Development of Antibody Isotype Responses to *Schistosoma mansoni* in an Immunologically Naive Immigrant Population: Influence of Infection Duration, Infection Intensity, and Host Age

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We have identified the influence of host and parasite factors that give rise to characteristic antibody isotype profiles with age seen in human populations living in different areas of schistosomiasis endemicity. This is important in the immunobiology of this disease. It is also of interest in the context of human responses to chronic antigen stimulation, vaccines, allergens, and other pathogens. In populations exposed to endemic schistosomiasis, factors such as intensity and duration of infection are age dependent. They therefore confound the influence of host age on antiparasite responses. Here, we resolved these confounding factors by comparing the developing antibody responses of an immunologically naive immigrant population as they acquired the infection for the first time with those of chronically infected resident inhabitants of the same region of *Schistosoma mansoni* endemicity in Kenya. Recent arrival in the area strongly favored immunoglobulin G3 (IgG3) responses against the parasite. The antibody isotype responses associated with human susceptibility to reinfection after chemotherapy were elevated in those suffering high intensities of infection (IgG4 responses against worm and egg antigens) or were characteristic responses of young children irrespective of the intensity or duration of infection (IgG2 responses against egg antigen). IgE responses against the adult worm, a response associated with resistance to reinfection after chemotherapy, increased with the ages of infected individuals and were also favored in those currently suffering higher intensities of infection.

Many infectious diseases show a marked age dependency in their prevalence, mean intensity, or severity. In the case of human helminth infections such as opistorchiasis (21), ascariasis (25), or trichuriasis (4), exposure of the parasite is often age dependent, resulting in characteristic age-related patterns of infection intensity. In childhood viral infections, it is generally supposed that this is simply the result of immunity acquired after first contact (33). With schistosomiasis infections, it is possible that the lower intensities of infection seen in adults may result from acquisition of immunity due to exposure to chronic antigen stimulation (6). However, it has also been proposed that naturally acquired immunity to schistosomiasis and malaria may be linked to key features of the immune system that change during normal human development and aging (2, 17).

An interesting immunological aspect of *Schistosoma mansoni* infections is the fact that among human populations living in areas of endemicity, specific antibody isotype responses against parasite worm and egg antigens are also characteristically dynamic with age. For instance, specific immunoglobulin E (IgE) responses against worm antigens, associated with protection against reinfection after treatment (12, 19), increase with age whereas specific IgG2 responses against egg antigens, associated with susceptibility to reinfection after treatment (5), decrease with age (5, 49). Confounding factors are major problems in examining the factors which influence these characteristic patterns of specific antibody responses with age; since both intensity and duration of infection for populations in which infection is endemic are themselves highly age dependent, it is impossible to distinguish their influences from those of age alone.

In the present study, we have focused on an area of low-intensity *S. mansoni* endemicity in Masingaleni, Kenya (35). In March 1992, an area adjacent to an established Kamba settlement, which had been exposed to endemic schistosomiasis for many years, was allocated to fellow Kamba tribe members who came from an area of nonendemicity. A cohort of immigrants was examined serologically and parasitologically soon after arrival and again after acquisition of infection. The levels in sera of a circulating worm antigen, circulating anodic antigen (CAA), were measured to obtain accurate estimates of intensities of infection synchronous with antibody isotype levels measured in the same sera. Age-related patterns of infection intensity and specific antibody responses against *S. mansoni* were compared to those of a cohort of the established community. Analysis of covariance was carried out to determine the influences of age, intensity of infection, and/or duration of infection/exposure on the various isotype responses.

**MATERIALS AND METHODS**

**Population.** The study took place in an area of low-intensity *S. mansoni* endemicity Masingaleni, Kenya. In March 1992, an area adjacent to an established settlement was allocated to a group of previously uninfected members of the same tribe. Details of the histories of both populations have been described by Ouma et al. (35). Random, age-stratified cohorts of both communities were selected for this study. Members of the immigrant cohort (n = 184; age, 5 to 59...
IgG4, IgM, and IgE against *S. mansoni* of serum (45). Adult worm antigen (AWA), and expressed as nanograms of CAA per milliliter were calculated as described previously, by using serial dilutions of TCA-treated the course of these assays to be shortened to 15 min (29). CAA concentrations in microtiter plates. The use of a shaking incubator allowed all incubation steps in Samples from the three bleeds were randomly distributed across the wells of the AP. The pretreated serum samples were tested in duplicate in a 1:4 dilution. Plates (Maxisorp; Nunc, Roskilde, Denmark) coated with the protein A-purified measured as described previously (8). Briefly, CAA was captured on microtiter pretreated with trichloroacetic acid (TCA) (9), and then serum CAA levels were measured to obtain an additional estimate of intensity of infection. To remove of the stool surveys and the times of the bleeds, CAA levels in serum were examined. *S. mansoni* eggs were counted in two 50-μl Kato smears from each stool sample, as described previously (43). The arithmetic means of the egg counts in the six smears were calculated and converted to eggs per gram of feces (EPG).

