

Factors Affecting Human IgE and IgG Responses to Allergen-Like *Schistosoma mansoni* Antigens: Molecular Structure and Patterns of in vivo Exposure

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Key Words

Schistosoma mansoni · IgE response, human

Abstract

Background: The human IgE response is associated with allergy and with host defence against parasitic worms. A response to Sm22.6, the dominant IgE antigen in adult *Schistosoma mansoni* worms, correlates with resistance to re-infection after treatment. Sm22.6 is one of a family of EF-hand containing parasite proteins with sequence similarity to dynein light chain (DLC) and with major non-parasite allergens. Here we compare human IgE and IgG responses to other family members, Sm20.8 and Sm21.7, as well as to SmDLC1, relating these to antigen structure and expression in parasite life stages. **Methods:** Recombinant antigens were used in ELISA to measure antibody isotype responses in 177 cases from an endemic area, before and 7 weeks after treatment. Parasite antigen expression was assessed by RT-PCR and Western blotting. **Results:** Levels of antibodies to Sm22.6 and Sm20.8 (but not to Sm21.7 or SmDLC1) showed post-

treatment increases in all but young children. Many produced IgE to Sm22.6 and Sm20.8 (2 EF-hands), few to Sm21.7 (1 EF-hand) or SmDLC1 (no EF-hands). Sm21.7 was expressed in cercariae, adults and eggs, Sm22.6 and Sm20.8 were concentrated in the adult. **Conclusions:** These studies suggest that IgE antigens Sm22.6 and Sm20.8 are only released to boost antibodies when adult worms die, whilst Sm21.7 and SmDLC1 are released constantly from eggs dying in host tissue. IgE responses to these allergen-like molecules may be influenced by patterns of exposure and the number of EF-hand motifs.

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Introduction

The production of IgE antibodies against otherwise harmless molecules is the definitive step in the development of type I hypersensitivity or allergy. The IgE response is, however, widely believed to have evolved as a host defence against metazoan parasites, and studies on

the immune responses to worm infections such as schistosomiasis have increased the understanding of IgE-mediated hypersensitivity as well as host defence [1].

In man, schistosomiasis is usually caused by one of three species of the parasite, *Schistosoma mansoni*, *Schistosoma haematobium* or *Schistosoma japonicum* [2]. The condition is contracted from water-borne larvae (cercariae) that penetrate the skin and develop into adult worms that live for many years in the bloodstream. Their vascular location depends on the species, but in *S. mansoni* the adults reside in the mesenteric veins of the intestine. In situ, mature females lay hundreds of eggs per day that either pass through into the host's gut for excretion and release or are washed away to the liver, where they become trapped and die.

Chemotherapy is available for schistosomiasis, but in areas of high transmission re-infection is very common. In endemic areas, reduced re-infection in adults is evidence that resistance can develop, and a number of immune responses have been associated with this resistance, in particular the production of parasite-specific IgE [3–5]. How this response could be protective in humans has not been fully established, although it has been shown that eosinophils, macrophages and platelets can kill schistosome larvae in an IgE-dependent manner in vitro [6]. The identity of IgE targets within the parasite has also been the subject of some investigation. In treatment studies carried out in an *S. mansoni*-endemic area of Kenya, Dunne et al. [3, 7] and Webster et al. [8] showed that relatively few worm molecules were recognized by IgE, and that a response to the dominant antigen, Sm22.6, was associated with reduced reinfection.

The protein Sm22.6, a major target for the human IgE response [3], shares structural features with calcium-binding allergens from fish and grass pollens [9]. It is a member of a gene family that includes orthologues Sj22.6 in *S. japonicum* and Sh22.6 in *S. haematobium*, which are also human IgE antigens [9, 10]. The family includes other characterized schistosome proteins Sm21.7, Sm20.8 Sj20.8 and Sj21.7 [11–16] and similar molecules in other trematodes [17, 18]. Members are characterized by the presence of EF-hand motifs (Pfam accession No. PF00036) at the N-terminal end and by a region similar to dynein light chain (DLC) at the C-terminal end [10–18]. Localization within the parasite has been studied for Sm22.6, Sj22.6, Sm20.8 and *Fasciola gigantica* calcium-binding protein 1. In each case the molecules were strongly associated with the outer tegument layer [7, 15–17, 19]. These proteins are sometimes termed the tegument-associated antigens.

From the epidemiological evidence it seems that the IgE response to Sm22.6 may be a factor in host defence or may be a marker for resistant individuals. In order to identify factors that may be important in this and perhaps other IgE responses, we have compared the human IgE and IgG responses to Sm22.6 with those to other members of the gene family, Sm20.8 and Sm21.7, and to those of the structurally related SmDLC1. Chemotherapy for schistosomiasis can boost antibodies to parasite antigens including those to Sm22.6 [20–22], and in the current study we assess IgG1, IgG4 and IgE responses both before and 7 weeks after praziquantel treatment in a fishing community in Uganda.

Materials and Methods

Study Population, Parasitology and Treatment

The study was conducted in the fishing village of Booma in Uganda. The area and population are described in detail elsewhere [23]. The study cohort consisted of 177 individuals aged 7–50 years. Ethical permission for this study was obtained from the ethical review committee of the Ugandan Ministry of Health. Before enrolment, informed consent was obtained from adults, and assent was obtained from parents or guardians of the children. Before treatment, 3 stool samples were collected on consecutive days from each participant and helminth eggs were counted using the Kato-Katz method [24]. After collection of the final sample, participants were treated with 40 mg/kg praziquantel and 2 weeks later they were re-treated at the same dose.

