

Report

Human IgE response to the *Schistosoma haematobium* 22·6 kDa antigen

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SUMMARY

In *Schistosoma mansoni* and *S. japonicum* infection, the 22·6 kDa tegumental antigens Sm22·6 and Sj22·6 are principal targets for the human IgE response, and levels of IgE to Sm22·6 have been correlated with resistance to re-infection after chemotherapy. *S. haematobium* is arguably a more important species in terms of human infection, and in this report we describe for the first time the molecular characterization of a cDNA from *S. haematobium* (Sh22·6) that is closely homologous to Sm22·6 and Sj22·6. As a member of the tegument-associated antigen family, Sh22·6 possesses EF-hand domains and regions homologous to the dynein light chain domains. We have expressed recombinant Sh22·6 and studied the IgE responses to the antigen in a group of 99 infected individuals (68 children and 31 adults) from an endemic area of Gabon who donated blood before and 5 weeks after praziquantel treatment. IgE to Sh22·6 was detected by ELISA in 18 subjects (18%), and in the majority of responders levels rose between pre- and post-treatment. Interestingly, the proportion of adults expressing IgE to Sh22·6 was 35·5%, significantly higher than the 10·3% seen in children. IgE from at least 10 of the 18 ELISA responders recognized Sh22·6 on Western blots of adult worm extract and recombinant antigen. These results demonstrate that like related molecules in other species, Sh22·6 is a target for the human IgE response. The data also indicate that changes in the IgE response occur with age or with progressive exposure to key antigens.

Keywords human IgE, *Schistosoma haematobium*, tegumental antigen

RESEARCH NOTE

A characteristic feature of schistosomiasis is the production of large amounts of specific IgE antibodies in the host (1). This is likely to be a defensive response. IgE-mediated killing of schistosome larvae has been observed *in vitro* and in animal experiments (1–3) and immunoepidemiological evidence suggests that elevated levels of parasite-specific IgE may be protective in humans. Most cases of human schistosomiasis are caused by the three species of the parasite, *Schistosoma mansoni*, *S. haematobium* and *S. japonicum*. Studies in areas endemic for each of the three have shown that higher plasma concentrations of parasite-specific IgE are correlated with lower levels of re-infection after treatment (4–7). Chemotherapy to remove infection can itself increase IgE specific for the adult worm stage (8,9), and it has been proposed that this may be due either to the removal of the immunomodulatory influence of the parasite (9) or to a boosting effect of antigen release as worms are killed (10).

In *S. mansoni* a major target for the human IgE response is a 22·6 kDa antigen termed Sm22·6 (5,11,12). This protein, of unknown function, is expressed in schistosomula as well as in adult worms, where it is strongly associated with the outer syncytial tegument (11). IgE against Sm22·6 has been shown to be common in the plasma of subjects with a strong IgE response to whole adult worm antigen preparations (13,14). What is more, in studies carried out in an endemic area of Kenya, individuals whose IgE recognized Sm22·6 had reduced incidence and intensity of re-infection when treated (5,11,12). This suggests that IgE to Sm22·6 may be involved in resistance or may be a marker of resistant individuals.

A homologue of Sm22·6 has been identified in *S. japonicum* (Sj22·6), and studies on infected communities in the Philippines show that this is also a major target for the human IgE response (13,14). To date, however, no equivalent antigen has been characterized in *S. haematobium*, the species that almost certainly causes most human schistosomiasis (15).

Note: GenBank accession number for Sh22·6 is AY851615.