Meetings were held to explain the proposed project, and both communities expressed their willingness to participate. Informed consent was obtained from each individual participating in the study. Treatment (praziquantel, 40 mg/kg of body weight) was offered to the whole community, regardless of participation, at the end of the study.

**Determination of CAA levels.** Since there were discrepancies between the times of the stool surveys and the times of the bleeds, CAA levels in serum were measured to obtain an additional estimate of intensity of infection. To remove interfering proteins and dissociate immune complexes, serum samples were pretreated with trichloroacetic acid (TCA) (9), and then serum CAA levels were measured as described previously (8). Briefly, CAA was captured on microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) coated with the protein A-purified monoclonal antibody (MAb) 120-1B10-A and detected with MAb 120-1B10-A-AP. The pretreated serum samples were tested in duplicate in a 1:4 dilution. Samples from the three bleeds were randomly distributed across the wells of the microtiter plates. The use of a shaking incubator allowed all incubation steps in the course of these assays to be shortened to 15 min (29). CAA concentrations were calculated as described previously, by using serial dilutions of TCA-treated adult worm antigen (AWA), and expressed as nanograms of CAA per milliliter of serum (45).

**Determinations of specific antibody levels.** Specific levels of IgG1, IgG2, IgG3, IgG4, IgM, and IgE against *S. mansoni* AWA and soluble egg antigen (SEA) were measured by enzyme-linked immunosorbent assay (ELISA), as described previously (12, 48). The following reagents were used and titrated for their optimal dilutions: mouse anti-human (mAb) IgG1 (1/1,000), mAb IgG2 (1/1,000), mAb IgG3 (1/8,000), and mAb IgG4 (1/2,000) (all reagents from Unipath); mAb IgE (1/8,000) (Calbiochem); and sheep anti-mouse biotinylated Ig (1/1,100) and streptavidin-biotinylated horseradish peroxidase complex (1/4,000) (both from Amersham). Sera from the two time points for the immigrants and the one time point for the established cohort were randomly distributed into the wells of microtiter plates to avoid the effects of any intra-assay variability. Sera were diluted to 1/200 for the detection of IgG1, IgG4, and IgM; 1/100 for IgG2 and IgG3; and 1/20 for IgE. Unpublished experiments from our group have determined that specific removal of other antibody isotypes before testing of sera for specific IgE does not significantly affect the results obtained by our IgE assay.

**Statistical analysis.** The cohorts were divided into the following age groups (in years): 5 to 9 (*n* = 26 [established] and 21 [immigrants]), 10 to 14 (*n* = 23 and 24), 15 to 19 (*n* = 24 and 22), 20 to 24 (*n* = 27 and 19), 25 to 29 (*n* = 23 and 21), 30 to 34 (*n* = 27 and 16), 35 to 39 (*n* = 24 and 22), 40 to 49 (*n* = 28 and 20), and 50 to 59 (*n* = 34 and 19). Throughout, serum CAA levels and egg counts (EPG) and specific antibody levels were log transformed (log [CAA or EPG + 1] and log [optical density (OD) + 0.12], respectively) in order to approximate a normal distribution.

The influences of age, intensity of infection (expressed as level of serum CAA), and/or duration of infection/exposure on isotype responses were estimated by calculating the partial *R*² derived from an analysis of covariance by using the general linear modelling command in Minitab. We directly compared either the three different bleeds or any combination of two bleeds. This analysis gave us the independent influences of age and intensity of infection as well as the confounding between these factors, expressed as percentages of the overall variance. A similar analysis gave the influence of bleed (effectively being the duration of exposure/infection) independently of age and intensity of infection as well as the influence of the interactions of bleed with age and infection intensity. Although data from the two bleeds of the immigrant cohort were not strictly independent, because the same individuals were bled on each occasion, when either bleed was dropped from the analysis similar results were obtained. For simplicity and since it did not alter the conclusions, this problem is ignored in the analysis presented here.