Native Parasite Antigens

The Puerto Rican strain of *S. mansoni* was maintained in outbred mice and *Biomphalaria glabrata*. Adult worms were recovered from mice by portal perfusion 6 weeks after infection. Cercariae were shed from snails phototrophically. Soluble worm antigen (SWA) and soluble cercarial antigen were prepared from frozen parasites in the presence of protease inhibitors, 1 mM TPCK and 1 mM PMSF (Sigma, UK), and parasite eggs were isolated from liver tissue, and saline soluble egg antigen (SEA) was prepared from frozen eggs, all as previously described [3, 7].

Recombinant Antigens

Sm22.6 accession No. M29837), Sm21.7 (M67507), Sm20.8 (U872430) and SmDLC1 (U55992) were cloned into pGEX-1 λ T expression vector (Pharmacia) as described previously [8, 13, 25]. The antigens were expressed as GST fusion proteins, cleaved and purified by preparative electrophoresis as described previously [10].

Enzyme-Linked Immunosorbent Assay

Plasma was prepared from venous blood samples. Human IgG1, IgG4 and IgE responses to *S. mansoni* SWA, SEA, recombinant (r) Sm22.6, rSm20.8, rSm21.7 and rSmDLC1 were measured using isotype-specific monoclonal antibodies as described previously [26]. Plasma was tested in triplicate at dilutions of 1/200 for IgG1, and IgG4, and 1/20 for IgE. Samples from healthy European volunteers were also assayed as negative controls. Specific isotype

Table 1. Percentage change in antibody isotype responses to recombinant schistosome antigens

Antigen	IgG1		IgG4		IgE	
	boost, %	p value	boost, %	p value	boost, %	p value
rSm22.6	164 (127–206)	<0.001	102 (72–136)	<0.001	53 (35–75)	<0.001
rSm20.8	31 (18–46)	<0.001	32 (17–48)	<0.001	42 (26–60)	<0.001
rSm21.7	10 (1–19)	0.030	7 (–2 to 17)	0.111	4 (–4 to 12)	0.299
rSmDLC1	17 (4–32)	0.012	–5 (–17 to 6)	0.362	1 (–5 to 7)	0.843

Boost: approximate percentage increase induced by praziquantel treatment averaged over all study subjects = $100 \times [\exp(\text{mean}[\ln(\text{OD}_{\text{posttreatment}} + 0.01) - \ln(\text{OD}_{\text{pretreatment}} + 0.01)]) - 1]$; p value: paired t test for $\ln(\text{OD}_{\text{posttreatment}} + 0.01)$ versus $\ln(\text{OD}_{\text{pretreatment}} + 0.01)$; OD = optical density. Figures in parentheses indicate 95% confidence intervals.

responses are abbreviated in the text so that, for example, the IgE response to Sm22.6 becomes Sm22.6-IgE.

Reverse-Transcription Polymerase Chain Reaction

The abundance of mRNA for *S. mansoni* α -tubulin and tegument-associated antigens in cercariae, adult worms and eggs was measured by isolating total RNA and performing RT-PCR as described [27] using the oligonucleotide primers

5'-CATTTAGCGCACCATCGAAGC-3' and 5'-GGCGGTG-GTACTGGTTCTGGG-3' for α -tubulin, 5'-CCATGGATAG-CAAGGTTTGA-3' and 5'-TGTTTTGGACATGGTTGCTG-3' for Sm22.6, 5'-TCAACAATGCCAAAACCAAA-3' and 5'-GCA-TTCACTTGCCATACACG-3' for Sm21.7, 5'-ATTGGGTTTGA-GTCCTGCAC-3' and 5'-ATGACAATGTGCCATGCTTT-3' for Sm20.8 and 5'-AAGAAATGCAAGAGGATGCAA-3' and 5'-GGAATGCTGATCATCCAAA-3' for SmDLC1. The annealing temperature was 53°C for α -tubulin PCR and 57°C for the others. Extension time was 1 min and the number of cycles varied as detailed in figure 4 and Results. For every cDNA preparation, a control sample was prepared excluding reverse transcriptase (RT minus). No PCR products were seen with any of these negative controls.

Statistical Analysis

Optical density values from ELISAs were analysed as the logarithm with 0.01 added to all readings to avoid logarithms of zero. All statistical analysis was performed using SPSS v11.

Results

Treatment Boosts Antibody Responses to Sm22.6 and Sm20.8 but Not to Sm21.7 or SmDLC1

Isotype-specific ELISAs were used to measure IgG1, IgG4 and IgE to antigens rSm22.6, rSm20.8, rSm21.7 and rSmDLC1 in the plasma of the 177 individuals from an *S. mansoni*-infected community. Figure 1 shows the levels of these antibodies before and 7 weeks after praziqu-

antel therapy in each individual, and table 1 shows the mean changes. Overall, treatment resulted in striking increases in the levels of all 3 isotypes to Sm22.6 and to less marked, but highly significant increases in all 3 responses to Sm20.8. Treatment produced only marginal increases in the IgG1 responses to Sm21.7 and SmDLC1 and no significant change in the other responses to these 2 antigens (fig. 1 and table 1).

