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Associations between specific antibody responses and resistance to reinfection in a Senegalese population recently exposed to *Schistosoma mansoni*

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Summary We examined associations between schistosome-specific antibody responses and reinfection in Senegalese individuals recently exposed to *Schistosoma mansoni*. The effects of treatment, age, intensity of infection and duration of exposure on schistosome-specific antibody responses were also investigated by comparing immune responses in individuals exposed for less than 3 years with responses in people exposed for more than 8 years. All individuals were bled before treatment as well as 6 and 12 weeks after. We used a statistical model that included interaction terms between time, age, infection intensity and duration of exposure. The overall patterns of most specific antibody responses by age were similar to those previously published for *S. mansoni*, *Schistosoma japonicum* and *Schistosoma haematobium* infections in different endemic areas. In general, a boost in specific antibody responses against adult worm antigen (SWA) was observed at 6 weeks after treatment whereas the majority of isotype responses against egg antigen (SEA) were not affected by treatment. Our analysis showed that the effect of treatment on schistosome-specific antibody responses is influenced by age, infection intensity and duration of exposure. We found no evidence that treatment matures the specific antibody response of children recently infected with *S. mansoni*. Our results indicate that the build-up of potentially protective immunoglobulin E (IgE) responses was associated with duration of exposure, or, in other words, experience of infection. Interestingly, in recently exposed individuals there was a significant association between IgA responses to SWA and resistance to reinfection. Resistance to reinfection and production of IgA-SWA was associated with adulthood independently of exposure patterns, suggesting that susceptibility to *S. mansoni* and the development of protective immune responses is age-dependent.

keywords Schistosomiasis, *schistosoma mansoni*, antibody responses, senegal, treatment, reinfection

Introduction

The peak of infection burden in populations living in areas where *Schistosoma mansoni* is endemic usually occurs in puberty and susceptibility to reinfection after treatment is greater in children than adults. This dissimilarity in susceptibility to infection cannot be explained by differences in exposure patterns (Kabatereine *et al.* 1999; Scott *et al.* 2003). This has led to a long and lively debate surrounding the question whether resistance to schistosomiasis is because of age-associated changes in immune responses and physiology of the host and/or can be explained by the slow built-up of protective immune responses which takes several years of experience of the infection (Gryseels 1994; Butterworth 1994; Woolhouse & Hagan 1999). People residing in endemic areas are in contact with the parasite all their lives and age and experience of infection are strongly associated. It is therefore important to study factors associated with resistance to reinfection in communities where age and experience are not associated. In northern Senegal, irrigation works have led to an epidemic of *Schistosoma mansoni* in an area where this parasite was not present before (Stelma *et al.* 1993). This setting is very suitable to assess the major differences that exist in the way host and schistosome interact in adults *vs.* children, independent of the confounding between age and experience of infection.

In this paper, we focus on the role of specific antibody responses in resistance to schistosomiasis in recently exposed adults and children from northern Senegal.

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Numerous *in vitro* studies on the immunology of schistosomiasis have clearly pointed out the essential role played by antibodies in various effector or regulatory mechanisms (Butterworth *et al.* 1977; Capron *et al.* 1984; Khalife *et al.* 1989). However, the underlying epidemiology of the humoral immune response against schistosomiasis is quite complicated. Infected individuals of all ages have high levels of circulating antibodies with anti-schistosome specificity and the class and subclass composition of these antibodies vary with age, sex and intensity of infection (van Dam *et al.* 1996; Mutapi *et al.* 1997; Webster *et al.* 1997; Naus *et al.* 1999, 2003). The class and subclass distribution of the antibody response is instrumental because each isotype has a specific biological function (Spiegelberg 1989). Schistosome-specific isotype responses have distinct associations with the intensity of reinfection. For instance, immunoglobulin E (IgE; Hagan *et al.* 1991; Dunne *et al.* 1992; Zhang *et al.* 1997)and IgA (Auriault *et al.* 1990; Grzych *et al.* 1993; Ali & Shaneen 1994) responses have been associated with resistance against reinfection, whereas, e.g. IgG2 (Butterworth *et al.* 1988) and IgG4 (Hagan *et al.* 1991) responses are thought to block protective antibody responses and correlate with susceptibility to reinfection. A key feature of humoral immunity against schistosomiasis is therefore not so much the presence or absence of specific antibody responses but rather the balance between them (Butterworth 1994).

Praziquantel kills adult worms and mature eggs, releasing antigens from both stages of the parasitic life cycle (Harnett & Kusel 1986; Giboda & Smith 1994). As expected, treatment of schistosomiasis induces changes in cellular and humoral immune responses against this parasite. In general, specific antibody responses against adult worm antigen tend to increase in the weeks following treatment (Grogan *et al.* 1996; Naus *et al.* 1998; Li *et al.* 2002) while responses against soluble egg antigens generally show little change during this period (Naus *et al.* 1998; Li *et al.* 2002; Shen *et al.* 2002). Drug treatment also has a significant long-term effect on the human immune response against schistosomiasis (Butterworth *et al.* 1988; Webster *et al.* 1996; Naus *et al.* 1998; Mutapi *et al.* 1998; Li *et al.* 2002). Previous studies on *Schistosoma haematobium* infection indicate that the effect of treatment on the humoral immune response is different in adults and children (Grogan *et al.* 1996; Mutapi *et al.* 1998). Here, we examine the effect of treatment on schistosome-specific antibody responses in individuals who have been exposed to *S. mansoni* for less than 3 years and compare their responses with people who have been exposed for approximately 8 years. None of these people have experienced the neonatal impact of maternal antibodies, which influences multiple parameters in the adult immune system (Lemke *et al.* 2004). Associations between specific antibody responses and the intensity of reinfection will also be examined. In addition, we are especially interested in the influence of age, intensity of infection before treatment and duration of exposure on the changes observed in specific antibody responses as a result of treatment and reinfection.

Materials and methods

Study population

The study was carried out in 1997 and 1998 in five villages, situated in the north of Senegal. A survey carried out in 1994 in the area around the villages Maraye, Ndigue, Rone and Ravette found no infected individuals, therefore we can assume that *S. mansoni* has been prevalent in this area for no more than 3 years (Ernould 1996). These villages will be referred to as the recent foci. The fifth village, Buntabat, has had cases of *S. mansoni* since the beginning of the outbreak of the infection in northern Senegal in 1989. This village will be referred to as the older focus. More details about the set up of the study and the water contact patterns of these communities have been described before (Scott *et al.* 2003). Intensity of infection was determined using the Kato-Katz method (Katz *et al.* 1972). All patients were negative for *S. haematobium* infection, which was determined by filtration of 10 ml of two urine samples collected on different days.

