range of activities found in the samples. Each standard serum was arbitrarily assigned 10^6 units (arbitrary units, AU/mL), and a duplicate dilution series was done to obtain the standard curve, which was fitted using four-parameter curve-fitting [26, 27]. The antibody activity of the samples was expressed as AU per milliliter of the respective standard serum. If the sample response exceeded the upper limit of the standard serum, the sample was retested in a higher dilution. All samples were tested in duplicate, and a reference serum was included in three positions on each plate to detect any systematic errors.

To obtain a crude estimation of the absolute concentrations of the specific antibody levels in the standard sera, purified immunoglobulins were coated in dilution series directly to the ELISA plates and the concentrations of anti-AWA, and anti-SEA antibodies in the standard sera were read against these dilution curves. The concentrations obtained for the individual standard sera were well within the ranges that could be expected. From these values, the specific antibody concentrations in the serum samples could be approximated.

To exclude possible bias by the four-parameter function data-transformation procedure, some of the associations were also investigated using only ΔA_{630} values after correction for interplate variation. As no significant differences were found, only results based on transformed data will be reported.

Statistical analysis. The relationships between the various antibody activities (isotypes and antigen specificity) and between antibodies and pretreatment EPG were examined after log-transformation of the data as they approximated a log-normal distribution.

Table 1. Specifications of the optimized ELISAs.

Isotype	Serum dilution AWA	Serum dilution SEA	Serum incubation	Primary conjugate* Preparation	Primary conjugate* Dilution	Primary conjugate* Incubation	Secondary conjugate†, incubation
IgA	1/100	1/100	1 h, 37°C	Goat anti-human IgA/biotin‡	1/1000	2 h, 37°C	1 h, 37°C
	1/100	1/100	ON, 4°C	Monoclonal anti-human IgA/HRP§	1/1000	2 h, 37°C	NA
IgE	1/30	1/30	ON, 4°C	Goat anti-human IgE/biotin‡	1/1000	ON, RT	4 h, 37°C
IgG	1/500	1/500	1 h, 37°C	Goat anti-human IgG/biotin‡	1/10,000	1 h, 37°C	1 h, 37°C
IgM	1/500	1/500	1 h, 37°C	Goat anti-human IgM/biotin‡	1/10,000	1 h, 37°C	1 h, 37°C
IgG1	1/100	1/500	ON, 4°C	Monoclonal anti-human IgG1/HRP§	1/3000	1 h, 37°C	NA
IgG2	1/100	1/100	ON, 4°C	Monoclonal anti-human IgG2/biotin‖	1/1000	1 h, 37°C	1 h, 37°C
	1/30	1/30	ON, 4°C	Monoclonal anti-human IgG2/HRP§	1/1000	1 h, 37°C	NA
IgG3	1/30	1/30	ON, 4°C	Monoclonal anti-human IgG3/biotin‖	1/1000	1 h, 37°C	1 h, 37°C
	1/30	1/30	ON, 4°C	Monoclonal anti-human IgG3/HRP§	1/1000	1 h, 37°C	NA
IgG4	1/100	1/100	1 h, 37°C	Monoclonal anti-human IgG4/biotin‖	1/1000	1 h, 37°C	1 h, 37°C
	1/100	1/100	1 h, 37°C	Monoclonal anti-human IgG4/biotin§	1/1000	1 h, 37°C	1 h, 37°C
	1/100	1/100	1 h, 37°C	Monoclonal anti-human IgG4/HRP§	1/3000	1 h, 37°C	NA

NOTE. ON, overnight; RT, room temperature; NA, not applicable; HRP, horseradish peroxidase.

* For practical reasons, some anti-human immunoglobulin conjugates were changed during testing, after it had been verified that this had no influence on antibody levels measured.

† Streptavidin/HRP (Dakopatts, Glostrup, Denmark), 1/3000 diluted.

‡ Vector Laboratories, Burlingame, California.

§ Central Laboratory for Blood Transfusion, Amsterdam.

‖ Sigma, St. Louis.

Pearson's product-moment correlation coefficients and geometric means (±95% confidence intervals) were used to describe quantitative relationships and associations between the different variables.