IgE Responses to Sm22.6 and Sm20.8 Are Common, Those to Sm21.7 and DLC Are Not

For any given antigen/isotype combination, even after treatment, many individuals did not produce detectable levels of specific antibody (i.e. levels above those in uninfected controls). Figure 2 shows the percentage of individuals that produced a posttreatment IgG1, IgG4 or IgE response to SWA or the 4 recombinant antigens. Plasma from approximately 60% of the study cohort contained detectable levels of Sm22.6-IgG1, and essentially the same individuals (data not shown) also produced IgG4 and IgE to this antigen. The pattern was markedly different for Sm21.7. The majority produced Sm21.7-IgG1 but a much smaller proportion produced Sm21.7-IgG4 or Sm21.7-IgE (fig. 2). Thus, although similar numbers responded with Sm22.6-IgG1 (62%) and Sm21.7-IgG1 (71%), significantly more produced Sm22.6-IgE (62%) than Sm21.7-IgE (18%; χ^2 test, $p < 0.001$). The pattern for Sm20.8 responders was similar to that for Sm22.6 in that most of those producing detectable levels of Sm20.8-IgG1 also produced Sm20.8-IgG4 and -IgE. In each case those responding to Sm20.8 were a subgroup of those responding to Sm22.6 (not shown). The pattern for SmDLC1 was similar to Sm21.7 in that many subjects produced SmDLC1-IgG1, but very few (4%, $n = 7$) responded with a detectable IgE response (fig. 2).

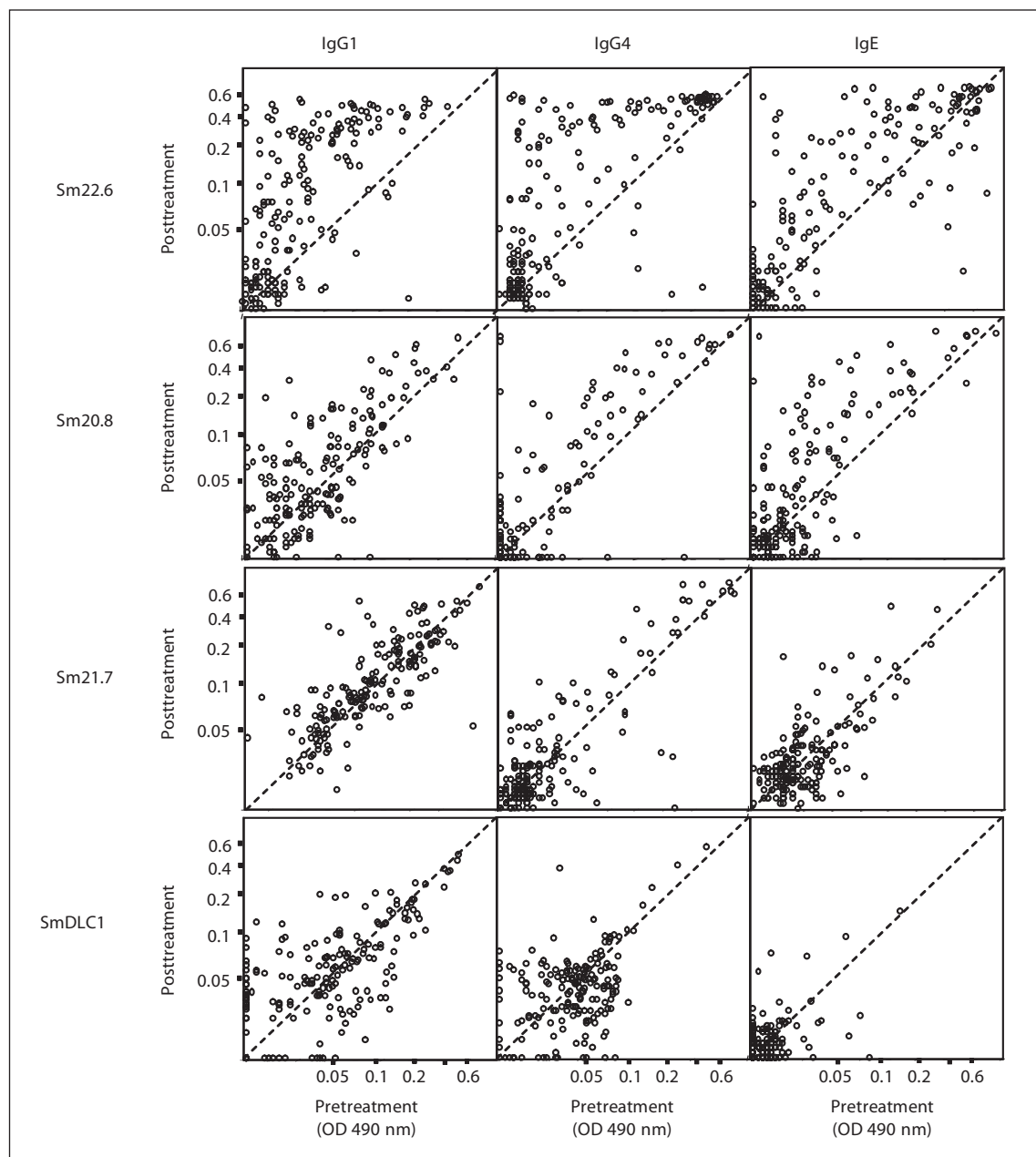


Fig. 1. Pretreatment versus posttreatment antibody responses to recombinant tegument-associated antigens. Blood samples were donated before (pretreatment) and 7 weeks after the first dose of praziquantel (posttreatment), and isotype-specific ELISAs were used to measure the plasma levels of IgG1, IgG4 and IgE to the 4

recombinant antigens indicated. Each scatter graph shows the data for 177 subjects. OD = Optical density. The dashed line indicates the no-change limit, and points above the line represent individuals whose antibody levels have increased following treatment.