Blood samples were taken before as well as 6 weeks and 12 months after treatment (40 mg/kg praziquantel). Only people who donated a blood sample at all three time points were selected for this study. In the recent foci, the study group consisted of 204 individuals, 143 children, aged 3–15 (71 females, 72 males) and 61 adults, aged 16–80 (51 females, 10 males), while 45 individuals (24 children, aged 6–15 (16 females, 8 males) and 21 adults, aged 17–60 (15 females, 6 males)) were included in the older focus. Peripheral blood plasma samples were stored at −30 °C and subsequently transported to Antwerp on dry ice.

All participants gave oral consent, and ethical permission for this study was obtained from the Ethical Committee of the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium and the Région Médicale, Saint Louis Sénégal, in accordance with the principles and practice of the Helsinki Declaration.

Specific antibody ELISA

Specific IgG1, IgG2, IgG3, IgG4, IgE, IgA and IgM responses to *S. mansoni* soluble worm antigen (SWA) and soluble egg antigen (SEA) were measured b.y enzyme-linked immunoassay technique (ELISA) using methods similar to those

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described previously (Naus *et al.* 1999). Both antigen preparations were purchased from Prof. A. Deelder (LUMC, Leiden, The Netherlands). All assays testing for one particular isotype response were carried out at the same time. Briefly, the antigen was coated overnight (ON) at a concentration of 5 μg/ml. The plates were washed six times in between each incubation step. Following the blocking step [1% bovine serum albumin (BSA) in phosphate-buffered saline, 1 h incubation at room temperature (RT)], the samples were randomly distributed into the wells of microtitre plates (Immulon 4HBX) and incubated for 1 h at RT for IgG1, IgG4, IgA and IgM or ON at 4 °C for IgG2, IgG3 and IgE. Sera dilutions were the following: 1/200: IgG1, IgG4, IgA, IgM; 1/50: IgG2, IgG3; and 1/20: IgE. All samples, which were tested in triplicate on different plates, had been treated to eliminate viral contamination hazard (Poulsen & Sorensen 1993). As detecting antibodies, mouse anti-human IgG1 diluted at 1/4000, IgG2 diluted at 1/4000, IgG3 diluted at 1/4000, IgG4 diluted at 1/2000 (all from Sanquin, Amsterdam, The Netherlands), IgE diluted at 1/4000 (Calbiochem), IgA-biotin, diluted at 1/2000 and IgM-biotin diluted at 1/2000 (both from Becton Dickinson (BD), Erembodegem, Belgium) were used. Again, for the IgG1, IgG4, IgA and IgM assays, the incubation time was 1 h at RT; the other assays were incubated overnight at 4 °C. The relevant assays were incubated with biotinylated anti-mouse Ig (1/2000, 1 h, RT, from BD). Finally, streptavidin horseradish peroxidase (1/4000, 1 h, RT, from BD) was used to amplify the signal. The assays were developed using tetramethylbenzidine (TMB), and stopped by adding 2 M sulphuric acid. Specific antibody responses were expressed as the mean optical density of the triplicate samples.

Statistical analysis

All data were analysed using SPSS 13.0 (SPSS, Chicago, USA). Egg counts per gram faeces (EPG) were log-transformed [log (EPG + 1)] while antibody isotype responses were converted to normal scores because they could not be normalized by log transformation. *P*-values were considered significant after Bonferroni correction ($P < 0.05/n$, where n is the number of comparisons).

The significance of changes in EPG levels as well as specific antibody responses in time (effect of treatment and subsequent reinfection) were determined by repeated measures general linear model (GLM) analysis. To this end, the population was divided into four groups based on age (children, younger than 16 *vs.* adults) and duration of exposure (recent foci *vs.* older focus). As information of individual duration of exposure (experience of infection) was not available, we used recently and chronically exposed villages as an indication of duration of exposure instead.

Differences in EPG and specific antibody response between adults and children and between recently and chronically infected individuals were determined by an independent *t*-test.

The effect of age, pre-treatment intensity of infection (pre-EPG) and duration of exposure (recent focus *vs.* older focus) on the change in specific antibody responses in time was determined using repeated measures GLM analysis. In this model, the within-subjects variable consisted of the isotype response at the three time points; duration of exposure, age and pre-treatment EPG were entered as between-subjects variable.

Associations between reinfection levels and specific antibody responses were examined using a Spearman correlation coefficient; significant associations were further examined by a univariate GLM analysis with the specific antibody responses as dependent variable, living in one of the recent foci or the older foci as the fixed factor, and intensity of reinfection, pre-treatment intensity of infection and age as independent variables.

Results

Intensity of infection

The prevalence of infection and range of *S. mansoni* eggs per gram faeces (EPG) at the three time points were the following: before treatment: recent focus: 81% (range 0–7120 EPG), older focus: 87% (range 0–11050 EPG); 6 weeks after treatment: recent focus: 30% (range 0–1560 EPG), older focus: 37% (range 0–8700 EPG); 12 months after treatment: recent focus: 81% (range 0–6320 EPG), older focus: 71% (range 0–1760 EPG). Intensity of infection peaked around the age of 12 in both the recently and chronically infected individuals. In both groups, the mean EPG decreased significantly after treatment in children ($P = 0.000$ in both foci) and adults ($P = 0.000$ in both foci). At 12 months after treatment, the mean EPG returned to pre-treatment levels in the recent foci only (children and adults: $P = 0.000$). In the older focus, the mean EPG at 12 months after treatment was significantly lower compared with the pre-treatment EPG (children: $P = 0.016$, adults: $P = 0.005$). There were no significant differences between EPG levels in either groups except for reinfection levels which were significantly lower in the older focus ($t = 4.162$, $P = 0.000$).

Differences in specific antibody responses in time

Figures 1, 2, 3 and 4 represent error bar charts of the mean specific antibody response against SWA and SEA at the three time points in children and adults from the recent and