Results

Intensity of infection versus age and antibody versus age profiles. Fecal egg counts of the group ($n = 289$) from which sera also were tested are shown in figure 1 for individuals and for age groups. The prevalence was almost 100% in all age groups >5 years (overall prevalence in this group, 96%), but egg counts declined strongly in adults. Patterns for men and women were not significantly different, although the peak intensity tended to be at a lower age for women (data not shown). More epidemiologic details are given by Stelma et al. [19].

For each antibody assay, the percentage of positive samples in the population is shown in figure 2. Generally, 75%–100% positivity was found in the anti-SEA assays, with the exception of IgA, which was positive up to 30% in the older age groups. In the anti-AWA response, particularly IgA and IgG2 showed very low positivity rates (12% and 28%, respectively), and only 52% of the samples were positive in the anti-AWA IgM assay. Positivity rates were not significantly associated with egg counts, except for the antibody isotypes that showed an overall correlation with EPG (table 2, e.g., anti-SEA IgG, IgG1, and IgG4).

In figure 2, the geometric mean antibody levels (±95% confidence interval) of IgA, IgE, IgG, IgM, IgG1, IgG2, IgG3, and IgG4 against AWA and SEA are plotted against age. With the exception of anti-AWA IgM and IgG3, all antibody patterns showed a peak in the younger age groups (5–20 years). For IgA, IgE, IgG2, and IgG3, a second increase of antibody activity (both against AWA and SEA) was observed in the older age groups. The overall shapes for the antibody-age profiles of the anti-AWA and anti-SEA reactivity were similar. For all isotypes except IgE, the antibody reactivities to SEA were markedly higher than to AWA if expressed as nontransformed ΔA_{630} values or in nanograms per milliliter using the crude estimation of the specific antibody concentrations in the standard sera. For IgE, however, the anti-AWA response was much higher.