Anti-Egg Response Correlated with Anti-Sm21.7 but Not with Anti-Sm22.6 Response

For the 4 recombinant antigens, posttreatment IgG1 was the only response present in sufficient subjects to allow comparisons between antigens to be made (fig. 2).

Some tegument-associated antigens have been shown to be components of the adult worm, and in the posttreatment data shown in table 2 it is clear that there was a strong correlation between levels of IgG1 to Sm22.6 and those to SWA. There was also a highly significant correla-

Table 2. Correlations of posttreatment IgG1 responses to recombinant tegument-associated antigens with posttreatment responses to native parasite extracts

	Sm22.6-IgG1	Sm20.8-IgG1	Sm21.7-IgG1	SmDLC1-IgG1
SWA-IgG1	0.82* p < 0.001	0.43* p < 0.001	0.24* p = 0.001	0.23* p = 0.001
SEA-IgG1	0.14 p = 0.069	0.17 p = 0.025	0.24* p = 0.001	0.17 p = 0.028

Estimates for Spearman's correlation coefficient for the whole study cohort (n = 177) of posttreatment levels [ln(OD + 0.01)] of IgG1 to each recombinant antigen against posttreatment levels of SWA-IgG1 and SEA-IgG1 [ln(OD + 0.01)].

* Significant correlation after Bonferroni correction applied.

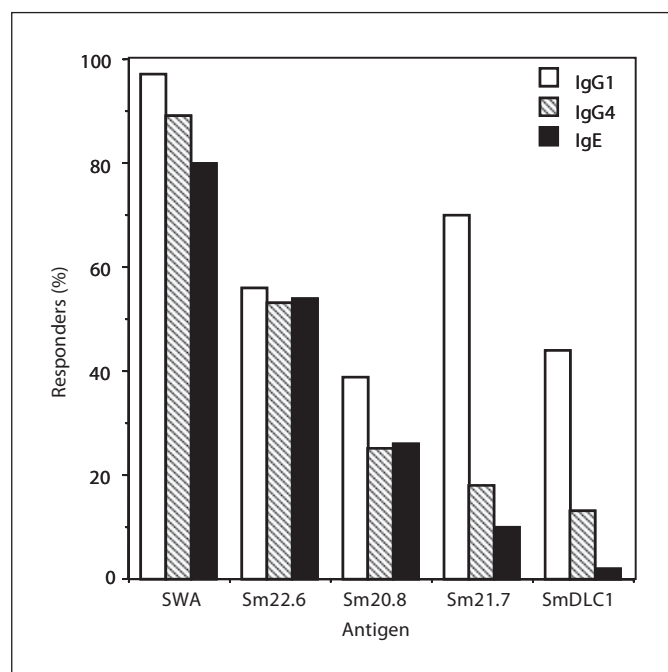


Fig. 2. Frequency of antibody isotype responses to SWA and tegument-associated antigens following treatment. The proportion of subjects whose posttreatment plasma contained detectable levels of IgG1, IgG4 or IgE to the antigens indicated. For each antigen and isotype, the detection threshold was defined as the mean $\pm$ 3 SD of uninfected control plasma samples (n = 22).

tion between levels of IgG1 to Sm20.8 and those to SWA. In contrast, the correlation between Sm21.7-IgG1 and SWA-IgG1 was relatively weak, as was the correlation between SmDLC1-IgG1 and SWA-IgG1. Notably, whilst there was no relationship between Sm22.6-IgG1 and SEA-IgG1, there was a significant correlation between

Sm21.7-IgG1 and SEA-IgG1 comparable to that seen between Sm21.7-IgG1 and SWA-IgG1 (table 2).

Intensity of Infection and Age May Increase Host Experience of Sm22.6 and Sm20.8

As IgG1 production was the predominant response to each of the antigens (fig. 2), we considered IgG1 levels to be the best indicator of in vivo exposure to an antigen and examined factors influencing IgG1 responses. For example, in the study population, the intensity of infection measured before treatment was correlated with the elevated posttreatment levels of Sm22.6-IgG1 (Spearman's coefficient $r_s = 0.46$, $p < 0.001$) and Sm20.8-IgG1 ($r_s = 0.26$, $p < 0.001$) and, importantly, was significantly correlated with the increase in Sm22.6-IgG1 induced by treatment ($r_s = 0.41$, $p < 0.001$).

In endemic areas, intensity of infection varies with age. Figure 3a shows that in agreement with other studies [26, 28], intensity of infection was low in children, peaked in adolescents and was relatively low in adults. Figure 3b–e shows the increases in IgG1 that occurred in the different age groups between pre- and posttreatment examinations. In parallel with the intensity of infection data, increases in Sm22.6-IgG1 were low in the youngest age group (7–9 years) and highest in the 13- to 17-year age group (fig. 3b). However, in contrast to the drop in infection intensity seen in older individuals, posttreatment increases in Sm22.6-IgG1 were significantly higher in adults than in young children.