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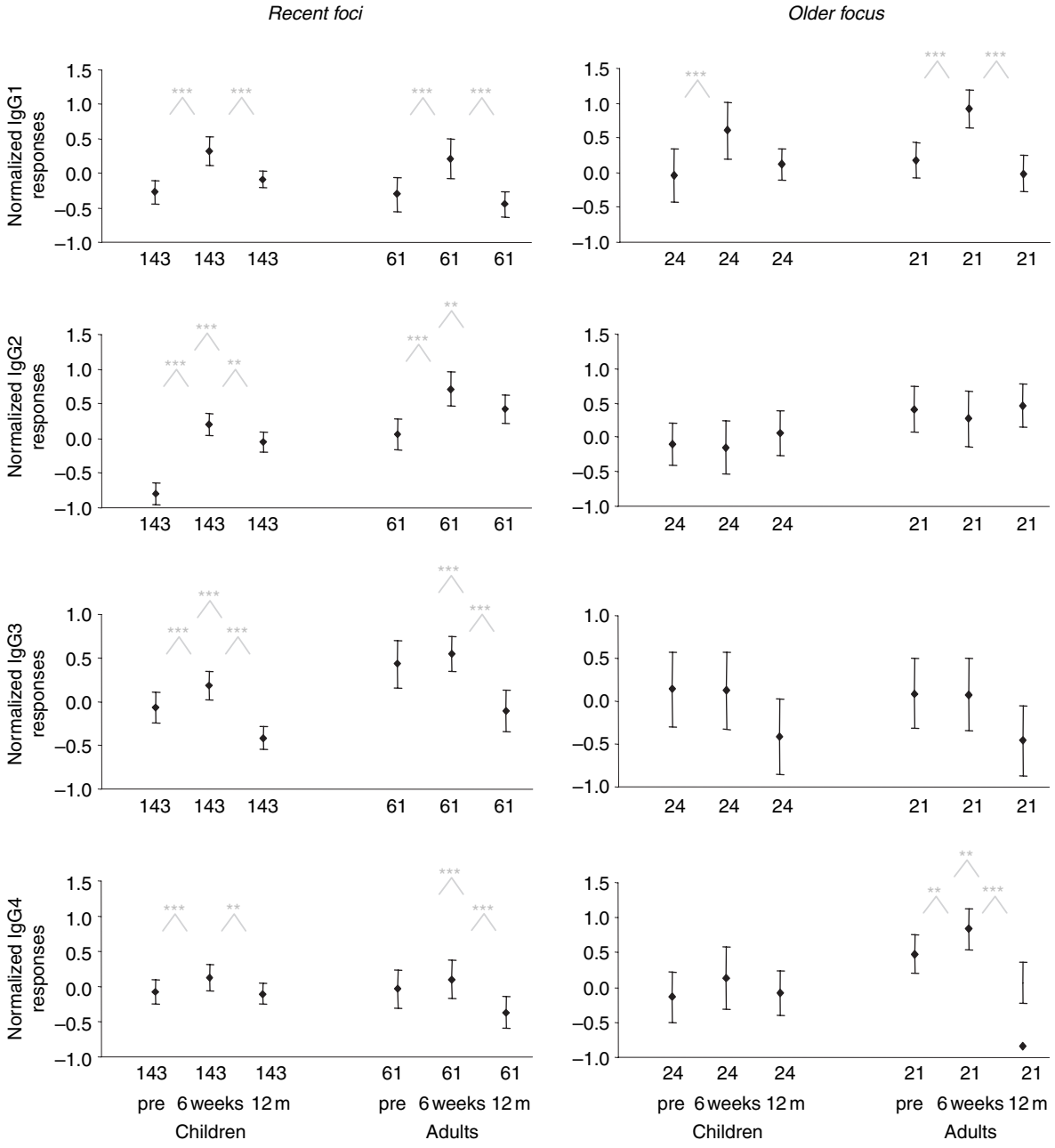

Figure 1 Specific immunoglobulin (Ig) G1, IgG2, IgG3 and IgG4 responses against *Schistosoma mansoni* soluble worm antigen (SWA) before treatment, 6 weeks after treatment and 12 months after treatment in children (aged 3–15) and adults (aged 16–80) residing in the recent foci or the older foci. Specific antibody responses are expressed as normalized optical density values. Depicted are the mean values with the 95% confidence interval. The hats represent significant differences between the respective time points; * = $0.01 < P < 0.05$; ** = $0.001 < P < 0.01$; *** = $P < 0.001$.

the older foci. The majority of specific antibody responses against SWA in children and adults from both the recent and older foci increased at 6 weeks after treatment, although this increase was not statistically significant for all isotypes (Figures 1 and 2). At 12 months after treatment, most of these responses tended to decrease again.

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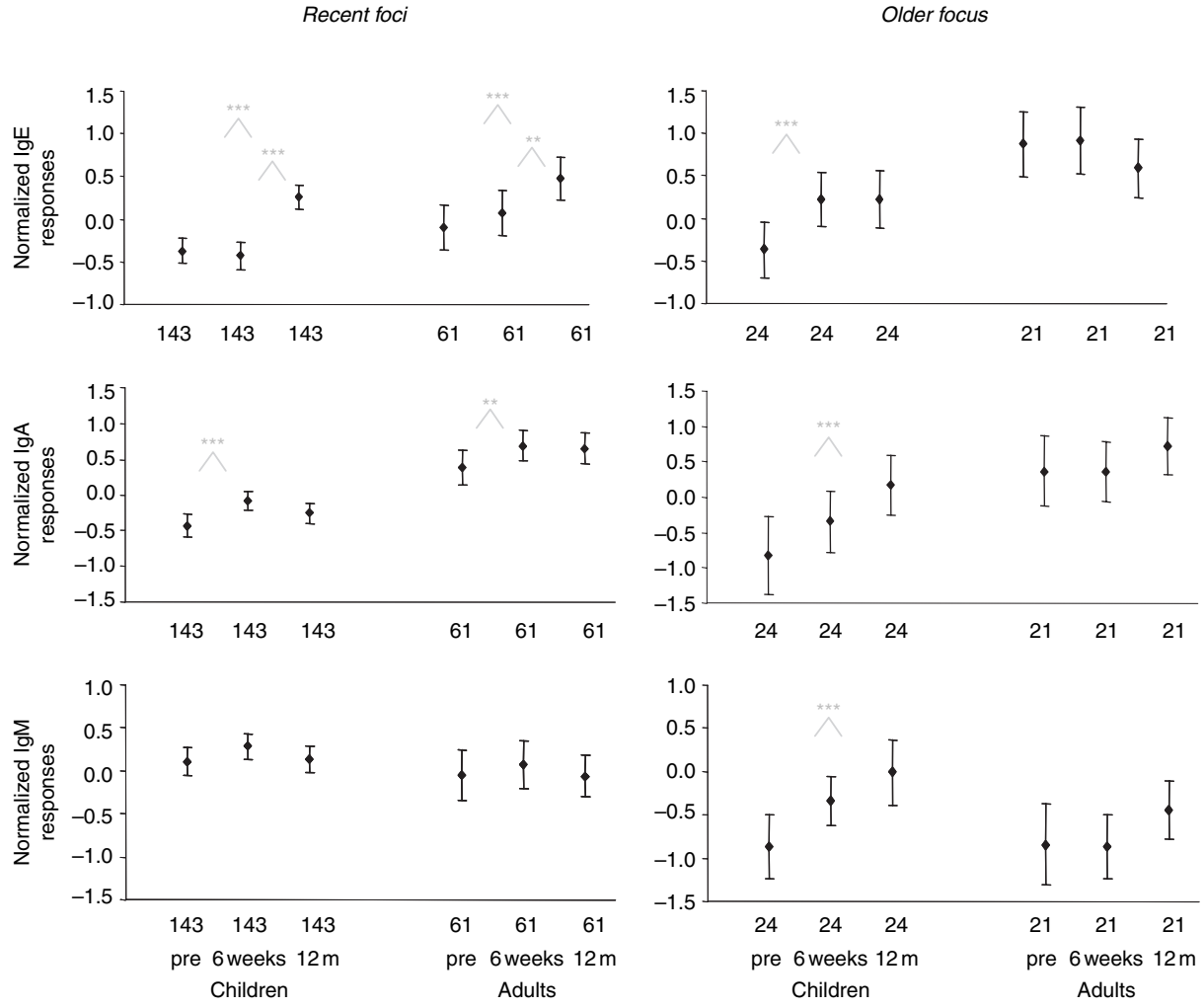

