

Mario Luis Santiago^a
Julius Clemence R. Hafalla^a
Jonathan D. Kurtis^b
Gemiliano L. Aligui^a
Peter M. Wiest^c
Remigio M. Olveda^a
G. Richard Olds^c
David W. Dunne^d
Bernadette L. Ramirez^a

Identification of the *Schistosoma japonicum* 22.6-kDa Antigen as a Major Target of the Human IgE Response: Similarity of IgE-Binding Epitopes to Allergen Peptides

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

^b International Health Institute,
Brown University, Providence, R. I., and

^c Department of Medicine,
MetroHealth Medical Center,
Case Western University,
Cleveland, Ohio, USA;

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

Abstract

Human resistance to reinfection with *Schistosoma mansoni* and *Schistosoma haematobium* correlates with elevated IgE titers against worm antigens (soluble worm antigen preparation, SWAP). In *S. mansoni* infection, low levels of reinfection following chemotherapy are associated with the recognition of a cloned tegumental protein Sm22.6. Because of potential species-specific differences in resistance to schistosomes, we attempted to identify *Schistosoma japonicum* antigens recognized by human IgE. Following a survey of 176 infected individuals in Leyte, Philippines, we show that IgE antibodies from the majority of older, high-IgE/SWAP responders recognize antigens in the 22 (Sj22)-, 45-, 78- and 97-kDa range in SWAP. Limited IgE cross-reactivity between Sj22 and Sm22 was observed following a comparison of Filipino IgE responses to these antigens. The antigen was cloned from an adult *S. japonicum* λ -ZAP cDNA library (Mindoro strain) by immunoscreening with pooled high-titer IgE antisera and a rabbit anti-Sj22 polyclonal antibody. The deduced amino acid sequence of the identified cDNA clone, MJ-1, showed significant homology to Sm22.6 (74%) and Sj22.6 (99%). Although the molecular sequence of Sj22.6 has already been reported, this is the first demonstration of its recognition by human IgE, thereby strengthening its potential as a vaccine candidate. Using an overlapping peptide approach, four IgE-binding epitopes were identified in Sj22.6, two of which exhibited similarities to known IgE-binding epitopes from codfish (Gad c 1) and β -lactoglobulin-related allergens. These findings suggest that allergy and protective immunity to helminth infection may be linked by the structural similarities of epitopes recognized by human IgE.

Key Words

Schistosoma japonicum
Sj22.6
IgE
Philippines
Vaccine
Allergen

Introduction

Schistosomiasis is a major cause of morbidity and mortality in the developing world, with an estimated 200 million people infected [1]. The disease is caused mainly by

three trematode species of the genus *Schistosoma*: *Schistosoma mansoni* in Africa and South America, *Schistosoma haematobium* in Africa and *Schistosoma japonicum* in Asia. In the Philippines, schistosomiasis japonicum is a major public health problem with an estimated 1 million people

infected and more than 2–10 million at risk [2, 3]. Population-based chemotherapy with praziquantel, the only effective drug against this parasite, resulted in a significant reduction in *S. japonicum*-associated morbidity and mortality in the Philippines and China. However, despite annual treatment, transmission cannot be eliminated [4, 5]. This may reflect, in part, extensive occupational exposure and the presence of nonhuman reservoirs [6]. Ongoing transmission, coupled with the threat of praziquantel resistance in *S. mansoni* [7] indicate that alternative strategies, such as a vaccine, will be necessary to control schistosomiasis.

Previous studies in *S. mansoni*- and *S. haematobium*-endemic populations have shown that resistance to reinfection following parasitologic cure correlates with older age and elevated IgE titers against worm antigens [8–10]. In *S. mansoni* infection, individuals whose IgE antibodies recognized antigen(s) in the 22-kDa range were reinfected less frequently and had lower intensities of reinfection [11]. The molecule responsible for this trend was cloned and shown to correspond to a previously identified tegumental protein Sm22.6 [12]. Based on homology, the corresponding antigen in *S. japonicum*, Sj22.6, was also cloned [13], but it is still unclear whether the human IgE response to this molecule parallels that observed for Sm22.6.

In a recent study of individuals chronically infected with *S. japonicum* in the Philippines [14], we have shown that elevated IgE titers against worm antigens are positively correlated with age, similar to earlier reports in the other human schistosomiasis. This observation, together with our long-term studies which support the development of age-acquired immunity [4, 15], suggested that IgE antibodies may also play a significant role in immunity to human schistosomiasis japonicum. Interestingly, antigen(s) in the 22-kDa range also appear to be the targets of this response [14].