The age profile for the IgG1 response to Sm20.8 (fig. 3d) was similar to that for Sm22.6 in that the adults experienced a significant increase in antibody levels following treatment, but children did not. None of the age groups showed significant increases in Sm21.7-IgG1 (fig. 3c) or SmDLC1-IgG1 (fig. 3e).

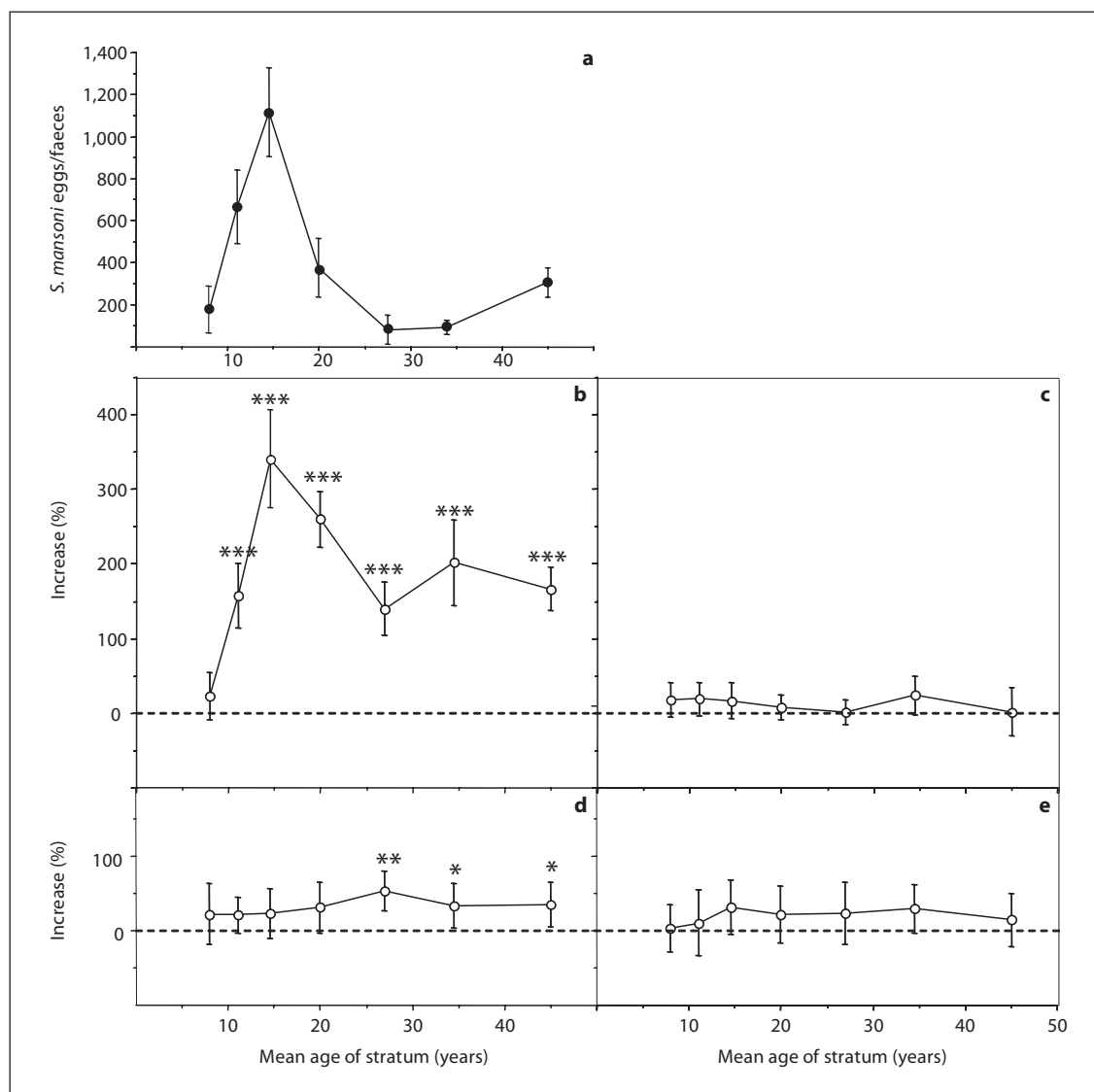


Fig. 3. Intensity of infection, age and the increase in IgG1 responses following treatment. The pretreatment intensity of infection (a) and the posttreatment increases in Sm22.6-IgG1 (b), Sm21.7-IgG1 (c), Sm20.8-IgG1 (d) and SmDLC1-IgG1 (e) are shown for each of 7 age groups: 7–9, 10–12, 13–16, 17–23, 24–30, 31–38 and 49–50

years. The percent increases between pretreatment and 7-week posttreatment antibody levels were calculated as detailed in the legend to table 1. *** $p < 0.001$, ** $p = 0.001-0.01$, * $p = 0.01-0.05$: groups showing a significant increase (paired t test).