**RESULTS**

**Intensity of infection versus age.** Infection intensity levels, expressed as geometric mean fecal egg counts and levels of
CAA in serum, versus age are shown in Fig. 1. The patterns of serum CAA levels with age were similar to those of fecal egg counts. The Spearman’s rank correlation coefficients between serum CAA and egg counts relevant to the times of the bleeds were 0.707, 0.500, and 0.424 for the established and first and second immigrant time points, respectively. Throughout this paper, serum CAA levels are used to describe intensity of infection. Overall prevalence of infection was 86% in the established cohort; in the immigrant cohort, it was 80% at the time of the first bleed and 94% at the time of the second bleed. The established cohort showed a peak in infection intensity around the age of 16 years, which was typical of previously described patterns in untreated populations in areas of endemicity (12, 16, 19, 31). The pattern of infection intensity with age in the immigrants upon first examination, within 1 year of their arrival, contrasted with that of the established cohort. In particular, very little infection was detected among immigrant children, who are usually the most heavily infected members of a community in an area of endemicity. The low levels of infection intensity among the immigrants peaked around the age of 30 years. When the immigrants were reexamined 2 years later, the children had acquired infection, moving the peak of infection intensity to around the age of 12 years. Levels of infection intensity (CAA) were significantly lower in the immigrant cohort than in the established cohort (results not shown).

Specific antibody levels against AWA versus age and infection intensity. Figure 2 shows the geometric means of the specific IgG1, IgG2, IgG3, IgG4, IgM, and IgE antibody levels against AWA versus age. Since the 95% confidence interval was found to be almost symmetrical, only the upper halves of the graphs are shown (to improve the clarity of the graphs). In the established cohort, the patterns showed that levels of specific IgG1 and IgG4 peaked around the age of 20 years and were followed by a decline later in adulthood, whereas levels of specific IgE were higher in adults, with a dramatic increase in IgE levels around the age of 13 years. Levels of specific IgG2, IgG3, and IgM were similar in all age groups. In contrast to the established cohort, initially the specific IgG1 and IgG4 levels in the immigrants were similar in all age groups. IgE levels were higher in the immigrant adults than in the children, but levels started to increase slowly in young adults. Levels of IgG2 and IgG3 were similar in all age groups, while specific IgM showed an increase with age. Surprisingly, given the increased level of infection in the immigrant children, there was relatively little difference between initial specific antibody levels and their responses 2 years later. Longitudinally, the immigrant children showed small increases in the levels of all isotypes examined, with the exception of IgG2. In the immigrants, levels of specific IgM and specific IgG3 were higher than those in the established cohort.
Figure 3 shows the geometric mean levels of IgG3, IgG4, and IgE against AWA in the immigrant (both time points) and established populations plotted against intensity of infection. This clearly shows that IgG4 and IgE responses increase as the intensity of infection increases, irrespective of the length of time spent in the area of endemicity. Similar results were found for IgG1 (results not shown). On the other hand, IgG3 responses (Fig. 3), as well as IgG2 and IgM responses (results not shown), show no relation to infection intensity; however, IgG3 and IgM anti-AWA responses were higher in the immigrant population than in the established population, irrespective of intensity of infection. This is particularly well illustrated for IgG3 in Fig. 3.

Specific antibody levels against SEA versus age and infection intensity. The geometric mean levels of specific IgG1, IgG2, IgG3, IgG4, IgM, and IgE against SEA versus age are shown in Fig. 4. In the established cohort, levels of specific IgG1 and IgG2 were highest in the younger children, followed by a steep decline with age. Specific IgG4 levels showed a peak between the ages of 16 and 20 years, while levels of specific IgE, IgM, and IgG3 were similar in all age groups. Like the established cohort, the levels of specific IgG1 and IgG2 in the initial immigrant bleed peaked in children and then declined steeply with age. Specific IgG4 levels, however, showed a contrasting pattern, with a peak around the age of 30 years. Initially, levels of IgG3 and IgM were similar in all immigrant age groups, while specific IgE levels were higher in the children and then declined with age. Interestingly, 2 years later, levels of all isotypes examined, except IgE, had increased in the youngest immigrant children, whereas levels in the other age groups remained similar. Levels of specific IgG3 were higher in the immigrants.

Regarding the pattern of isotype responses against SEA versus infection intensity, only levels of IgG4 increased with increasing intensity of infection (Fig. 3). The geometric mean levels of IgG3 and IgE (Fig. 3), as well as of IgG1, IgG2, and IgM, showed no relation to infection intensity. Levels of specific IgG3 against SEA were, like IgG3 anti-AWA, higher in the immigrants.