Figure 2 Specific immunoglobulin (Ig) E, IgM and IgA responses against *Schistosoma mansoni* soluble worm antigen (SWA) before treatment, 6 weeks after treatment and 12 months after treatment in children (aged 3–15) and adults (aged 16–80) residing in the recent foci or the older foci. Specific antibody responses are expressed as normalized optical density values. Depicted are the mean values with the 95% confidence interval. The hats represent significant differences between the respective time points; * = $0.01 < P < 0.05$; ** = $0.001 < P < 0.01$; *** = $P < 0.001$.

Exceptions were IgM from recently infected individuals, IgG2 and IgG3 from chronically infected individuals, IgG4 in children and IgE, IgA and IgM in adults of the older foci, which remained unchanged over time. IgA and IgM responses from children in the older focus and IgE responses of adults of the recent focus significantly increased over time.

Specific antibody responses against SEA (Figures 3 and 4) were not affected at 6 weeks after treatment and remained similar to pre-treatment values with the exception of IgE in chronically infected individuals, IgG2 in recently infected individuals and IgA in children from the recent foci, which all increased significantly. At 12 months after treatment, the majority of SEA-specific isotype responses remained unchanged. Exceptions were IgG4 responses, which decreased over time in adults; IgG2 and IgA significantly increased in children from both foci, and IgE and IgM in children from the older focus.

Difference in specific antibody responses between children and adults

Differences in specific antibody responses between children and adults of the recent foci or children and adults of the

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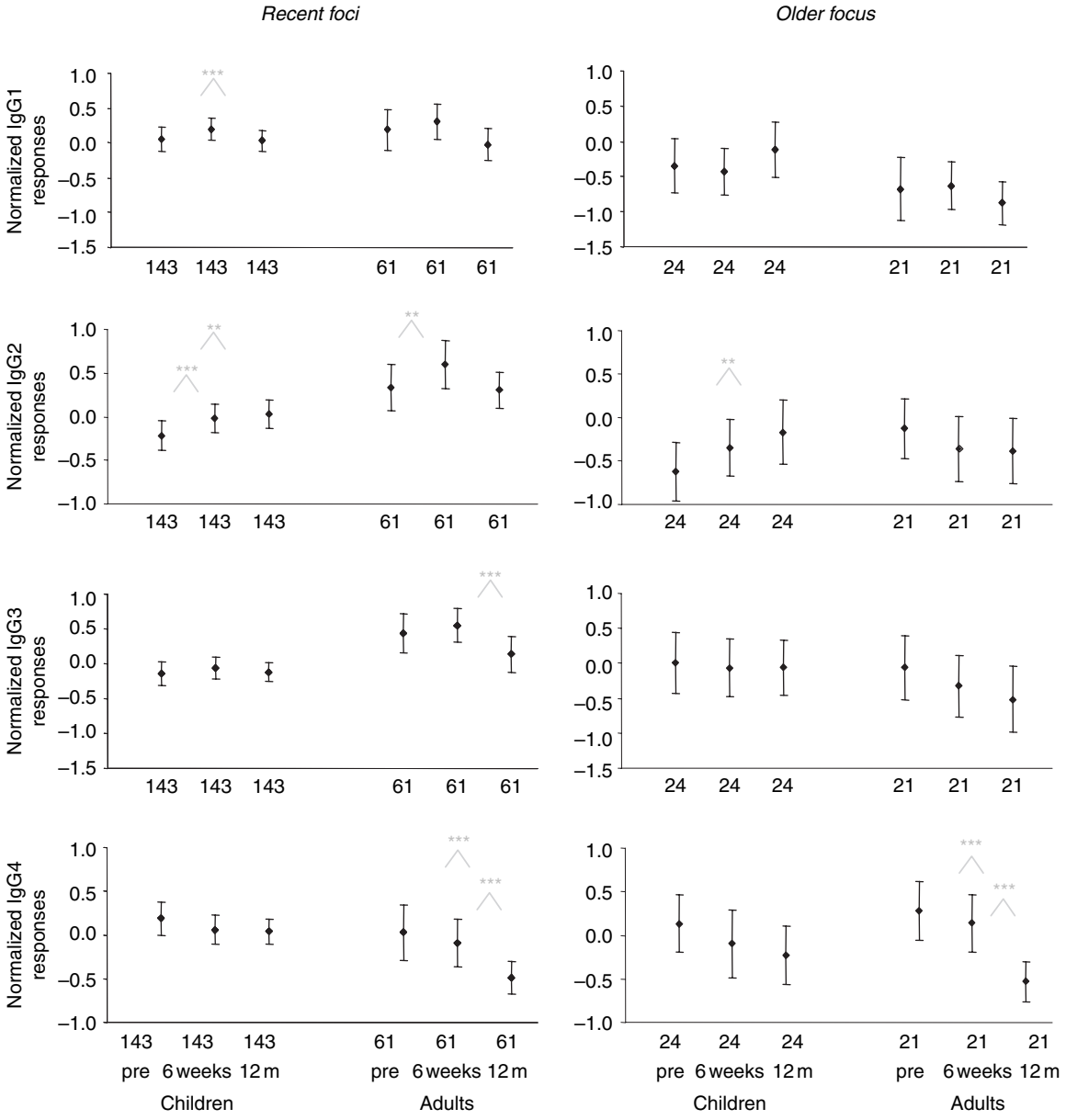

Figure 3 Specific immunoglobulin (Ig) G1, IgG2, IgG3 and IgG4 responses against *Schistosoma mansoni* soluble egg antigen (SEA) before treatment, 6 weeks after treatment and 12 months after treatment in children (aged 3–15) and adults (aged 16–80) residing in the recent foci or the older foci. Specific antibody responses are expressed as normalized optical density values. Depicted are the mean values with the 95% confidence interval. The hats represent significant differences between the respective time points; * = 0.01 < P < 0.05; ** = 0.001< P < 0.01; *** = P < 0.001.

older foci were examined by an independent sample *t*-test. The results are displayed in Table 1. Interesting observations from this table are that the majority of IgG2 and IgG3 responses against both SWA and SEA were significantly lower in children than in adults from the recent foci whereas there was no age difference in most of these specific isotype responses in the older foci. Furthermore, IgE and IgA responses against both antigens tended to be lower in children than in adults of both foci, although this decrease was not significant at all time points.