Sm21.7 Is Expressed in Eggs and Worms, Sm22.6 and Sm20.8 in Worms Only

In order to determine whether the differences in antibody responses could be explained by different expression patterns, expression of the 4 antigens was measured in isolated cercariae, adult worms and eggs. RT-PCR demonstrated that although transcription of Sm22.6 occurred in cercariae, it was greater in adult worms (fig. 4a). Transcription of Sm22.6 was minimal in parasite eggs; it was

not detected by RT-PCR under the conditions shown in figure 4a, but was seen at a very high cycle number (39 cycles, not shown). Transcription of Sm20.8 was also observed in cercariae and adults but was not detected in eggs. In contrast to these first 2 antigens, transcription of Sm21.7 was maintained at similar levels in all 3 life cycle stages (fig. 4a). Transcription of SmDLC1, although highest in adult worms, was also seen in both cercariae and eggs.

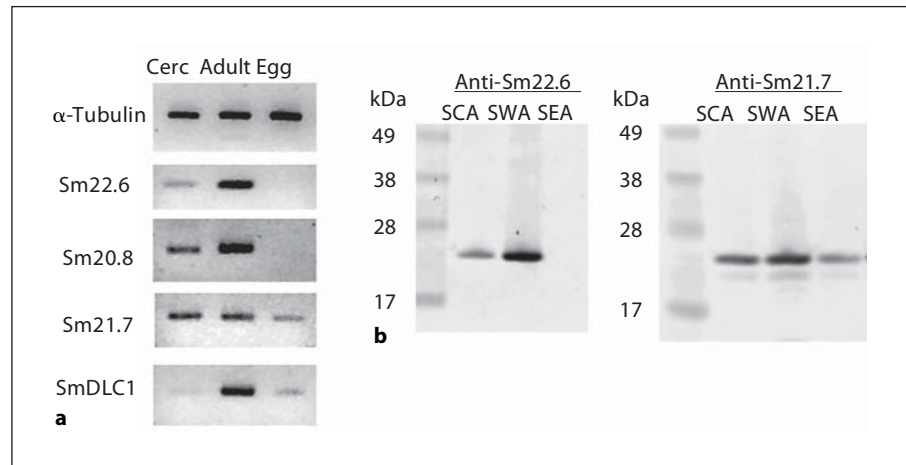


Fig. 4. Expression of tegument-associated antigens in cercariae, adult worms and eggs. **a** Total RNA was isolated from cercariae, 7-week adult worms and eggs and used to prepare cDNA for RT-PCR using specific primers and an optimized cycle number for Sm α-tubulin (26 cycles), Sm22.6 (27 cycles), Sm20.8 (22 cycles), Sm21.7 (27 cycles) and SmDLC1 (22 cycles) as indicated. PCR products were separated on a 2.0% agarose gel and detected using ethidium bromide. **b** Soluble extracts of cercariae (SCA), 7-week

adult worms (SWA) and eggs (SEA) were run on 4–12% acrylamide SDS-PAGE (10 µg protein/lane), electroblotted onto nitrocellulose, probed with rabbit antisera against Sm22.6 or Sm21.7 as indicated and visualized with horseradish-peroxidase-conjugated anti-rabbit IgG and diaminobenzidine/hydrogen peroxide. Molecular-weight markers were also run as shown. (Antisera to Sm22.6 or Sm21.7 were raised by immunizing rabbits with electro-eluted antigen in adjuvant as described previously [7].)

Protein levels of Sm22.6 and Sm21.7 were assessed in soluble extracts of cercariae (soluble cercarial antigen), adult worm (SWA) and egg (SEA) by Western blotting. Figure 4b shows that mirroring the mRNA levels, Sm22.6 protein was present in cercarial and worm extract but was absent in egg extract. Sm21.7 protein was present in all 3 stages (fig. 4b).

Discussion

In this cohort, living in an *S. mansoni*-endemic area, treatment to kill intravenous parasites caused pronounced increases in antibody responses to the allergen-like molecules Sm22.6 and Sm20.8. Adult schistosomes live in the bloodstream, employing immune evasion strategies and exposing few antigens to the immune system [29]. It seems possible that Sm22.6 and Sm20.8 could be cryptic worm antigens, hidden during active infection but released from dying parasites during treatment. It has been proposed [22, 30] that such a release would vaccinate the host and boost antibody levels as a result of primary or secondary responses to these antigens. The absence of any change in the responses to the closely related Sm21.7 suggests either that it is not released during treatment, or that death of worms is not required for exposure of this

antigen. Adult *S. mansoni* can live for many years [31], whereas the survival of eggs trapped in the tissue ranges from a few days to several weeks [32]. If Sm21.7 were a component of parasite eggs, the constant trapping and dying of eggs in the liver that occurs during *S. mansoni* infection would expose this antigen to the immune system continuously.

Expression studies on the parasite in this investigation showed that Sm21.7 was indeed transcribed and translated in schistosome eggs, whilst Sm22.6 and Sm20.8 were not. In fact, expression of Sm21.7 appeared to be relatively constant throughout those life stages present in the host, whereas expressions of Sm22.6 and Sm20.8 were mostly concentrated in the adult worm. Earlier expression studies, although not entirely consistent, do support our findings that transcription and translation of Sm22.6 and Sm20.8 are high in adult worms and absent in eggs [11, 13, 16, 33]. Furthermore, the ubiquitous nature of Sm21.7 is supported by previous Northern blotting experiments on Sm21.7 [12] which showed that mRNA was equally abundant in snail-stage sporocysts, schistosomula and adult worms. The abundance in eggs was not reported.

