

Short communication

Molecular identification of a 21.7 kDa *Schistosoma japonicum* antigen as a target of the human IgE response[☆]

Julius Clemence R. Hafalla ^{a,*}, Judith G. Alamares II ^a, Luz P. Acosta ^a,
David W. Dunne ^b, Bernadette L. Ramirez ^a, Mario Luis Santiago ^{a,1}

^a *Schistosomiasis Study Group, Research Institute for Tropical Medicine, Alabang, Muntinlupa, Philippines*

^b *Microbiology and Parasitology Division, Department of Pathology, Cambridge University, Cambridge, UK*

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Vaccine development remains to be an important long-term goal in the control of schistosomiasis. Hence, extensive efforts were directed towards the identification of antigens recognized by protective immune responses in different animal models and in humans [1]. In longitudinal studies in naturally-exposed human populations, elevated IgE titers against soluble worm antigens (SWAP) were shown to correlate with resistance to reinfection following praziquantel cure [2–4]. Hence, the molecular targets of IgE antibodies from resistant individuals may be important candidates for a vaccine. In line with this view, IgE-recognition of an *Schistosoma mansoni* 22.6 kDa tegumental antigen correlated with low levels of reinfection after treatment, suggesting that IgE responses to this antigen may be protective [5].

Initial studies in *Schistosoma japonicum*-endemic populations in the Philippines [6] showed strikingly similar patterns of isotype responses against SWAP and egg antigens (SEA) when compared to *Schistosoma mansoni*- and *Schistosoma haematobium*-endemic populations in Kenya [3,6], Brazil [7], Gambia [2], and Zimbabwe [8]. Similarity in isotype response patterns, as well as trends in age-acquired immunity [2–10], suggest that similar immune mechanisms operate against the three major schistosome species in geographically-

* Corresponding author. Present address: Department of Medical and Molecular Parasitology New York University School of Medicine, 341 East 25th Street, OPH 107, New York, NY 10010, USA. Fax: +1-212-263-8116; e-mail: hafalj01@popmail.med.nyu.edu.

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¹ Present address: Department of Microbiology, LHRB 622, 701 19th Street South, University of Alabama at Birmingham, Birmingham, AL 35294, USA.

isolated human populations. Hence, in an effort to identify potential vaccine candidates against human schistosomiasis japonica, we selected 15 high-IgE/SWAP responders from a survey of 176 individuals in Leyte, the Philippines [6,11]. These individuals were demographically similar to resistant individuals in Kenya, since they were older and had higher IgE titers to SWAP than the rest of the population. These individuals also had lower egg loads, but was not statistically-significant, potentially due to the inaccuracy of single-time point stool examinations in assessing the intensity of *S. japonicum* infections [9,10].

In IgE-blot against SWAP, multiple antigens were recognized by these individuals, including an immunodominant doublet at the 22 kDa range. In an effort to clone these antigens, a λ -ZAP adult *S. japonicum* cDNA library was IgE-screened using pooled antisera, from which 9 cDNA clones were identified [11]. The upper 22 kDa band was determined as Sj22.6 after identifying the corresponding cDNA clone, MJ-1, with rabbit polyclonal antisera against the upper 22 kDa band [11]. This finding was intriguing, since Sj22.6 is the homologue of the resistance marker Sm22.6 [3,5,6]. In this study, we report the molecular nature of MJ-4, one of the cDNA clones identified in the IgE-screen.

Following three rounds of screening with IgE, the MJ-4 cDNA clone was excised as a construct in pBluescript SK+ vector and transformed into XL-1 blue cells (Stratagene, La Jolla, CA). DNA sequencing from the 5' and 3' ends using T3 and T7 primers suggested a complete open reading frame. The full-length sequence was obtained by using TaqFS polymerase and fluorescent dideoxy-terminators then analyzed using an automated Applied Biosystems 377 DNA sequencer.

The complete MJ-4 cDNA clone contained an 85 bp 5' UTR, 558 bp ORF, 314 bp 3' UTR and 17 bp poly-A tract. The ORF potentially encodes a 21.7 kDa protein of 185 amino acids which was highly hydrophilic based on Kyte and Doolittle predictions (data not shown). The deduced amino acid sequence exhibited significant similarity (74%) and identity (64%) to the