Influences of age, intensity of infection, and/or duration of infection/exposure on isotype responses. Table 1 shows the results of the analysis of covariance examining the influences of age, infection intensity (CAA), and bleed/duration of exposure on specific isotype responses. Only results with all three bleeds are shown (Table 1), as the use of any combination of any two bleeds gave a similar outcome. Results of the analysis confirm what is illustrated in Fig. 2, 3, and 4 and show that age explains a high percentage of the overall variance in specific IgG1 and IgG2 responses against SEA, while the specific IgE response against AWA seems to be influenced by both age and intensity of infection. The overall variance in specific IgG4 responses against AWA and SEA, as well as specific IgG1 responses.
against AWA, can be largely explained by infection intensity. Levels of specific IgG3 against AWA and SEA, as well as of IgM against AWA, are strongly associated with bleed, i.e., duration of exposure/infection, when the established bleed and either of the immigrant bleeds are compared; when the two immigrant bleeds are compared with each other, no association is found (results not shown).

**DISCUSSION**

Human populations living in areas in which schistosomiasis is endemic develop antiparasite antibody isotype responses which may have distinct roles in immunity to infection and reinfection. For example, IgE is associated with resistance to reinfection after treatment and may therefore be involved in protective immunity, whereas, IgM, IgG2, and IgG4 are associated with susceptibility to reinfection after treatment (5, 10, 12, 13, 19, 23, 24, 38). Schistosomiasis-specific antibody isotype responses, like observed levels of infection intensity, show similar dynamic patterns with age in human populations living in different parts of the world in which schistosomiasis is endemic (5, 12, 19, 31, 48). It is of great interest to examine which host or parasite factors influence these specific antibody responses in schistosomiasis infections. However, since both intensity and duration of infection in endemic populations are themselves highly age dependent, it is impossible to distinguish their influences from those of age alone. Several studies have tried to elucidate this complex situation. Recently, specific antibody response patterns with age have been reported in a study of an epidemic outbreak of *S. mansoni* in Richard-Toll, Senegal (44). This study, of a previously uninfected population, showed antibody-age patterns similar to those reported previously for situations involving chronic endemicity (12, 19, 20, 31, 48), suggesting that patterns of responses seen in populations in areas of endemicity are not dependent on life-long exposure to infection. However, the Senegalese study started some 3 to 4 years after the estimated time when the epidemic began and after a pattern of infection intensity with age, similar to those in areas of endemicity, had become established in the study population (37, 44). It is difficult to ascertain from that study, therefore, whether intensity of infection or age per se is the primary factor that induces the characteristic age-patterns of antibody isotype responses seen in populations in which schistosomiasis is endemic.

This study in Masongaleni allowed us to overcome the confounding factors of age, duration of infection, and infection intensity by examining schistosome-specific antibody responses in an immigrant cohort beginning shortly after initial exposure, before the usual patterns of infection intensity with age common to areas of chronic endemicity had been established, and...
again 2 years later. These results could then be compared directly to those of established residents living in the same area and showing patterns of infection intensity and specific antibody responses with age that are characteristic of an untreated population in an area of S. mansoni endemicity (12, 48).

The measurement of CAA in all sera permitted an accurate estimation of the intensity of infection at the exact time of each bleed. CAA is regurgitated from the gut of live adult worms and correlates significantly with the actual worm load (1). Levels decrease rapidly after treatment (7, 45). The patterns of infection intensity with age seen in the cohorts described here are consistent with those reported by Ouma and colleagues, who described the development of infection in the Masongaleni area in a larger community-based parasitological study (35). They proposed that the interesting observation that initially only the adult immigrants were infected was the result of exposure in the Masongaleni area, although they did not completely exclude the possibility that some of these individuals may have been infected elsewhere in childhood (35).