Correlations between antibody responses provided additional evidence for the different exposure patterns of the antigens. IgG1 is the dominant subclass in human plasma preceding IgG4 and IgE in the immune response

[34] and is therefore a good indication of exposure to an antigen. In this study, posttreatment levels of IgG1 to the worm-associated antigen Sm22.6 showed a strong correlation with the anti-worm response (SWA-IgG1), but no correlation with anti-egg response SEA-IgG1 (also true for Sm20.8). This contrasted with the IgG1 response to the ubiquitous Sm21.7, which showed a weak but significant correlation with both SWA-IgG1 and SEA-IgG1.

For any individual, the pretreatment intensity of infection should affect the amount of cryptic antigen released during treatment, and this is likely to influence the magnitude of any boost in antibody levels. Indeed, this is supported by the Sm22.6-IgG1 data for children in this study. Very young individuals had the lowest intensity of infection and showed no boost in Sm22.6-IgG1, whilst adolescents were most infected and had the greatest boost. The situation was different in adults. They had a relatively low infection intensity but still showed pronounced boosts in Sm22.6-IgG1 after treatment. In this endemic area, these marked increases in older subjects suggest that a history of repeated experience of cryptic antigens may prime, so that a strong secondary antibody response occurs with a relatively small release of antigen. This age-dependent experience does not appear to derive from the small amounts of antigen released from invading cercariae dying in the skin, as this would occur in adults and children, but is more likely to develop gradually through the infrequent natural death of mature worms in the blood. The age profile for the Sm21.7-IgG1 response contrasts with that for Sm22.6-IgG1, in that it does not boost in any age group. Altogether these data are consistent with the hypothesis that Sm21.7 is released from dying eggs in all infected individuals regardless of age whereas release of Sm22.6 requires the death of adult worms. In support of this, studies of chronic *S. mansoni* infection in mice have shown that high levels of IgG to Sm21.7 appear in the plasma as soon as egg deposition begins, whereas IgG to Sm22.6 remains very low unless the animals are treated, re-infected and then treated again [Frances Jones, unpubl. data].

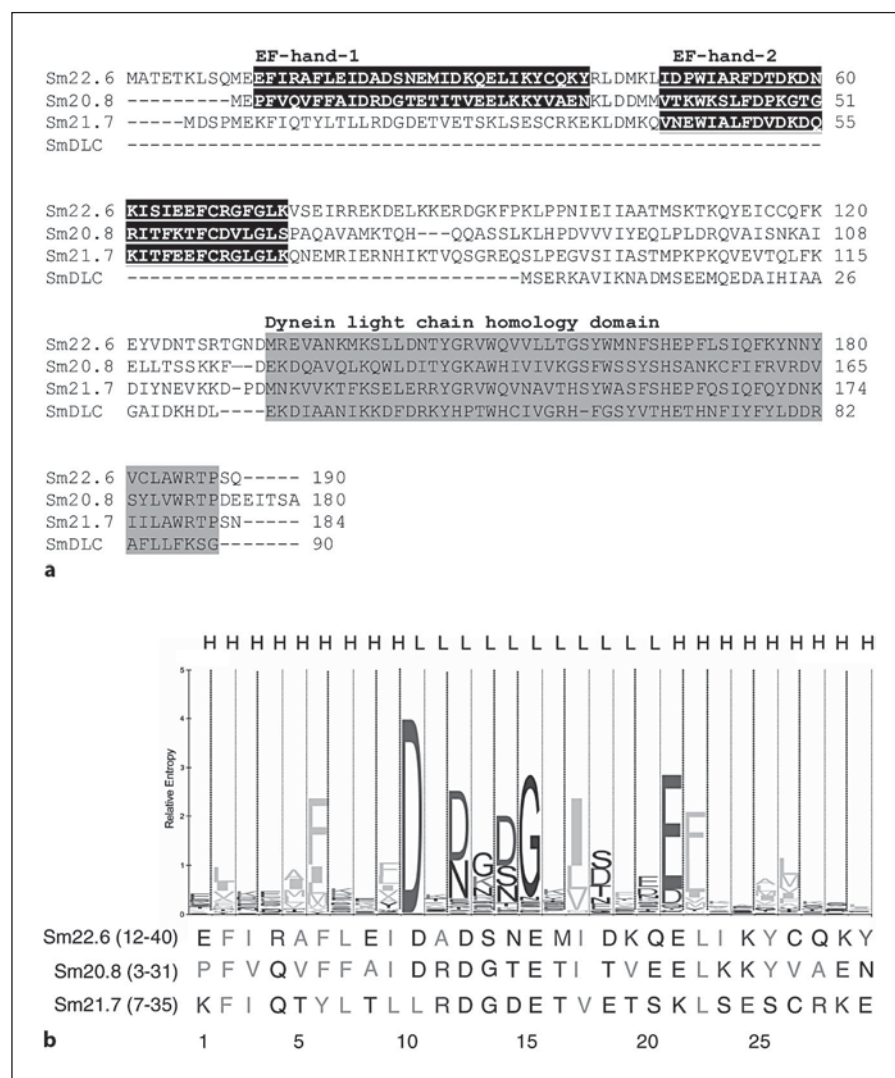
Some of the most interesting observations in this study concerned the antibody isotype patterns to the different antigens. As observed previously [7], specific antibody responses to Sm22.6 were only detected in a proportion of the population. Importantly, those that did respond to Sm22.6 produced both IgG1 and IgE to the antigen. This was also the case with those that responded to Sm20.8. However, a different isotype pattern was seen in response to Sm21.7. Whilst a majority of the cohort produced Sm21.7-IgG1, an IgE response was relatively rare. This