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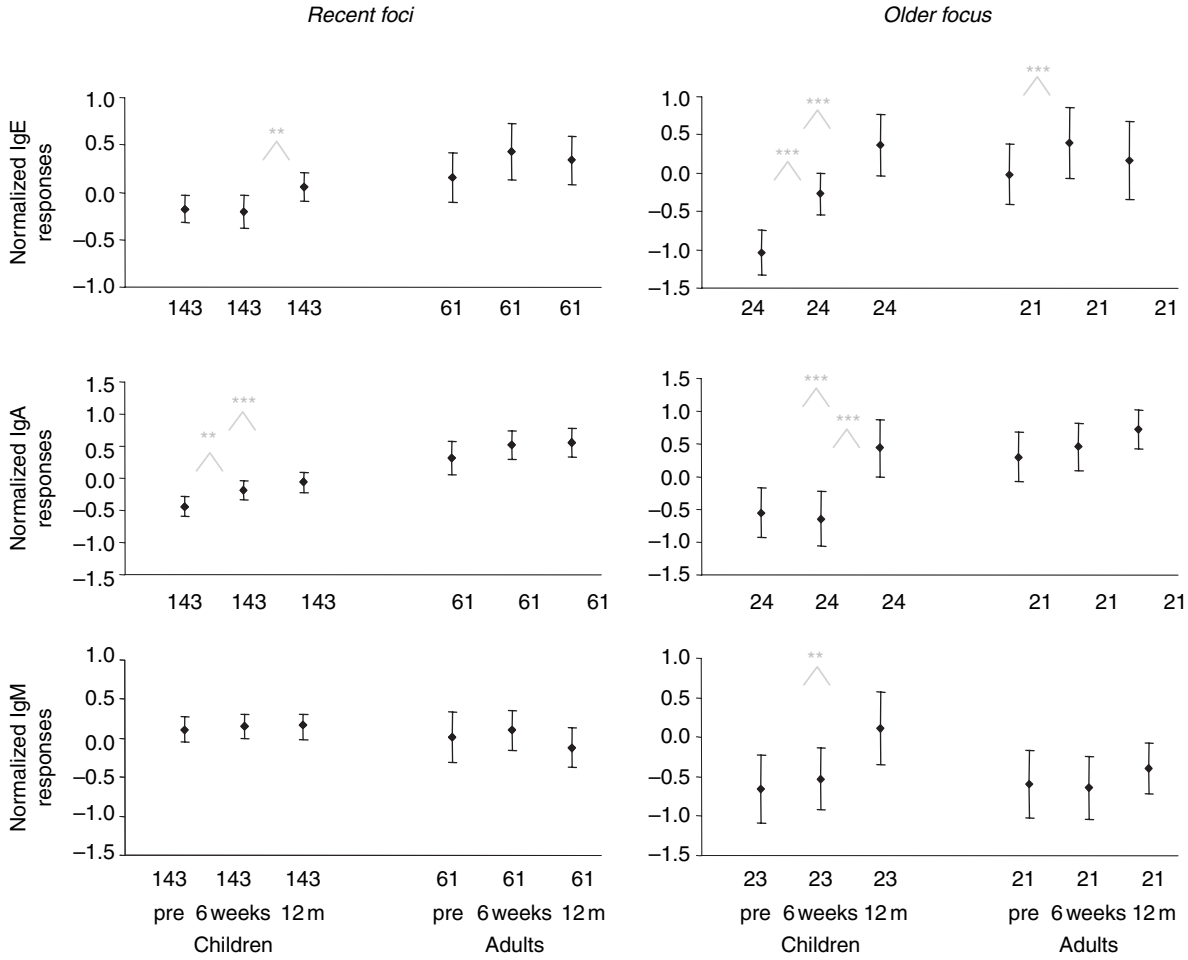

Figure 4 Specific immunoglobulin (Ig) E, IgM and IgA responses against *Schistosoma mansoni* soluble egg antigen (SEA) before treatment, 6 weeks after treatment and 12 months after treatment in children (aged 3–15) and adults (aged 16–80) residing in the recent foci or the older foci. Specific antibody responses are expressed as normalized optical density values. Depicted are the mean values with the 95% confidence interval. The hats represent significant differences between the respective time points; * = $0.01 < P < 0.05$; ** = $0.001 < P < 0.01$; *** = $P < 0.001$; + = number of people is 23 instead of 24.

At 12 months after treatment no differences, except for the IgG1 response against SEA, were observed between children and adults in the older focus.

Difference in specific antibody responses in individuals from the recent foci *vs.* the older focus

The differences in mean specific antibody responses against SWA and SEA between individuals (children and adults) from the recent foci *vs.* the older foci are described in Table 2. Differences in SWA-specific IgG1 and IgG4 responses were observed for the adults only; these responses were higher in the older foci than in the recent foci. IgE-SWA responses were also higher in individuals from the older focus; for adults, a significant difference was observed before and at 6 weeks after treatment; for the children, the difference was significant at 6 weeks after treatment only. Interestingly, IgE-SEA reponses were significantly lower in children from the older foci but only prior to treatment. SWA and SEA-specific IgM responses tended to be higher in participants from the recent foci. A similar observation was made for IgG1 and IgG2-SEA, especially among the adults. Finally, SWA- and SEA-specific IgA responses were comparable between residents from the recent foci and the older focus prior to treatment. However, 6 weeks after treatment SEA-specific IgA was significantly higher in children in the recent foci whereas both SEA- and SWA-specific IgA were lower in the new foci after 12 months.