21.7 kDa antigen of *S. mansoni* [12]. The encoded MJ-4 ORF also exhibited similarity to other members of the 20–22 kDa family of trematode tegumental antigens which include Sm22.6 [14], Sj22.6 [11,15], Fh22.2 (Machin et al., unpublished) and Fh22 (de Eugino et al., unpublished) from *Fasciola hepatica* and Sm20.8 [13] (Fig. 1A). Phylogenetic analyses using these amino acid sequences showed separate clustering of MJ-4 and Sm21.7 from Sj22.6 and Sm22.6 (data not shown), arguing that MJ-4 is the homologue of Sm21.7. Hence, we subsequently refer to the MJ-4 ORF as Sj21.7. Consistent with this analysis, the level of homology between Sj21.7 and Sm21.7 was similar to that observed between Sm22.6 and Sj22.6 [11]. Like Sj22.6, Sj21.7 was also one amino acid longer than the corresponding *S. mansoni* homologue. Interestingly, the C-terminus of Sj21.7 showed similarity to dynein light chains (DLCs) from lower eukaryotes (Fig. 1B). While the precise mechanism of DLC action is still unknown, they are considered as important components of dynein motor complexes in different organisms, which function in diverse cytoskeletal events such as cytokinesis, vesicular and/or organellar transport and flagellar movement [16].

Motif analyses identified a putative EF-hand calcium-binding domain in Sj21.7 (amino acid (aa) 50–62), making this molecule the second of 4 EF-hand containing proteins identified in the IgE-screen [11] (Alamanes et al., unpublished, Kilayko et al., unpublished). Moreover, we have also identified an Asn-glycosylation site (aa 159–162) and interestingly, 2 myristylation motifs (aa 67–72 and 122–127), suggesting that Sj21.7 may directly interact with the schistosome membrane (Fig. 1A). The potential glycosylation site and the second myristylation motif were not found in Sm21.7. Sj21.7, like Sm21.7 and Sm22.6, lack the invariant glycine in the EF hand motif, suggesting that it may also be incapable of binding calcium. The charge-dense region [15] was not found in this molecule.

The moderate (69%) similarity between Sj21.7 and Sj22.6 suggest that IgE-crossreactivity between these molecules exist. However, this may not be extensive, as observed between Sm22 and

A

Sj21.7	MAST-----MEQFITAYLTITVRDGDDEVDDKELIDYCRKEKLDMMVDTWLSHFDVDKD	54
Sm21.7	MDSP-----MEKFIQTTLTLLRDGDETVEVTSKISESCRKEKLDMMKOVNEWIALFDVDKD	54
Sm22.6	MAT-ETKLSOMEEFIRAFLEIDADSNEIMDKQELIKYCOQYRLDMKLIIDPWIARFDTDKD	59
Sj22.6	MATTEYRLSLMEQFIRAFTEIDKNNELIDKQELTKYCOQNMMDKQIDPWIARFDTDKD	60
Fh22.2	M-TTAYKLHDVESLIDWFMELDKNNDRADVDDKELTAYYKSKGVSDSKINEWMEHFDADSD	59
Sm20.8	M-----EPEVQVEFAIDRDGTETITVEELKKYVAENKLDMMVTWKSLFDPKGT	50
Sj21.7	NRITLEEFCRGLGLKANEMRIERNHIKAVQSGRPKIFDGVETIASTMPLEPROVEVTQLY	114
Sm21.7	OKITFEFCRGLGLKQNEMRIERNHIKTVOGREQSLHEGVSTIASTMPKPKOVEVTOLF	114
Sm22.6	NKISIEEFCRGEGLKVSEIRREKDELKKERDGFEPKLPENIEIIAATMSKTKQYEICCOF	119
Sj22.6	GKVSLEEFCRGEGLKVWEVREKEELKKDKEGKVSTLHLDIQIIAATMSKAKQYNICCKF	120
Fh22.2	GKITLMEFCRGLGLRIDEVRVEQKOPPLORAGTAPALGTDIEMIATMSQPROVEVTEKF	119
Sm20.8	GRITFKTFCDVLGLSPAQAVAMKIQ--HQQASSLKIHDPDVVVIYEQLPLDROVAISNKA	107
Sj21.7	KDLIPKSGGHEADMRKVANDFKIKLEERYERVWQVVLTLTGSYWMNFSHEPLOSIOQFKVDK	174
Sm21.7	KDIY-NEVKKDPDMNKVVKTFKSELERRYGRVWQVNAVTHSYWASF SHEPLOSIOQFOYDN	173
Sm22.6	KEYVDNTRIGNDMRELVANKMSLLDNTYGRVWQVVLTLTGSYWMNFSHEPFLSIOQFKYNN	179
Sj22.6	KELIDKTSRIGDEVRAVANDLKAFLDSEYGRVWQVVIILTGSYWMNFSHEPFLSMQFKYSN	180
Fh22.2	KSLVIAHNGKDEEMKIVAHKLTFLDDTYGRVWQCVILTGSYWMQFSHEPFLSIOQFRYGR	179
Sm20.8	TELLTSSKKFDEKDAQV--QLKQWLDITYGKAWHIVIVKGSFWSYSHSANKCFIERVRD	165
Sj21.7	NIILAWRTP-----NN	Similarity (%) Identity (%) 185
Sm21.7	KIILAWRTP-----SN	74 64 184
Sm22.6	YVCLAWRTP-----SQ	72 53 190
Sj22.6	YVCLWRTP-----SS	69 48 191
Fh22.2	YICLAWRTP-----RG	66 46 190
Sm20.8	VSYLVWRTEDEEITSA	48 33 181