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	EF-hand 1	EF-hand 2	
Sh22·6	MSTETRLSSME EFIRAFLEMDADNNEMIDKQELIKYCQKNRLDMRLIDPWIA RFDTDKD		59
Sm22·6	-MATETKLSQME EFIRAFLEIDADS NEMIDKQELIKYCQKYRLDMKLIDPWIARFDTDKD		59
Sj22·6	MATTEYRLSLME QFIRAFIEIDKDNNELIDKQELTKYCQONQMDMKQIDPWIA RFDTDKD		60
Sm21·7	-----MDSPMEKFIQTYLTLLRDGDETVEVTSKLSSECRKEKLD MQVNEWIALFDVDKD		54
Sj21·7	-----MASTMEQFITAYLTIYRDGDEVVDKKELIDYCRKEKLD MQVDTWLSHFDVDKD		54
Sm20·8	-----ME PFVQVFFAIDRDGTETITVEELKKYVAEN KLDDMM VTWKWSLFD PKGT		50
Sj20·8	-----ME PFVQVFFAIDKDETETISIDELKSYVAANN LDEM MTKWQTLFD PNRT		50
Sh22·6	NKISIEEFCRGFGLK VSEIRREKEELKKEKRGKVS KLFFKVEIIAATMSKTKQYDICYQF		119
Sm22·6	NKISIEEFCRGFGLK VSEIRREKDELKKERDGKFPKLPPNIEIIAATMSKTKQYEIC CCQF		119
Sj22·6	GKVSLEEFCRGFGLK VWEVRREKEELKKDKEGKVSTLPLDIQIIAATMSKAKQYN ICCKF		120
Sm21·7	QKITFEEFCRGLGLK QNEMRIERNHIKTVQSGREQSLPEGVSI IASTMPKPKQVEVTQLF		114
Sj21·7	NRITLFEFCRGLGLK ANEMRIERNHIKAVQSGRVPKIPDGVEII IASTMP LPRQVEVTQ LY		114
Sm20·8	GRITFKTFCDVLGLS PAQAVAMKTQHQQASS---LKLHPDVVVI YEQ LPLDRQVAIS NA		107
Sj20·8	GKITFKKFCEVLGLS PAQAVAMKTQHHSAT---MKLHPDVTVI YEQ LPLDKQIAIS NKT		106
	Dynein light chain homology domain		
Sh22·6	KEYIDNSSRTNKD IKEVANKMKTLLDNTYGRVWQVVILTG SYWMNFS SHEPFLS IQFKYNN		179
Sm22·6	KEYVDNTSRTGND MREVANKMKSLLDNTYGRVWQVVLLTG SYWMNFS SHEPFLS IQFKYNN		179
Sj22·6	KELLDKTSRTGDE VRAVANDLKAFLDSEYGRVWQVIILTG SYWMNFS SHEPFLS MQFKYSN		180
Sm21·7	KDIYNEVK-KDPD MNKVVKTFKSELERRYGRVWQVNAVTHSYWASFS SHEPFQ SIQFQYDN		173
Sj21·7	KDLLPKSGGHEAD MRKVANDFKIKLEERYERVWQVVLLTG SYWMNFS SHEPLQS IQFKVDK		174
Sm20·8	IELLTSSK--KFDE EKDQAVQLKQWLDITYGKAWHIVIVKGSFWSSY SHSANKCF IFRVRD		165
Sj20·8	IELAKSTK--KLDE EKDQAVQLKQWLDQTYGKAWHVVIVRGSFWSSY SHSANKCF MYRIYD		164
Sh22·6	LVCLAWRTPSQ ----- 190	Similar(%)	Identical(%)
Sm22·6	YVCLAWRTPSQ ----- 190	95	77
Sj22·6	YVCLLWRTPSS ----- 191	86	64
Sm21·7	KIILAWRTPSN ----- 184	63	44
Sj21·7	NIILAWRTPNN ----- 185	69	47
Sm20·8	VSYLWVRTPDEEITSA 181	48	29
Sj20·8	FSYLWVRTPDEELTGE 180	47	30

Figure 1 Alignment of Sh22·6 with Sm22·6 ([AAA29856](#)), Sj22·6 ([AAC67308](#)), Sm21·7 ([AAA29923](#)), Sj21·7 ([AAD13338·1](#)), Sm20·8 ([AAC791301·1](#)) and Sj20·8 ([AA059421·2](#)). Residues identical to Sh22·6 are shaded grey. Predicted EF-hand domain (Pfam database www.sanger.ac.uk) are indicated in bold and underlined as are the dynein light chain homology domains (18). The alignment was generated by version 1·82 of ClustalW.

In the work described here, we have molecularly identified a cDNA from *S. haematobium* (Sh22·6) that shares close homology to Sm22·6 and Sj22·6. We have subsequently expressed recombinant Sh22·6 in *E. coli*, to study the IgE responses to the antigen in a cohort of infected subjects, both before and 5 weeks after treatment.

To identify Sh22·6, a *S. haematobium* cDNA library was first constructed in λ -ZAP from adult *S. haematobium* mRNA (a gift from V. Gutsman, Imperial College, London) using a Stratagene λ -ZAP cDNA cloning kit. Clones were screened with rabbit antiserum raised against native *S. mansoni* 22·6 purified from worm extracts (11). After two further rounds

of screening the Sh22·6 clone was excised as a construct in pBluescribe SK+ vector, and the nucleic acid sequence was determined by the dideoxy chain termination method of Sanger *et al.* (16).