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Table 1 *t*-values expressing the differences between the mean specific antibody responses in children (aged 3–15) *vs.* adults (aged 16–80) from the recent foci and the older foci at the three time points (pre, pre-treatment; 6w, 6 weeks after treatment; 12 m, 12 months after treatment)

	Differences between children and adults					
	Recent foci			Older foci		
	Pre	6w	12m	Pre	6w	12m
Against SWA						
Ig G1	ns	ns	3.31***	ns	ns	ns
IgG2	−5.94***	−3.49***	−3.82***	−2.30*	ns	ns
IgG3	−3.08**	−2.57*	−2.42*	ns	ns	ns
IgG4	ns	ns	ns	−2.76**	−2.72*	ns
IgE	ns	−3.27***	ns	−5.16***	−2.85**	ns
IgA	−5.46***	−6.56***	−6.88***	−3.32**	−2.37*	ns
IgM	ns	ns	ns	ns	2.34*	ns
Against SEA						
IgG1	ns	ns	ns	ns	ns	3.07**
IgG2	−3.50***	−3.87***	ns	−2.12*	ns	ns
IgG3	−3.59***	−4.23***	ns	ns	ns	ns
IgG4	ns	ns	4.25***	ns	ns	ns
IgE	−2.34*	−3.88***	ns	−4.39***	−2.73**	ns
IgA	−5.15***	−5.22***	−4.29***	−3.27**	−4.04***	ns
IgM	ns	ns	ns	ns	ns	ns

*$P < 0.05$.
**$0.01 < P < 0.001$.
***$P < 0.001$.
ns, not significant; SWA, soluble worm antigen; SEA, soluble egg antigen; Ig, immunoglobulin.

Table 2 *t*-values expressing the differences between the mean specific antibody responses in children (aged 3–15) from the recent foci *vs.* the older foci and adults (aged 16–80) from the recent foci *vs.* the older foci at the three time points (pre, pre-treatment; 6w, 6 weeks after treatment; 12 m, 12 months after treatment)

	Differences in residents from the recent foci vs. the older focus					
	Children			Adults		
	Pre	6w	12m	Pre	6w	12m
Against SWA						
IgG1	ns	ns	ns	−2.78**	−3.68***	−2.49*
IgG2	−3.31***	ns	ns	ns	ns	ns
IgG3	ns	ns	ns	ns	2.25*	ns
IgG4	ns	ns	ns	−2.70**	−3.77***	−2.09*
IgE	ns	−3.11**	ns	−3.87***	−3.31***	ns
IgA	ns	ns	−2.14*	ns	ns	ns
IgM	4.61***	4.05***	ns	2.81**	3.61***	ns
Against SEA						
IgG1	ns	3.00**	ns	3.11**	3.98***	3.88***
IgG2	ns	ns	ns	ns	3.68***	3.39***
IgG3	ns	ns	ns	ns	3.72***	2.55*
IgG4	ns	ns	ns	ns	ns	ns
IgE	4.58***	ns	ns	ns	ns	ns
IgA	ns	2.27*	−2.30*	ns	ns	ns
IgM	3.43***	3.32***	ns	ns	3.02**	ns

*$P < 0.05$.
**$0.01 < P < 0.001$.
***$P < 0.001$.
ns, not significant; SWA, soluble worm antigen; SEA, soluble egg antigen; Ig, immunoglobulin.

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Influence of age, infection intensity and residence in a recent or older focus on the changes in antibody responses between the three different time points

The influence of various factors on individual changes in specific antibody responses in time was examined by repeated measures GLM analysis. The results are described in Table 3. Changes in the IgG subclass responses to SWA were associated with treatment and subsequent reinfection (time) with age (except IgG3), and with pre-treatment intensity of infection (except IgG2). Only changes in IgG2 responses were influenced by place of residence. Changes in the IgG subclass responses to SEA were influenced by the effects of pre-treatment intensities of infection and age (except IgG1). They were not affected by place of residence. Changes in IgE responses to SWA and SEA were associated with place of residence; IgE responses to SEA were also related to age and pre-treatment infection intensity. Changes in IgA responses to SWA and SEA were associated with place of residence only. Finally, changes in IgM responses to SWA and SEA could be contributed to all factors, except to age.

Associations between specific antibody responses and the intensity of infection at 12 months after treatment

A negative association between the EPG at 12 months after treatment and pre-treatment-specific antibody responses was observed for SWA-specific IgG2 ($R = -0.187$, $P = 0.005$), IgE ($R = -0.183$, $P = 0.006$), IgA ($R = -0.335$, $P = 0.000$) and SEA-specific IgA ($R = -0.218$, $P = 0.001$). Negative associations were also observed for 6 weeks post-treatment SWA-specific IgE ($R = -0.170$, $P = 0.011$), as well as for 12 months post-treatment IgA against SWA ($R = -0.233$, $P = 0.000$) and SEA ($R = -0.152$, $P = 0.023$).

Positive correlations were observed with pre- and 6 weeks post-treatment IgM responses against both antigens (pre-SWA: $R = 0.149$, $P = 0.026$) and (pre-SEA: $R = 0.145$, $P = 0.030$); 6 weeks SWA ($R = 0.239$, $P = 0.000$) and 6 weeks SEA ($R = 0.188$, $P = 0.005$), as well as with 6 weeks post-treatment IgG4 responses against SEA ($R = 0.170$, $P = 0.011$). At 12 months post-treatment, positive correlations were found with SWA-specific IgG1 ($R = 0.405$, $P = 0.000$), IgG4 ($R = 0.382$, $P = 0.000$) and IgM ($R = 0.150$ $P = 0.025$), as well as SEA-specific IgG1 ($R = 0.254$, $P = 0.000$), IgG3 ($R = 0.167$ p = 0.012), IgG4 ($R = 0.582$, $P = 0.000$) and IgM ($R = 0.273$, $P = 0.000$).

After correction for the confounding influences of age, place of residence and pre-treatment intensity of infection in GLM analysis, a negative association with EPG 12 months after treatment remained significant for pre-treatment IgA-SWA ($F = 10.075$, $P = 0.002$). A positive association remained significant at 12 months post-treatment IgG1-SWA ($F = 37.818$, $P = 0.000$), IgG4-SWA ($F = 33.008$, $P = 0.000$), IgG1-SEA ($F = 16.734$, $P = 0.000$), IgG3-SEA ($F = 9.546$, $P = 0.002$), IgG4-SEA ($F = 62.962$, $P = 0.000$) and IgM-SEA ($F = 10.699$, $P = 0.001$).

Table 3 Results of the repeated measures general linear model (GLM) analysis examining the effect of age, pre-treatment intensity of infection (pre-EPG) and residence in one of the recent foci or the older foci on the change in specific antibody responses against soluble worm antigen (SWA) and soluble egg antigen (SEA) in time

F value	Interaction between time and age	Interaction between time and pre-EPG	Interaction between time and residence in recent or older foci
Responses to SWA			
IgG1	8.65***	40.20***	ns
IgG2	3.25*	ns	20.26***
IgG3	ns	10.78***	ns
IgG4	8.42***	17.02***	ns
IgE	ns	ns	11.07***
IgA	ns	ns	8.67***
IgM	ns	7.01***	11.80***
Responses to SEA			
IgG1	ns	4.50*	ns
IgG2	5.45**	6.59**	ns
IgG3	3.52*	3.32*	ns
IgG4	10.89***	41.51***	ns
IgE	3.23*	3.16*	10.12***
IgA	ns	ns	8.30***
IgM	ns	6.85***	8.53***

*$P < 0.05$.
**$0.01 < P < 0.001$.
***$P < 0.001$.
ns, not significant; Ig, immunoglobulin.