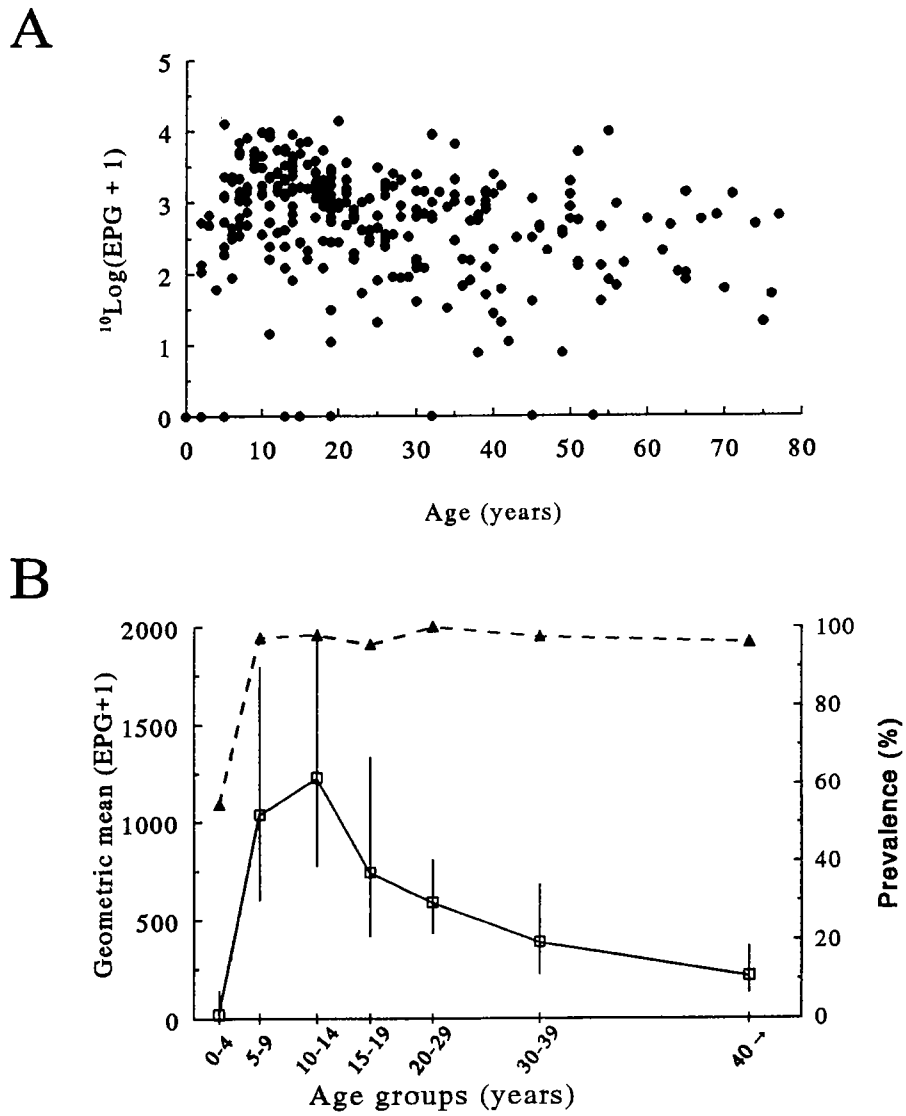

Figure 1. Population pretreatment intensities of infection and prevalence patterns by age. **A**, Egg counts of individual subjects (n = 289); values are expressed as $\log_{10}$(eggs per gram [EPG] + 1). **B**, Prevalence (▲) and intensity of infection (□) of subjects grouped by age. Data are geometric mean ± 95% confidence interval.

Associations between intensities of infection, age, and antibody. The relationships of intensities of infection and age with antibody levels were initially examined by calculating Pearson's product-moment correlation coefficients using log-transformed EPG + 1 and antibody values (table 2). As antibody levels and EPG showed a peak in the lower age groups, the study population was divided into younger (<16 years) and older (≥16 years) groups, and correlations between antibody levels and egg counts were calculated (data not shown). No age effect was observed for any of the isotypes, except that anti-AWA IgA antibodies were not significantly associated with EPG in young persons but in the older group showed a significantly negative correlation.

The similarities between the age-related EPG and antibody patterns (figures 1, 2) suggest a strong correlation between IgG4 antibodies and infection intensity. This was reflected in a highly significant correlation coefficient with EPG (table 2, figure 3A); after dividing the population into <16 and ≥16 years, this association was still highly significant. To a lesser extent, but still significant, this was also observed for IgG1. The correlation with EPG was absent for anti-AWA IgE, while for anti-SEA IgE, a weak negative association was found only in the older group (r = −.15, P = .05, n = 169).

Associations between anti-AWA and anti-SEA response. All antibody isotypes showed a highly significant relationship (P < .001) between the responses against AWA and SEA, most prominently for IgG4 (n = 258, r = .80; figure 3B).

Associations between responses of immunoglobulin classes (IgA, IgE, IgG, and IgM) to AWA and SEA. Table 3 shows the Pearson's correlation coefficients between the responses of the major immunoglobulin classes against AWA and SEA. Strong correla-