Figure 1 shows the predicted 190 amino acid sequence of Sh22·6, aligned with those of Sm22·6 and Sj22·6, as well as the other members of the 20–23 kDa schistosome tegument-associated antigen family (17–19). This family of structurally related proteins appears to be developmentally regulated (17–19), but as yet no function has been assigned to any of the members. The marked similarities between Sh22·6 and the other two 22·6 kDa species are clear from the sequences

shown in Figure 1. Sh22-6 is 88% identical to Sm22-6 and 75% identical to Sj22-6. The homology is even higher within certain recognized domains. For example, the tegument-associated antigen family members are all characterized by the presence of EF-hand motifs in the N-terminal half of the molecule. The predicted Sh22-6 sequence contains two potential EF-hands (Figure 1) and these regions are very highly conserved between Sh22-6, Sm22-6 and Sj22-6. Notably, the second EF-hand domain in Sh22-6 (residues 47–73) is 100% identical to the equivalent region of Sm22-6 and 93% identical to the region in Sj22-6. Indeed, the structure of this domain is conserved across the whole antigen family (Figure 1).

It was noted by Hoffmann and Strand (18), that the C-terminal half of Sm20-8 showed marked homology to a domain present in members of the dynein light chain (DLC) family. Figure 1 shows that this region is highly conserved across the tegument-associated antigens, with the DLC domain of Sh22-6 (residues 131–187) sharing 93%, 58% and 37% identity with the equivalent regions of Sm22-6, Sm21-7 and Sm20-8, respectively. If conservative substitutions are taken into consideration these values rise to 96%, 77% and 66%, respectively.

Given that both Sm22-6 and Sj22-6 have been shown to be targets for the human IgE response (12,14), we next examined the IgE responses to Sh22-6 in a cohort infected with *S. haematobium*. First, recombinant Sh22-6 was expressed in bacteria. The coding region was directionally cloned into the prokaryotic expression vector pTrcHis (Invitrogen, Paisley, UK) and over-expressed in *E. coli* as polyhistidine (6xHis) tagged-fusion protein. The protein was isolated using nickel affinity chromatography (20) and purified by preparative SDS PAGE (12). The purified product was then coated onto plates for use in isotype-specific antibody ELISAs (5).

Plasma antibody levels were measured in a group of 99 infected individuals from Lambarene, Gabon, who had donated blood before and 5 weeks after praziquantel treatment. These 99 subjects formed part of a slightly larger cohort ($n = 106$) whose IgE and IgG4 responses to adult worm antigen (AWA) and soluble egg antigen (SEA) have been reported previously (9). The current subgroup, 55 females and 44 males, ranged from 5 to 48 years (median age 13), and the intensity of infection ranged from 1 to 12 450 eggs/mL urine (median value 102).

As was observed when measuring IgE responses to Sm22-6 and Sj22-6 in populations infected with *S. mansoni* and *S. japonicum* (14), IgE to Sh22-6 was (a) only detected in a proportion of subjects and (b) restricted to individuals with relatively high levels of IgE to AWA (data not shown). This restriction was not simply a concentration effect, however, as most of the subjects with high levels of IgE to AWA did not produce IgE to Sh22-6. IgE to Sh22-6 was detected (OD > 0.03) in 10 subjects before treatment and 18 subjects

(18%) at 5 weeks post-treatment (Figure 2a). In the majority of these 18 responders the levels of IgE to Sh22-6 rose between pre- and post-treatment, so that across the group there was a significant increase (paired *t*-test, $P = 0.004$). Neither the level of IgE to Sh22-6 at either time-point nor the observed increases, were correlated with the initial intensity of infection ($n = 18$).

The cohort consisted of 68 children (5–14 years) and 31 adults (15–48 years), as defined in the original report on this population (9). Adults had been selected on the basis of pre-treatment urinary egg counts, so that the child and adult groups would be matched for intensity of infection. In the current study, a post-treatment increase in the levels of IgE to Sh22-6 was observed in both children ($P = 0.028$) and adults ($P = 0.018$). However, as Figure 2(a) shows, the IgE response to Sh22-6 was more pronounced in adults. The proportion of adults expressing IgE to the recombinant antigen was 35.5% (11/31), significantly higher (χ^2 test, $P = 0.022$) than the 10.3% (7/68) seen in children. A similar adult bias was seen with IgG4 responses to Sh22-6, where, following treatment, the proportion of responders was 5.8% (4/68) in children and 25.8% (8/31) in adults (data not shown). This was not the case for IgG1, where 23.5% (16/68) of children responded to Sh22-6 and only 19.4% (6/31) of adults.