Based on these observations, we have undertaken an independent approach for identifying candidate *S. japonicum* vaccine antigens. This involved the identification of 'high-IgE/soluble worm antigen preparation (SWAP) responders', whose IgE antibodies were used as probes for screening a cDNA expression library. Using this strategy, we identified several antigens and cDNA clones recognized by human IgE. Moreover, we confirm that the majority of high-IgE/SWAP responders recognized antigen(s) in the 22-kDa range (Sj22), demonstrate limited IgE cross-reactivity between Sj22 and Sm22 and show for the first time that the antigen recognized by IgE in Sj22 is Sj22.6. Finally, we demonstrate the presence of four linear IgE-binding epitopes in this molecule, two of which exhibited similarity to known allergen epitopes.

Materials and Methods

Antigen Preparation

Adult *S. japonicum* worms (Mindoro Island, Philippines) were obtained by perfusion of the hepatic portal system of experimentally infected rabbits [16]. SWAP was prepared by grinding worms in liquid nitrogen and homogenization in buffer with Triton X-100 [17]. Adult worm tegumental (AWT) antigen was prepared by recovering teguments following a 37°C incubation of whole worms in CaCl₂ and MgCl₂ [18]. Denuded worm antigen (DWA) was prepared by first incubating freshly isolated worms in PBS supplemented with 1 mM N-tosyl-L-phenyl alaninechloromethylketone (TPCK), 1 mM phenylmethylsulfonylfluoride (PMSF) and 1% Non-Idet P-40 (NP-40) for 30 min at room temperature to remove the tegument. The denuded worms were removed, frozen in liquid nitrogen then pulverized in a mortar and pestle. The extract was suspended in 1% NP-40, then centrifuged at 10,000 *g* for 1 h at 4°C. Protein concentrations were determined using bovine serum albumin as standard [19]. Sj22 and Sm22 were eluted from polyacrylamide gels as described [14].

Human Antisera

Sera from 176 individuals infected with *S. japonicum* living in two villages in Leyte, the Philippines (Caninoan and Lanauan) were obtained as part of a comparative study of sex/antibody isotype responses between Kenya and the Philippines [14]. Isotype-specific ELISAs against SWAP, Sj22 and Sm22 eluted from polyacrylamide gels were performed as described [8, 14].

Immunoblot Analysis

SWAP (30 µg) was separated in 10% polyacrylamide gels by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose, then probed for IgE recognition [8, 11]. Briefly, strips were incubated with blocking solution of 5% skimmed milk in Tris-buffered saline with Tween-20 for 2 h then incubated with sera from infected individuals diluted 1:20 in blocking solution for 24 h in a flat rotary shaker at 4°C. The strips were washed, then incubated with 1:1,000 goat antihuman IgE conjugated to alkaline phosphatase (Sigma, St. Louis, Mo.) for 22 h. Color development utilized the 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium substrate system.

Polyclonal Antibody Production

Polyclonal antibodies were prepared by immunizing a New Zealand rabbit with unstained nitrocellulose-immobilized antigen recognized by pooled high-titer IgE antisera in AWT blots (Sj22). Nitrocellulose containing Sj22 was excised from 8 strips, shredded in 0.5 ml phosphate-buffered saline, emulsified in 0.5 ml complete Freund's adjuvant and injected subcutaneously in approximately 2–4 sites. On day 21, the rabbit was boosted with the same antigen emulsified in incomplete Freund's adjuvant. Mouse anti-Sj22 was also prepared by immunizing Balb/c mice with the same antigen preparation. Polyclonal antibody specificity was monitored through immunoblot analysis. Prior to immunoscreening, rabbit anti-Sj22 polyclonal antisera were preadsorbed in filters containing *Escherichia coli* lysate [20].

cDNA Library Screening

Approximately 5 × 10⁵ clones from an adult *S. japonicum* (Mindoro strain) λZAPII cDNA library [17] were screened using pooled high-titer IgE antisera as described in the IgE blots. The purity of cDNA clones cored from the tertiary screen was confirmed by the poly-

merase chain reaction [21] using M13 forward and reverse primers. Clones recognized by human IgE were subscreened with rabbit polyclonal anti-Sj22 antisera. Dually reactive cDNA clones were excised and the constructs sequenced with Sequenase Version 2.0 Kit (USB, Arlington Heights, Ill.). The full-length gene was obtained using 5'-RACE (Boehringer Mannheim, Indianapolis, Ind.). Sequences were analyzed using the BLASTP (NCBI, NIH, Bethesda, Md.) and MOTIF program using the PROSITE network server at EMBL. Nucleotide sequences were submitted to Genbank with Accession No. AF030404.