We and others have found consistently that levels of specific IgG1 against worm and IgG4 against both worm and egg antigens correlate with infection intensity (12, 39, 44, 46). Similar findings have been reported for Schistosoma haematobium infections (18, 30, 31) and for other helminth infections such as Bancroftian filariasis and hookworm (3, 4, 36, 42). Here, in both the immigrants and the established cohort, the peak levels of specific IgG1 against worm and IgG4 against both worm and egg antigens with age coincided with their respective peaks of infection intensity with age. The peak of these specific antibody levels increased with infection intensity whereas isotypes associated to age or to intensity of infection. A relevant factor might be that different IgG subclasses have been shown to be induced by antigens with different physiochemical properties (27, 28, 40). For instance, IgG4 is primarily induced by peptide antigens and IgG2 mainly recognizes polysaccharide antigens, while IgG1 and IgG3 can respond to both types of antigens (28). Our results could be interpreted as showing that antibody isotypes associated with responses to peptide antigens increased with infection intensity whereas isotypes associated with responses to polysaccharide antigens were age related. It was suggested previously that children in areas of schistosomiasis endemicity had higher antibody responses against carbohydrates than did adults (5, 11). A similar effect might be occurring in infections with the human whipworm Trichuris trichiura (32).

In Masongaleni, the pattern of IgE against worm antigen is associated with both age and infection intensity (Fig. 2 and 4; Table 1). Previous studies on S. mansoni in Senegal and Kenya (13, 44) showed no association between specific IgG responses and pretreatment infection intensity, while in studies in Brazil and the Sudan (39, 49), a positive correlation was found. In S. haematobium infections, a negative correlation between IgE against SEA and infection intensity was found, and IgE against AWA showed no significant association (30, 31). Webster and colleagues reported that IgE responses to rSm22.6, a recombinant worm antigen which has been associated with protection to reinfection (14, 47), are associated with infection intensity rather than age (50). Since we were unable to measure reinfection in these populations, we cannot make any comments about the role of IgE in protection. Our results support the suggestion, based on the results of the epidemic outbreak of S. mansoni in Senegal (44), that IgE responses to S. mansoni adult worm may not depend on prolonged and cumulative

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**TABLE 1. Results of analysis of covariance**

<table>
<thead>
<tr>
<th>Specific antibody response</th>
<th>Main effect of age independent of intensity</th>
<th>Main effect of intensity independent of age</th>
<th>Confounding between age and intensity main effects</th>
<th>Main effect of bleed independent of age and intensity</th>
<th>Interaction between bleed/duration and age</th>
<th>Interaction between bleed/duration and intensity</th>
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<td>1 **</td>
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<td>4</td>
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<td>1 *</td>
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<td>Against SEA</td>
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</table>

* Results of analysis of covariance illustrate the influences of age, intensity of infection (CAA level), and/or bleed/duration of infection/exposure on specific antibody responses against AWA and SEA.

* Values are percent partial R², indicating the influence of the various factors as a percentage of the overall variance. *, 0.05 > P > 0.01; **, 0.01 > P > 0.001; ***, P < 0.001.
exposure to infection but might be intrinsically age dependent and regulated by age-related physiological processes. However, our results also suggest that infection intensity is an important influence on antiparasite IgE expression.

An interesting, if somewhat puzzling, observation was that the immigrants had higher levels of IgG3 than the established cohort irrespective of age and intensity of infection (Fig. 2 to 4). This could be interpreted as an acute response of a naive population, on the basis of the responses measured within 6 months of first exposure. However, these elevated IgG3 levels were still present in the immigrants some 2 years later. Others have found no differences in IgG3 responses between acute and chronic schistosomiasis patients (22, 41) or between normally exposed individuals and more intensely exposed canal cleaners (39), although, compared to noninfected controls, specific IgG3 levels (39) and total IgG3 levels (22) are elevated in infected individuals. The significance of this response in a newly exposed immunologically naïve population remains to be determined, but the results reported here indicate that IgG3 responses were affected by duration of infection rather than intensity of infection or age per se (Table 1).

It is possible that factors associated with length of time spent in the Masongaleni area, other than actual duration of infection, might influence these antiparasite responses. For instance, unlike the majority of the immigrant children, the established children were probably born of infected mothers. It has been shown that children born of mothers with chronic infections such as schistosomiasis undergo natural in utero idiotypic changes and are born possessing cellular anti-Id reactivity (15, 26, 34). Other differences between the populations might include nutritional status and history of exposure to other pathogens (35).

In summary, by studying immigrant and established populations in an area of endemicity, we have been able to separate the usually confounding influences of age, duration of exposure/infection, and intensity of infection on specific antibody responses to schistosomiasis mansoni. Interestingly, specific IgG1 and IgG4 responses against worm antigen, as well as IgG4 responses against egg antigen, are strongly associated with intensity of infection, while specific IgG1 and IgG2 responses against egg antigen decrease with age. IgE responses against worm antigen showed an association with both age and intensity of infection. Finally, specific IgG3 responses were related to duration of exposure/infection and showed no association with either infection intensity or age.

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