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Discussion

Examination of specific immune responses against schistosomiasis in recently exposed communities has led to new insights into host- and parasite-derived factors that are associated with the production of potentially protective immune responses. However, many issues regarding the influence of age and experience of infection on the development of protective immune responses are still being debated. It has been suggested that the increased susceptibility to infection observed in children may be because of the fact that their immune system has had less encounters with dead worms (Woolhouse & Hagan 1999). If lack of antigen processing, or in other words shorter experience of infection, is indeed responsible for the observed differences in susceptibility to reinfection between adults and children, treatment may have a vaccinating effect by speeding up the development of protective immune responses after treatment and inducing adult-like specific antibody responses in children (Mutapi *et al.* 1998). Support for a potential immunizing effect of treatment comes from a study comparing specific antibody responses in individuals that were recently and chronically exposed to *Schistosoma japonicum* (Li *et al.* 2002). The results of this study led to the suggestion that multiple episodes of infection may be required to generate antibody isotype responses associated with resistance to reinfection. However, results from a study on Gabonese schoolchildren infected with *S. haematobium*, showed that repeated treatment did not result in the production of protective cellular immune responses and children that were treated repeatedly did not have a higher resistance to reinfection compared with the control groups (van den Biggelaar *et al.* 2002). Here, we examined the effect of treatment on schistosome-specific antibody responses as well as associations between these responses and reinfection in individuals who have been exposed to *S. mansoni* for less than 3 years. The theory that treatment may induce adult-like immune responses in children has not been tested yet in a community where the experience of infection is equal in all residents, irrespective of age.

As information of individual duration of exposure was not available, we used recently and chronically exposed villages as an indication of duration of exposure. We are aware that other factors related to these villages may also be influential, such as treatment history. To reduce as much as possible the potential impact of village-specific factors, the data from the four recently exposed villages were pooled; the chronic exposure data however originate from one single village. Although we have no reason to assume any important differences between the older focus and the newer foci other than duration of exposure, we cannot totally exclude them either. Nevertheless, we consider the present approach justified.

In general, a boost in specific antibody responses against SWA was observed at 6 weeks after treatment while the majority of isotype responses against SEA were not affected by treatment. This boost effect was much less pronounced in the older focus. As observed previously, IgG1 and IgG4 responses to SWA as well as IgG4-SEA showed a similar age profile as levels of intensity of infection, while IgE and IgA responses to SWA and SEA increased with age and SWA- and SEA-specific IgM responses were more or less similar in adults and children (van Dam *et al.* 1996; Ndhlovu *et al.* 1996; Webster *et al.* 1997; Naus *et al.* 1999, 2003;). In contrast to previous studies on populations that were recently and chronically exposed to *S. mansoni*, IgG2 and IgG3 responses against SWA and SEA tended to increased with age (Webster *et al.* 1997; Naus *et al.* 1999, 2003). On the other hand, our results were in concordance with those of van Dam *et al.* (1996) in a recent focus in Senegal.

This study confirms the results of previous studies on specific antibody responses in recently exposed communities by demonstrating that age, and/or intensity of infection and/or experience of infection influence specific antibody isotype responses against schistosome antigens, depending on the isotype (van Dam *et al.* 1996; Naus *et al.* 1999). In addition, we demonstrated that treatment and subsequent re-exposure and reinfection affect anti-schistosome antibody responses and that the effect of treatment on the humoral immune response is influenced by age, intensity of infection and duration of exposure. Results from a study on chronic *S. haematobium* infection indicated that treatment may induce adult-like immune responses in children: in treated children, a switch was observed from a predominantly IgA-specific antibody response to a predominantly IgG1 response (Mutapi *et al.* 1998). Our results show that at 12 months after treatment IgG1-SWA measured in individuals from the recent foci and IgG1-SEA in individuals of the older focus remained age-dependent. For IgG1-SEA in the recent foci and IgG1-SWA in the older foci no significant differences were observed between children and adults, but these responses were actually already similar before treatment (Table 1). Also for the other antibody isotypes we found no clear indication that treatment matures the specific antibody response of *S. mansoni*-infected children.

An interesting observation was that IgE, IgM and IgA trends were often very similar within each age group, with hardly any changes over time in adults but important changes after 12 months in children. Almost all SWA/SEA-specific IgA, IgM and IgE levels increased significantly in

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children in the older foci, whereas in children in the recent foci only SWA/SEA-specific IgE and SEA-specific IgA increased. The increase of both IgM and IgE in children of the older foci is a somewhat puzzling observation, in particular because of the distinct roles of these antibody isotypes in immunity to infection. In human schistosomiasis, IgE is usually associated with resistance to reinfection after treatment and IgM with susceptibility to reinfection after treatment (Butterworth *et al.* 1988; Dunne *et al.* 1992). A more recent study, however, also associates higher anti-SWA IgM responses with resistance to *S. mansoni* infection (Caldas *et al.* 2000). It is not clear which factors are responsible for the differences in observations between children of recent and older foci. They could be linked to the significantly lower intensity of reinfection in children of the older foci, but other unknown village-specific factors may have played a role as well.

Besides studying the effect of treatment on specific antibody responses in adults and children we also assessed associations between these responses and the intensity of infection at 12 months after treatment. Pre- and 6 weeks post-treatment IgE-SWA responses correlated negatively with reinfection. However, this association was no longer significant after correcting for the confounding influences of age, pre-treatment intensity of infection and place of residence in the GLM analysis. Schistosome-specific IgE responses have been associated with resistance to reinfection, independent of confounding factors (Hagan *et al.* 1991; Dunne *et al.* 1992; Zhang *et al.* 1997) and it is assumed that IgE antibodies are able to act against new invading larvae by activating eosinophil-mediated killing of schistosomulae (Capron *et al.* 1984, 1995). The results of our study are in agreement with those of other studies on recent exposed individuals by demonstrating that adults produce higher levels of IgE but that experience of infection also contributes to the build-up of this isotype response (van Dam *et al.* 1996; Naus *et al.* 1999; Li *et al.* 2002).