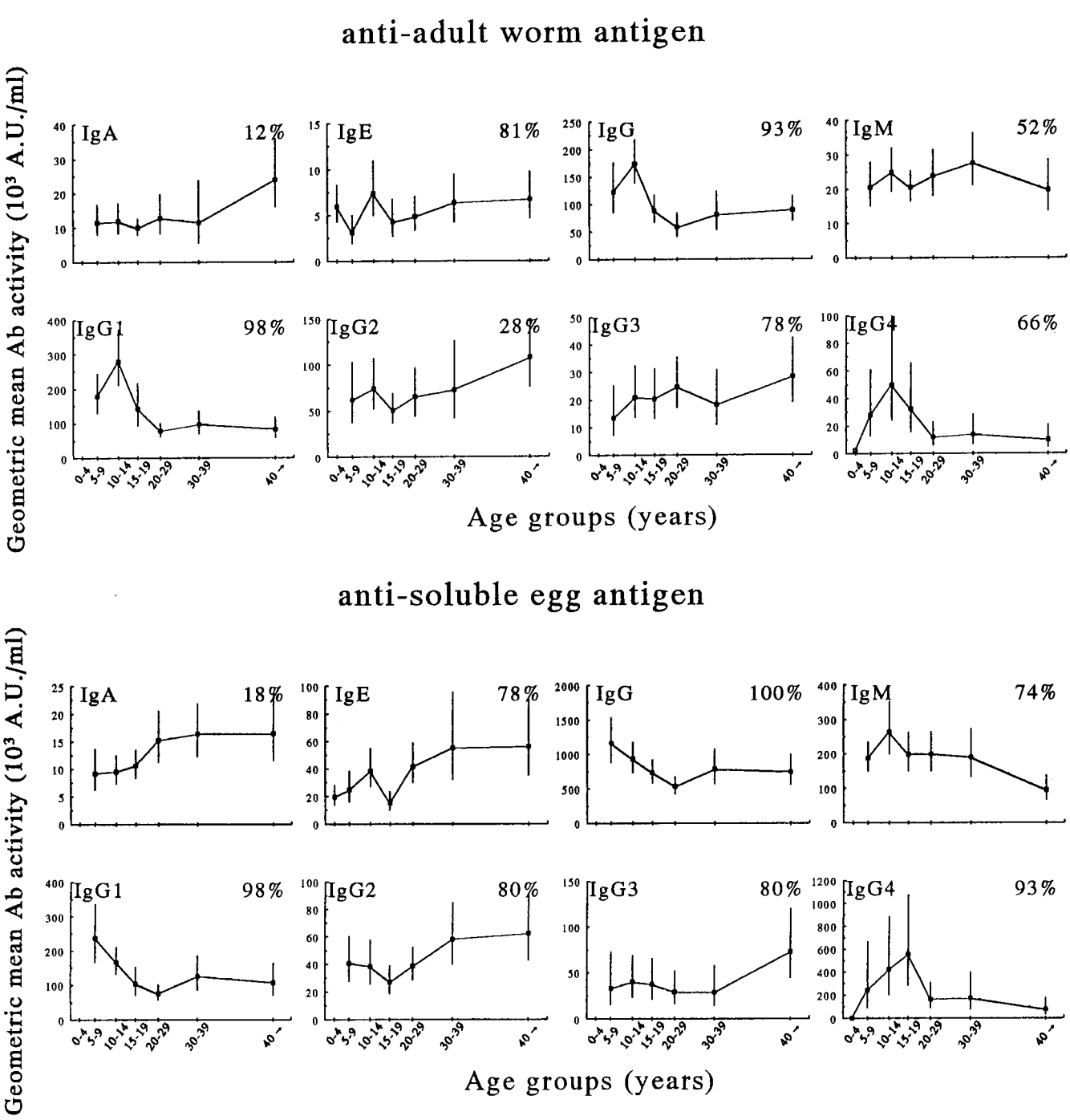

Figure 2. Antibody (Ab) activities by age (geometric mean ± 95% confidence interval) to adult worm antigen and soluble egg antigen; AU, arbitrary units. %s are of positive samples (values higher than mean + 3 SD of 20 negative sera).

tions were observed between the responses to AWA and to SEA for each antibody class, between anti-AWA IgM and the anti-SEA responses of the other antibody classes, and between IgE and IgG against AWA or SEA.

Associations between IgG (sub)class responses to AWA and SEA. Table 4 shows the correlations between the individual IgG (sub)class responses to AWA and to SEA. The responses to AWA correlated well with those to SEA, and anti-AWA IgG1 correlated well with the other (sub)classes. Anti-AWA IgG2 showed no correlation with other subclasses, but it did correlate with anti-SEA IgG2. In the response to SEA, all of the (sub)classes correlated well with each other, with the exception of IgG4, which showed association neither with the other subclasses nor with IgG as a whole.

Table 2. Correlations between antibody responses against adult worm antigen (AWA) or soluble egg antigen (SEA) and intensities of infection or age.

	Anti-AWA		Anti-SEA	
Isotype	Log(EPG + 1)	Age	Log(EPG + 1)	Age
IgA	−.11	.16	−.11	.18
	(P = .09)	(P = .02)	(P = .08)	(P = .004)
IgE	.03	.11	−.09	.22
	(P = .6)	(P = .06)	(P = .2)	(P < .001)
IgG	.22	−.12	.24	−.06
	(P = .001)	(P = .06)	(P < .001)	(P = .3)
IgM	−.18	−.01	.07	−.26
	(P = .003)	(P = .9)	(P = .3)	(P < .001)
IgG1	.31	−.27	.25	−.12
	(P < .001)	(P < .001)	(P < .001)	(P = .08)
IgG2	.07	.14	−.15	.22
	(P = .3)	(P = .03)	(P = .02)	(P = .001)
IgG3	.02	.14	.05	.13
	(P = .7)	(P = .03)	(P = .4)	(P = .04)
IgG4	.42	−.16	.48	−.08
	(P < .001)	(P = .008)	(P < .001)	(P = .19)

NOTE. Data are Pearson's product-moment correlation coefficients, with n between 233 and 285. EPG, eggs/g of feces.