B

117	182
Sj21.7	DLLPKSGGHEADMRKVANDFKIKLEERYERVWQVVLTLT--GSYWMNFSHEPLOSIOQFKVDKNIIILAWRT
AtDLC	DLLLQAFEKYSVEKDIAENIKKEFDKKHGATWHCIVGRNFGSY---VTHETNHFVYFYLDQKAVLLFKS
1	66
110	182
Sj21.7	EVTQLYKDLIPKSGGHEADMRKVANDFKIKLEERYERVWQVVLTLT--GSYWMNFSHEPLOSIOQFKVDKNIIILAWRT
SLC1	DILTISKDALDK---YQLE-RDIAGTVKKQLDVKYGNVHIVGKNFGSY---VTHEKGHFVYFYIGPLAFLVFKT
23	91

Fig. 1. Comparison of Sj21.7 with (A) the 20–22 kDa family of trematode tegumental proteins, and (B) with dynein light chains (DLC). Panel A: Underlined Sj22.6 peptides correspond to previously-identified IgE-binding epitopes [11]. The first epitope corresponds to the EF-hand domain [11]. Triple bars refer to potential myristylation sites, while the arrows correspond to the putative Asn-glycosylation site. Panel B: Like Sm20.8 [13] and Sj22.6 [11], the C-terminal half of Sj21.7 exhibited similarity to DLCs, in this case, from *Arabidopsis thaliana* (AtDLC, 52% with respect to the whole molecule) and yeast (SLC1, 48% in a 74 aa stretch). Bold letters refer to conservative amino acid substitutions, those underlined were identical. Genbank accession numbers: Sm21.7, P32070 [12]; Sm22.6, P14202 [14]; Sj22.6, AF030404 [11]; Fh22.2, AJ003821 (Machin and Parra, unpublished); Sm20.8, U86673 [13]; *A. thaliana* DLC, Q02442; and *S. cerevisiae* DLC, Q02647. Similarity searches utilized different versions of the BLASTP program, as compiled (BCM Search Launcher, Human Genome Center, TX).

Sj22 [11], and as suggested by the fact that Sj21.7 was not recognized by the rabbit anti-Sj22 which identified Sj22.6 [11]. Moreover, only the EF-hand motif epitope [11] in Sj22.6 appears to be conserved in Sj21.7 (Fig. 1A). These data suggest that IgE-recognition of Sj21.7 may be independent of Sj22.6, hence, the pattern of recognition of this antigen at the population level may be different. The molecular size of Sj21.7, however, leads us to suspect that it may correspond to the lower 22 kDa band in the doublet identified in the IgE-blots [11]. Like Sj22.6, this band was also recognized by the majority of high-IgE/SWAP responders (12/15).

Among the current set of trematode tegumental antigens, Sj21.7 is the first non-*S. mansoni* molecule identified aside from the classic 22.6 kDa antigens. Since *S. japonicum* is highly divergent from *S. mansoni* based on phylogenetic analyses [17], its identification argues that a diverse set of such antigens also exist in the other schistosome species. Such evolutionary conservation of a 20–22 kDa tegumental antigen set would suggest that these proteins have independent biological functions, as supported by their developmental expression [12–14]. Given their homology to DLCs [11,13] (Fig. 1B), potential involvement in dynein interactions near the tegument [13], and recent evidence for dynein complex diversity in mammalian systems partially due to DLC heterogeneity [18], it is tempting to speculate that these molecules are components of a heterogeneous set of stage-specific dynein complexes which mediate cytoskeletal rearrangements during transitions to different stages of the schistosome life cycle. Further studies will be needed to test this hypothesis.

In conclusion, we show that a novel *S. japonicum* molecule, Sj21.7, is a target of IgE antibodies from high-IgE/SWAP responders, indicating that it may be an important vaccine candidate against human schistosomiasis japonica. Its identification suggests that a 20–22 kDa tegumental antigen 'set' is evolutionarily conserved among schistosome species. Further studies should yield more insight on the vaccine potential of these antigens and their mechanism of action.

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