suggests that although Sm22.6 and Sm21.7 were equally likely to evoke an antibody response, Sm22.6 was a much more potent IgE antigen than the highly similar Sm21.7. This difference may be due to the different expression patterns of the 2 antigens. For example, the continuous and chronic release of Sm21.7 from dying eggs could influence antibody responses to Sm21.7 in a manner similar to the desensitization achieved in allergen immunotherapy. The mechanism by which increasing doses of IgE antigen inhibit allergy is not fully understood, although a role for IL-10 production by T-regulatory cells has been proposed [35–38].

Another reason why Sm21.7 is less likely to evoke an IgE response than either Sm22.6 or Sm20.8 may lie in the small but important structural differences between these antigens. In figure 5a the sequences of the 3 tegument-associated antigens and SmDLC1 are aligned. Significantly, whilst Sm22.6 and Sm20.8 each contain 2 EF-hands, Sm21.7 contains only 1 sequence that conforms to the structural requirements for an EF-hand motif (Pfam, www.sanger.ac.uk) and SmDLC1 does not contain any. The typical EF-hand is a 29-residue helix-loop-helix structure and in figure 5b the Hidden Markov Model (HMM) logo summary for EF-hand domains is aligned with the N-terminal EF-hand region of Sm22.6 and Sm20.8 and the equivalent region of Sm21.7. It is clear from figure 5b that the highly conserved residues D10 and E21 are substituted non-conservatively in Sm21.7. This marked difference in structure may be very important given that typical EF-hand motifs are a feature of many IgE antigens [39] and linear IgE epitopes have been recognized within EF-hand domains [9, 40]. Notably an IgE binding site has been identified in Sj22.6 that comprises the loop of an EF-hand motif and which shares marked sequence similarity with linear IgE epitopes in EF-hand loops within fish and pollen allergens [9, 41]. Perhaps more importantly, allergen EF-hand motifs usually occur in pairs folded into what is essentially a single compact [EF-hand]₂ domain [39]. The absence of one of the motifs in Sm21.7 would significantly alter the tertiary structure of the domain and potentially eliminate key conformational IgE epitopes in this antigen.

Why some molecules are IgE antigens and others are not has been the subject of much discussion. There is evidence that the IgE response against helminth infection is usually directed at a restricted number of antigens within the parasite [3, 10, 42], and this mirrors the observation that allergens generally come from a relatively small group of protein families, including the EF-hand family [39, 43]. In allergy and in helminth infection [44],

Fig. 5. Domain structures of *S. mansoni* tegument-associated antigens and DLC. The full amino acid sequences (**a**) of Sm22.6 (M29837), Sm20.8 (U87243), Sm21.7 (M67506) and SmDLC1 (U55992) are aligned to show homologous domains. Also, the Hidden Markov Model logo [39] for the EF-hand motif (Pfam accession No. PF00036) is aligned (**b**) using Clustal W with the N-terminal EF-hand (EF-hand-1) motifs in Sm22.6 and Sm20.8 and with the equivalent region of Sm21.7. In the logo, the height of each letter reflects the frequency of the amino acid at that position relative to background. Amino acids forming the 29-residue helix-loop-helix of the EF-hand motif are indicated (H and L).



the induction of T helper lymphocyte type 2 (Th2) responses culminates in IgE production, and it appears that some molecules are particularly suitable as targets for this IgE response [45]. It is not clear however which structural or functional characteristics are required for this property.

One of the most intriguing aspects of the IgE response to Sm22.6 is its association with resistance to re-infection in studies carried out in *S.-mansoni*-endemic areas [3, 7, 8]. Experiments in vitro and in animal models suggest that resistance may involve an antibody-mediated attack on invading larvae [6] and it is possible that an individual IgE response to highly 'allergenic' molecules such as Sm22.6 is a measure of their more general antiparasite IgE response. In *S.-mansoni*-endemic areas, the levels of

antiparasite IgE depend partly on age [46] but there is also a genetic component. It has been known for some time that the levels of total IgE are under genetic control [47] and a study of the HLA polymorphisms in the current study cohort revealed very strong associations between carriage of allele DRB1*13 and the IgE responses to SWA and to Sm22.6. Significantly, carriage of the allele was also associated with resistance to reinfection [48].

Finally, another reason why an IgE response to cryptic antigens such as Sm22.6 could be detrimental to invading larvae is the possibility of bystander effects. Studies in this laboratory have shown that recombinant Sm22.6 can bind to IgE on human basophils resulting in histamine release [Pierre Cahen, pers. commun.]. It is possible that Sm22.6 released by schistosomes dying in the tissue

could interact with IgE on effector cells, causing inflammatory release and making the environment hostile for other invading larvae. This current study indicates that the antigen structure and patterns of exposure can influence the IgE response and thus ultimately the contribution of IgE-dependent effector cells. This is important not only in host defence against worms but also in allergy and merits further investigation.

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