Discussion

The Ndombo project offers the opportunity to study the evolution of humoral immune responses to *S. mansoni* in a recently exposed population and also to evaluate these in the context of current hypotheses on the development of acquired immunity. These hypotheses are generally based on observations in populations that have been exposed to infection from an early age. The development of a specific IgE response is thought to occur very slowly, reaching protective levels only at adolescence. On the other hand, evidence has been presented for regulatory immune mechanisms in which the protective effect of specific IgE was counteracted by IgG4 directed against the same epitopes [5, 10, 23]. A similar mechanism for IgG4 competing with IgE has been suggested to occur in allergic patients [28–31].

The study population can be considered to be naive with respect to schistosomiasis, as *S. mansoni* was not endemic in the area before the dam was built [32, 33]. Only *S. haematobium* was focally present in some villages but was never found in the study village. Ndombo is an old village with a traditional structure and little or no immigration. The adult population can therefore be considered to consist of native villagers with no previous exposure to *Schistosoma* organisms.

The prevalence patterns as shown by EPG were most closely reflected by total IgG and IgG1 against AWA and SEA and IgG4 against SEA. IgE levels were positive in >75% of the cases, but total IgG approximated 100% positivity. IgA positivity rates were very low, which might partly be due to high background reactivity in the assays. In general, the antibody responses to SEA were much higher than those to AWA, except for IgE, for which the reverse was true. Such patterns have been found in other populations (in which schistosomiasis is endemic) as well [2, 10, 34], although sometimes other findings were described [35, 36]. The observation that IgE was mainly directed against AWA and that IgG4 was directed mainly against SEA suggests that the production of specific IgE and IgG4 is not stimulated by the same antigens. It might also be that the time scale of specific antibody production is antigen-dependent and different for IgE and IgG4, although it is known that both are stimulated by interleukin-4 [37–39]. It has been proposed that the IgG4 response against SEA probably precedes the IgG4 response against AWA [10].

Of the antibodies against AWA, only total IgG, IgG1, and IgG4 followed the age-related patterns similar to egg counts (figure 1 and 2), and IgG1 and IgG4 also showed the strongest correlations with EPG (table 2). These findings coincide with immunoepidemiologic studies in chronically infected popula-

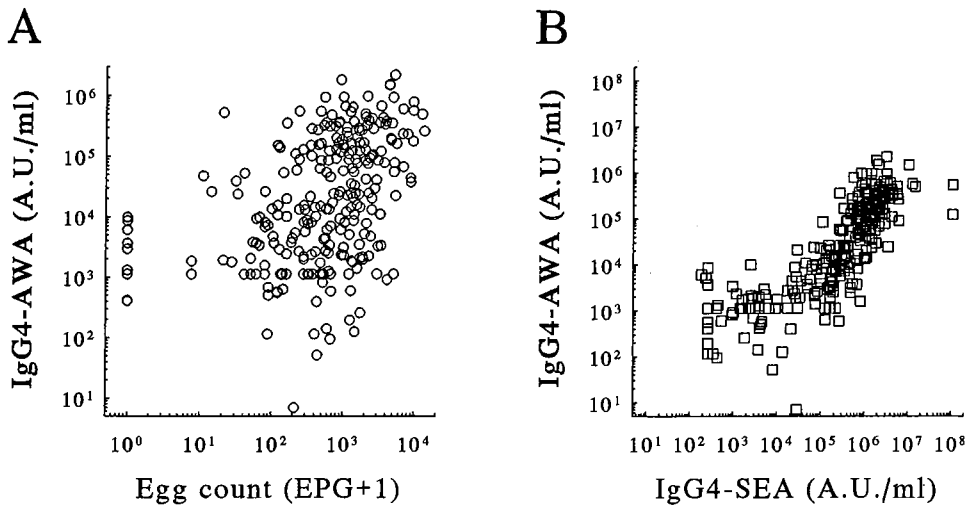

Figure 3. Example of data points used to obtain Pearson's product-moment correlation coefficients; individual values of anti–adult worm antigen IgG4 levels (in arbitrary units [AU]) plotted on logarithmic scales against values for egg counts (as eggs/g [EPG] + 1; **A**) and anti-soluble egg antigen [SEA] IgG4 levels (**B**).