Thus, in a cohort living in an area endemic for *S. haematobium*, the IgE response to Sh22-6 was more prevalent in adults than in children, with the highest post-treatment levels seen in people over 14 (Figure 2a). This is similar to findings in an *S. mansoni* endemic area in Kenya, where levels of IgE to Sm22-6 were shown to be higher in older subjects (12). Together these results indicate that qualitative changes in the IgE response occur either with increasing age or with progressive exposure to key antigens. Immune localization studies shows that neither Sm22-6 nor Sj22-6 are exposed on the surface of the adult worms (12,21). It may be that the host only encounters the 22.6 kDa tegumental molecule after damage to, or disruption of, the parasite tegument. Exposure of the antigen in this way could occur after death of migrating larvae or natural worm death over time. In endemic areas, the more pronounced IgE response to the 22.6 kDa species in adult subjects is likely to represent greater exposure to antigen with increasing age. This is supported by the results of a study in a previously non-endemic area of Senegal, where the population suffered an outbreak of *S. mansoni* following a damming project (10). The epidemic nature of that infection meant that individuals had their first exposure at different ages. There, IgE responses to Sm22-6 were correlated with pre-treatment intensity of infection and not with age. This indicated that the IgE response to the 22.6 kDa species was related to the extent to which an individual was exposed to the molecule, rather than to any age-specific effects.