In contrast to IgE-SWA, pre-IgA responses against SWA were associated with resistance to reinfection even after correcting for the confounding influences of place of residence, age and pre-treatment intensity of infection. Others have also found associations between schistosome-specific IgA responses and lower levels of infection intensity (Auriault *et al.* 1990; Grzych *et al.* 1993; Ali & Shaneen 1994); however, as far as we are aware this is the first time a negative association between pre-treatment SWA-specific IgA responses and the intensity of reinfection has been described. Two potential mechanisms for the role of IgA in protective immunity have been proposed. Receptors for IgA are present on the surface of the eosinophils (Capron *et al.* 1988) and coating of eosinophils with this antibody isotype leads to their activation and degranulation (Abu-Ghazaleh *et al.* 1989). Therefore, IgA may play a direct role in antibody-dependent cell-mediated-cytotoxicity (ADCC). In addition, it has been suggested that specific IgA responses against the vaccine candidate Sm28GST are able to neutralize the enzymatic properties of the native schistosome GST enzyme, resulting in impaired fecundity by limiting egg laying as well as the hatching capacity of eggs into viable miracidia (Grzych *et al.* 1993). IgA-SWA responses increased with age and after treatment in the older foci as described previously for *S. mansoni* and *S. haematobium* infections (van Dam *et al.* 1996; Ndhlovu *et al.* 1996).

To summarize, we found no evidence that treatment induces adult-like responses in children in a study population that was recently infected with *S. mansoni*, although it is possible that this effect only becomes visible after multiple treatment rounds. In addition, our results indicate that the potentially protective IgE response was associated with duration of exposure/experience of infection. Moreover, an association between SWA-specific IgA responses and resistance to reinfection was observed. Levels of IgA-SWA were higher in the adult population whereas the intensity of reinfection was lower, suggesting that resistance to reinfection and the development of protective immune responses are strongly age-associated.

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Association entre les réponses anticorps spécifiques et la résistance à la réinfection dans une population sénégalaise récemment exposée à *Schistosoma mansoni*

Nous avons examiné les associations entre les réponses anticorps spécifiques du schistosome et la réinfection chez des individus sénégalais récemment exposés à *Schistosoma mansoni*. Les effets du traitement, de l'âge, de l'intensité de l'infection et de la durée de l'exposition sur la réponse anticorps spécifiques du schistosome ont également été étudiés en comparant les réponses immunitaires chez des individus exposés pendant plus de 3 ans, à celles survenant chez des individus exposés pendant plus de 8 ans. Des prélèvements sanguins ont été effectués auprès de tous les participants à l'étude avant le traitement ainsi qu'à 6 et 12 semaines post thérapie. Les données ont été analysées à l'aide d'un modèle statistique prenant en compte les interactions entre le temps, l'âge et la durée du traitement. Dans l'ensemble, les profils de réponses anticorps les plus spécifiques par groupe d'âge, étaient similaires à ceux précédemment décrits pour les infections à *S. mansoni*, *S. japonicum* et *S. heamatobium* dans différentes régions endémiques. En général, une poussée de réponse anticorps spécifique dirigée contre l'antigène de ver adulte (SWA) était observée 6 semaines après le traitement, tandis que la majorité des réponses d'isotypes dirigées contre l'antigène de l'œuf (SEA) n'étaient pas affectées par le traitement. Notre analyse a montré que l'effet du traitement sur les réponses anticorps spécifiques du Schistosome est influencé par l'âge, l'intensité de l'infection et la durée de l'exposition. Nous n'avons trouvé aucune évidence que le traitement favorise la maturation la réponse anticorps spécifique chez les enfants récemment infectés par *S. mansoni*. Nos résultats indiquent que le développement d'une réponse d'immunoglobuline E (IgE) potentiellement protectrice était associé avec la durée de l'exposition ou en d'autres termes avec l'expérience de l'infection. Il était intéressant de constater que chez les individus récemment exposés, il existait une association significative entre les réponses IgA spécifiques du SWA et la résistance à la réinfection. Chez l'adulte, la résistance à la réinfection et la production d'IgA contre le SWA étaient associées, indépendamment du type d'exposition. Ceci suggère que la susceptibilité envers *S. mansoni* et le développement de réponses immunitaires protectrices sont dépendantes de l'âge.

mots clés Schistosomiase, *Schistosoma mansoni*, réponses d'anticorps, sénégal, traitement, réinfection

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Asociaciones entre respuestas de anticuerpos específicas y resistencia a la reinfección en una población senegalesa recientemente expuesta a *Schistosoma mansoni*

Hemos examinado las asociaciones entre las respuestas de anticuerpos específicos para esquistosoma y la reinfección en individuos senegaleses expuestos recientemente a *S. mansoni*. El efecto del tratamiento, la edad, la intensidad de infección y la duración de la exposición sobre respuestas de anticuerpos específicos para esquistosoma también fueron estudiadas mediante la comparación de la respuesta inmune en indiv iduos expuestos durante menos de 3 años, con las respuestas en personas expuestas por más de 8 años. Se tomó una muestra de sangre a todos los individuos antes del tratamiento, así como 6 y 12 semanas des pués del mismo. Utilizamos un modelo estadístico que incluye los términos de interacción entre tiempo, edad, intensidad de infección y duración de la exposición. Los patrones totales de las respuestas de anticuerpos más específicas por edad eran similares a aquellas previamente publicadas para infecciones por *S. mansoni*, *S. japonicum*, y *S. haematobium* en diferentes áreas endémicas. En general se observó un incremento en la respuesta específica de anticuerpos contra el antígeno del gusano adulto (AGA) después de 6 semanas de tratamiento, mientras que la mayoría de respuestas de is otipos contra el antígeno del huevo (AAH) no se vieron afectadas por el tratamiento. Nuestro análisis muestra que el efecto del tratamiento sobre las respuestas de anticuerpos específicos para esquistosoma está influenciado por la edad, intensidad de infección y duración de la exposición. No hemos encontrado evidencia de que el tratamiento madure la respuesta específica de anticuerpos en niños recientemente infectados con *S. mansoni*. Nuestros resultados indican que el aumento de una respuesta IgE potencialmente protectora, está asociada con la duración de la exposición o, en otras palabras, con la experiencia de infección. Es interesante que, en individuos con una exposición reciente, se observa una asociación significativa entre la respuesta IgA - AGA y la resistencia frente a una reinfección. La resistencia frente a la reinfección y la producción de IgA-AGA estaba asociada con la edad adulta, independientemente de los patrones de exposición, sugiriendo que la susceptibilidad a *S.mansoni* y el desarrollo de respuestas inmunes protectoras son dependientes de la edad.

palabras clave esqu istosomiasis, *Schistosoma mansoni*, respuestas de anticuerpos, respuestas anticuerpos, senegal, tratamiento, reinfección

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