Table 3. Correlations between antibody responses of different isotype classes against adult worm antigen (AWA) and soluble egg antigen (SEA).

	Anti-AWA				Anti-SEA		
	IgA	IgE	IgG	IgM	IgA	IgE	IgG
Anti-AWA							
IgE	−.003						
IgG	.01	.28*					
IgM	.21†	.07	.17				
Anti-SEA							
IgA	.40*	.12	−.02	.30*			
IgE	.12	.62*	.12	.26*	.31*		
IgG	.08	.17	.62*	.30*	.21†	.19†	
IgM	.18†	.05	.24*	.50*	.31*	.08	.33*

NOTE. Data are Pearson's product-moment correlation coefficients, with n between 221 and 261.
* $P < .001$.
† $P < .01$.

tions, in which IgG4 has also been found to follow the age-related egg count patterns and to be the antibody isotype most closely associated with infection intensity [4, 10, 13, 15, 34]. IgG4 is associated with chronic antigen stimulation [10, 29, 34, 40, 41], and the recognition patterns of IgG4 usually are restricted to peptide epitopes [41–43]. Interestingly, however, in schistosomiasis the antibody response to egg antigens generally appears to be dominated by carbohydrate epitopes [44–46].

IgE levels appeared to increase with age (figure 2), but no significant correlations were found with egg counts (table 2). Multivariate linear regression analysis confirmed the correlation of IgG4 and IgG1 with EPG independent of age but did not reveal any association of IgE. A strong serum inhibition in ELISA of specific IgE by competing IgG4 has been described [23] but is considered to be unlikely in the present study for two reasons: Anti-AWA IgE strongly correlates positively with IgG4 (anti-AWA or anti-SEA), and as mentioned above, the development of specific IgE and IgG4 levels appears to be differentially regulated. This also implies that the decreased egg counts and circulating antigen levels as observed in the older age groups within this population [19, 47] might not be the direct result of an increased protective IgE response and decreased levels of blocking IgG4, as is postulated for chronically infected populations in The Gambia *(S. haematobium)* or in Kenya *(S. mansoni)* [2, 4]. It has been suggested (partially on the basis of the Ndombo study) that the acquired resistance, generally believed to be IgE-mediated and to develop slowly over 10–15 years, does not depend on prolonged and cumulative exposure to the relevant antigen but might

Table 4. Correlations between antibody responses of different IgG (sub)classes against adult worm antigen (AWA) and soluble egg antigen (SEA).

	Anti-AWA					Anti-SEA			
	IgG1	IgG2	IgG3	IgG4	IgG	IgG1	IgG2	IgG3	IgG4
Anti-AWA									
IgG2	.19*								
IgG3	.16	−.05							
IgG4	.42†	.11	.03						
IgG	.68†	.05	.30†	.29†					
Anti-SEA									
IgG1	.60†	.07	.17	.14	.56†				
IgG2	.16	.22†	.07	−.18	.14	.35†			
IgG3	.26†	−.02	.53†	.11	.26†	.32†	.26†		
IgG4	.31†	.10	−.004	.80†	.15	.11	−.16	.05	
IgG	.50†	.10	.21*	.06	.62†	.80†	.40†	.31†	.03

NOTE. Data are Pearson's product-moment correlation coefficients, with n between 214 and 259.
* $P < .01$.
† $P < .001$.

Table 5. Correlations between IgG (sub)class responses to soluble egg antigen (SEA) and total IgG responses against SEA and adult worm antigen (AWA) in chronically and recently infected populations (ages, 9–16 years).

	Anti-SEA								Anti-AWA, IgG	
	IgG1		IgG2		IgG3		IgG4			
Anti-SEA	C	R	C	R	C	R	C	R	C	R
IgG1									.12	.46*
IgG2	.42*	.40*							.09	.15
IgG3	.33	.14*	.40*	.26					.29*	.06
IgG4	−.01	−.14	−.06	−.24	−.06	−.15			.36*	.11
IgG	.34*	.67*	.42*	.49*	.27†	.13	.44*	−.001	.44*	.38†

NOTE. C = chronic; chronically infected population of Kenyan schoolchildren [6]. R = recent; recently infected population from this study of same ages (n = 46–57). Data are Pearson's product-moment correlation coefficients. Underlining indicates major differences between antibody responses in these 2 populations.