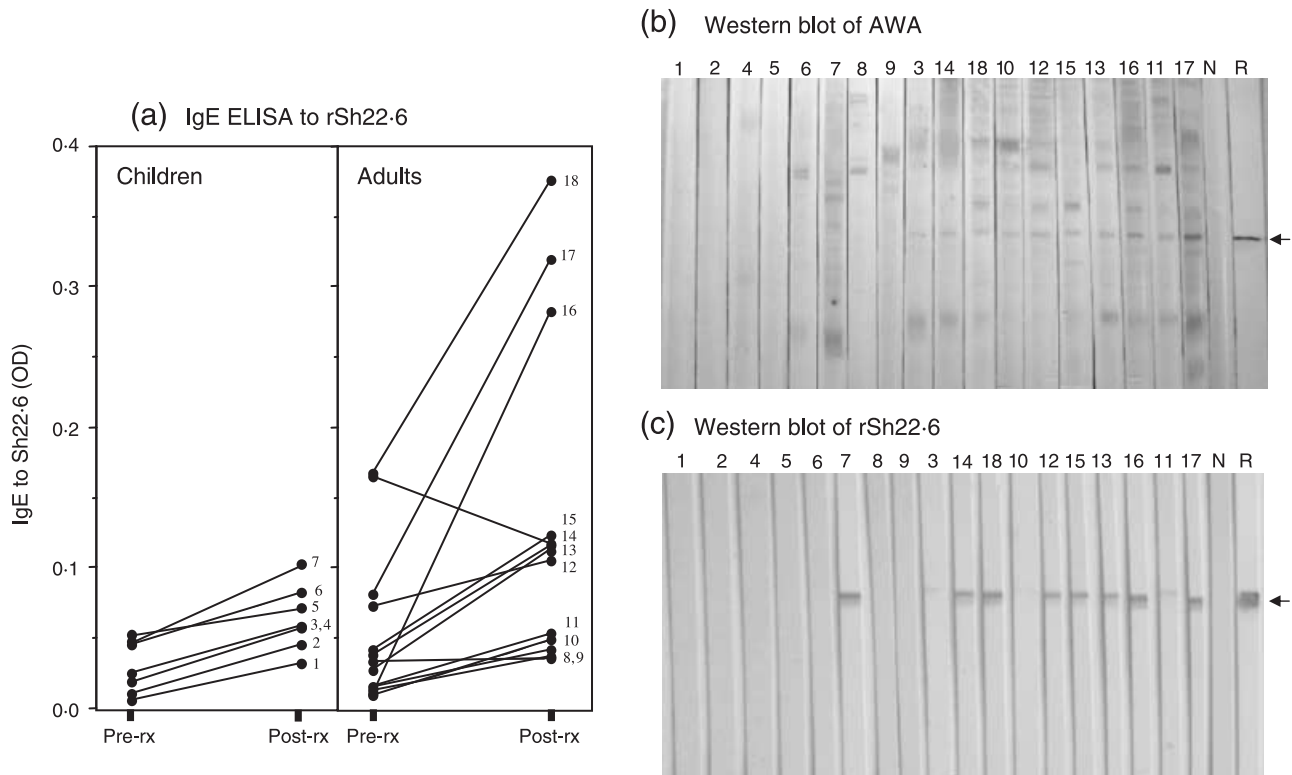


Figure 2 IgE response to Sh22-6 in infected subjects. (a) Plasma levels of IgE to recombinant Sh22-6 before and 5 weeks after treatment in the 18 subjects (7 of the 68 children and 11 of the 31 adults) that produced a response ($OD > 0.03$) to Sh22-6 at either time point. Values represent the mean of duplicate OD measurements in an ELISA specific for human IgE (5). Each of the 18 responders is numbered. (b) IgE Western blot of 200 μ g *S. haematobium* adult worm antigen (AWA) prepared as previously described (5) for *S. mansoni*. (c) IgE Western blot of 40 μ g recombinant Sh22-6. Samples were run on 15% SDS PAGE, electroblotted and probed as described (5), with post-treatment plasma (1 : 20) of those subjects who showed an IgE response to Sh22-6 in ELISA (numbered as in Figure 2a). Other lanes were incubated with non-infected control plasma (N) or rabbit antiserum against Sm22-6 (R) as indicated. Specific bands were detected using biotinylated mouse anti-human IgE (BD Biosciences, clone G7-26) and streptavidin-poly HRP (CLB, Amsterdam) or rabbit anti-Sm22-6 antisera (11) and HRP-conjugated anti-rabbit IgG (Sigma), as appropriate.

In the original description of antibody responses in the present cohort (9), it was reported that levels of IgE to AWA increased after treatment in children but not in adults. It was postulated that this might have been because the adult response to AWA was already maximal before treatment. In the current study, however, we note that (in those that responded) there were significant increases in IgE to Sh22-6 in both children and adults. The evidence that AWA and Sh22-6 have different antibody response profiles is not surprising. AWA is a complex mixture of molecules, some of which may have been secreted throughout infection (22). Sh22-6, on the other hand, is an individual, potentially cryptic antigen recognized by a particular subgroup of infected people.

In the current study, the evidence that Sh22-6 was a target antigen for human IgE was supported by Western blot analysis. Figure 2(b) shows that after treatment, IgE from at least 10 of the 18 ELISA responders recognized a band in

blots of AWA that coincided with Sh22-6. Further analysis confirmed that IgE from the same subjects recognized Sh22-6 on a Western blot of recombinant antigen (Figure 2c). Previous blotting studies have indicated that, compared with IgG, only a limited number of antigens are recognized by human IgE in *S. mansoni* and *S. japonicum* extracts, and dominant among these are tegument-associated antigens (5,14,19). It is interesting then, that due to the presence of EF-hand domains, these antigens share structural features with a number of IgE-binding allergens. The EF-hand is a helix-loop-helix domain, present in a large number of proteins. Previously it was shown (14) that a linear IgE epitope comprising the loop of the second EF-hand of Sj22-6 (residues 56–70) was remarkably similar to an IgE epitope in an EF-hand loop in Gad c1, the cod allergen (23). This 14-residue schistosome sequence, which, apart from two conservative substitutions, is identical in Sh22-6 and Sj22-6, also shows marked similarity

to an IgE epitope on an EF-hand loop in the grass pollen allergen Cyn d 7 (24).

The properties of an antigen that are involved in the induction of an IgE response have been the subject of much discussion. Allergenic molecules themselves may have an intrinsic capacity to induce IgE synthesis (25), and similarly it has been shown that recombinant Sj22-6 can, when vaccinated alone, induce specific IgE in mice (26). Virtanen and colleagues (25) have proposed that 'foreign' molecules, such as parasite and allergen proteins that contain sequences homologous to those in 'self' proteins, may specifically evoke a Th2 rather than a Th1 response. Given that EF-hand sequences are present in many host (self) proteins (27), the presence of very similar sequences in schistosome antigens may influence the responses to those antigens towards specific IgE production.

In this report we describe the molecular identification of a cDNA from *S. haematobium* that is highly homologous to the tegumental antigens Sm22-6 and Sj22-6. Our data suggest that, in common with Sm22-6, the *haematobium* antigen can induce an IgE response, but that this only occurs after many years of living in an endemic area. The onset of IgE recognition of molecules, such as the 22-6 kDa antigens, indicates qualitative changes in the IgE response. Evidence from *S. mansoni* infections suggest that this change may be associated with phenomena such as resistance to re-infection, and this merits further study.

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