* $P < .001$.

† $P < .01$.

instead be intrinsically age-dependent and regulated by other age-related physiologic processes [18, 48, 49].

We found only a weak, negative association of anti-SEA IgG2 and no association of anti-SEA IgM with egg counts (table 2). Previously, in chronically infected schoolchildren, the presence of IgG2 and IgM antibodies recognizing carbohydrate epitopes on both egg and schistosomulum antigens was positively correlated with reinfection intensity and therefore associated with the blocking of protective immune mechanisms [6, 13, 15]. Further follow-up of the IgG2 and IgM patterns in consecutive cohorts and their relation to reinfection patterns might clarify whether the difference in these observations is indeed due to the duration of exposure.

An interesting observation was made when the influence of sex on the antibody response was investigated. No differences in egg counts were observed between males and females, but anti-AWA or anti-SEA IgG4 levels were significantly higher in males (by t test, P = .003 and P = .007, respectively) in the older age group (≥16 years). Also exclusively in the older age group, IgM antibodies tend to be higher in females (t test, P = .02 and P = .04 for anti-AWA and anti-SEA antibodies, respectively).

Table 5 summarizes in more detail the correlations between IgG (sub)class responses to SEA and IgG to AWA compared with those described by Dunne et al. [6] for a chronically infected population. The patient group in this study was a group

Table 6. Correlations between IgG, IgG4, and IgE responses to adult worm antigen (AWA) and soluble egg antigen (SEA) in Egyptian *S. mansoni*– or *Schistosoma haematobium*–infected and recently *S. mansoni*–infected persons of all ages.

	Anti-AWA						Anti-SEA			
	IgG		IgG4		IgE		IgG		IgG4	
	E	S	E	S	E	S	E	S	E	S
Anti-AWA										
IgG4	.75	.29*								
IgE	.34	.28*	.62	.27*						
Anti-SEA										
IgG	.80	.62*	.45	.06	.17	.17†				
IgG4	.67	.15	.66	.80*	.25	.17†	.70	.03		
IgE	.20	.12	.39	.05	.69	.62*	.21	.19†	.25	.04

NOTE. E = Egyptian; 34 patients were tested for IgG, 40 for IgG4 and IgE; no significance levels were given [10]. S = Senegal; population recently infected with *S. mansoni* (this study; n = 222–269). Data are Pearson's product-moment correlation coefficients. Underlining indicates major differences between antibody responses in these 2 populations.

* $P < .001$.

† $P < .01$.

of Kenyan schoolchildren aged 9–16 years, and therefore we also selected only those in this age group for comparison. The main difference between the antibody responses in the two populations is found in the IgG4 subclass (underscores). In the chronically infected population, all of the individual IgG subclasses correlated well with total IgG, but in recently infected persons, IgG4 responses appeared to be dissociated from total IgG. In both situations, IgG4 was not associated with any of the other IgG subclasses. With regard to the anti-AWA IgG response, however, major differences were found for anti-SEA IgG1 and IgG4. In the chronic population, total anti-AWA IgG appeared to be related predominantly to IgG4, while in the recently infected patient group, only an association with IgG1 was found. Table 6 shows that a similar pattern with respect to IgG4 and total IgG (underscores) was found for a group of chronically infected Egyptian patients of all ages ([10] and this study).

Although care must be taken in assigning causality to the observed associations, the differences may reflect the immature IgG4 response in the recent group and the total IgG response being dominated by IgG1, while in the chronic group IgG4 makes up a larger portion of the total IgG antibodies. This interpretation is supported by several reports showing evidence for early production of specific IgG1 shifting to IgG4 in response to immunizations or infections [29, 41, 42] and generally increased levels of specific IgG4 in chronic parasitic infections [10, 50]. The study of the levels of IgG4 and other isotypes in the consecutive cohorts of this longitudinal Ndombo study might shed more light on the development and maturation of the humoral immune response.

Further studies in subsequent cohorts are in progress, in collaboration with other groups, by which we might be able to show the transition in immune response from a recent to a more chronic situation. Thus far, however, the epidemiologic results presented previously [18, 19, 49] as well as the immunologic results herein do not support the current concept of IgE-mediated acquired immunity in age-related resistance by prolonged exposure to *Schistosoma* antigens as such.

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