Expression Lysates

cDNA inserts in pBluescript plasmids were expressed as fusion proteins with a 31-aa fragment of β -galactosidase following transformation into XL-1 Blue MRF'. Transformed cells were grown in LB broth with 100 μ g/ml ampicillin, 12.5 μ g/ml tetracycline and 50 mM glucose to an OD₆₀₀ of 0.5, induced for 60 min with 0.5 mM isopropyl- β -thio-galactopyranoside, centrifuged at 3,000 *g* for 15 min, then resuspended in 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 10 mM β -mercaptoethanol, 50 mM NaCl, 1 mM iodoacetic acid, 1 mM PMSF, 0.1% SDS and 0.1% NP-40. Samples were freeze/thawed 3 times, sonicated for 30 s at 90% duty cycle and stored at -70°C. IgE blots of expressed lysates were performed against high-IgE/SWAP responder antisera and the rabbit and mouse anti-Sj22 antisera to confirm the immunoscreen reactivities.

Epitope Mapping

Based on the deduced amino acid sequence of the MJ-1 cDNA clone, 15-amino-acid peptides (overlapping at 7–8 residues) were synthesized using standard Fmoc chemistry, then covalently bound to a hydrophilic membrane (Peptides on Paper, Research Genetics, Huntsville, Ala.). The peptides were probed in duplicate with pooled high-titer IgE antisera using conditions specified in the IgE-specific immunoblots. Similarity searches were performed with each IgE-binding epitope using the BLASTP software with BEAUTY post-processing against >300,000 entries in the Swissprot, PDB, nrdb and CRSeqAnnot databases (Human Genome Center, Baylor College of Medicine, Tex.).

Statistical Analysis

Covariance analysis (STAT A software) was utilized to compare the age, high-IgE/SWAP responder status and IgE titers to SWAP, Sj22 and Sm22. Titers were transformed to log values to fit statistical assumptions for linear regression. Results with $p < 0.05$ were considered significant.

Results

SWAP/IgE Profiles of S. japonicum-Infected High-IgE/SWAP Responders

The study population included 176 individuals who had a positive egg count in duplicate stool examinations. IgE titers against SWAP among these individuals were ranked, and values above the 85th percentile ($n = 25$) were identified for the subsequent studies. However, sera from 10 individuals were already exhausted, hence only the remaining

15 individuals were considered as high-IgE/SWAP responders. High-IgE/SWAP responders ($n = 15$) were older than the rest of the population ($n = 176$, $p < 0.05$).

To identify common antigens recognized by these fifteen high-IgE/SWAP responders, individual antisera were used in an IgE-specific immunoblot with SWAP. Results confirmed the IgE recognition of antigen(s) in the 22-kDa range (Sj22) by the majority of the high-IgE/SWAP responders (13/15) [14]. However, in this particular SWAP preparation the band appeared as a doublet (fig. 1). Other antigens of higher molecular weight were also observed, in contrast to a previously reported blot [14]. Sj22-reactive antisera from 13 high-IgE/SWAP responders were pooled for the subsequent experiments.

In IgE blots with different parasite preparations, the pooled antisera recognized four distinct bands in DWA corresponding to antigens of 22, 45, 78 and 97 kDa (fig. 2, lane C). The 78- and 97-kDa antigens appear to be degraded in SWAP, while only the 22-kDa band was recognized in AWT preparation (fig. 2, lanes D and B, respectively). The lower 22-kDa band was not observed in AWT or DWA, suggesting that this may also be a degradation product. Alternatively, the band may correspond to a different antigen of similar molecular weight.

Filipino IgE responses to native Sj22 and Sm22 were subsequently compared (fig. 3). In our previous report [14], most individuals do not respond to Sj22, however, individuals who had IgE/SWAP titers above an arbitrary cutoff value ($A_{260} = 0.4$) were more likely to have higher IgE titers to Sj22. Confirming this trend, the high-IgE/SWAP responders identified for this study also had higher IgE responses to Sj22 than the rest of the population ($F_{4,154} = 19.68$, $n = 159$, $p < 0.001$). Interestingly, these individuals also had higher IgE responses to Sm22, but to a lesser extent ($F_{4,136} = 2.56$, $n = 141$, $p < 0.01$). A positive correlation between IgE titers to Sj22 and Sm22 was also observed ($\rho = 0.287$, $n = 136$, $p < 0.001$), suggesting IgE cross-reactivity between the molecules they contain. However, the threshold appears to be more pronounced for Sj22 than for Sm22, since IgE-Sm22 responses of high-IgE/SWAP responders are more disperse and difficult to segregate from IgE responses of the rest of the population (fig. 3). While some high-IgE/SWAP responders have high IgE titers to both Sj22 and Sm22 (patients 3 and 8), some (patients 2, 5 and 6) had lower IgE/Sm22 titers. These data suggest that the observed cross-reactivity may be limited to conserved epitopes.

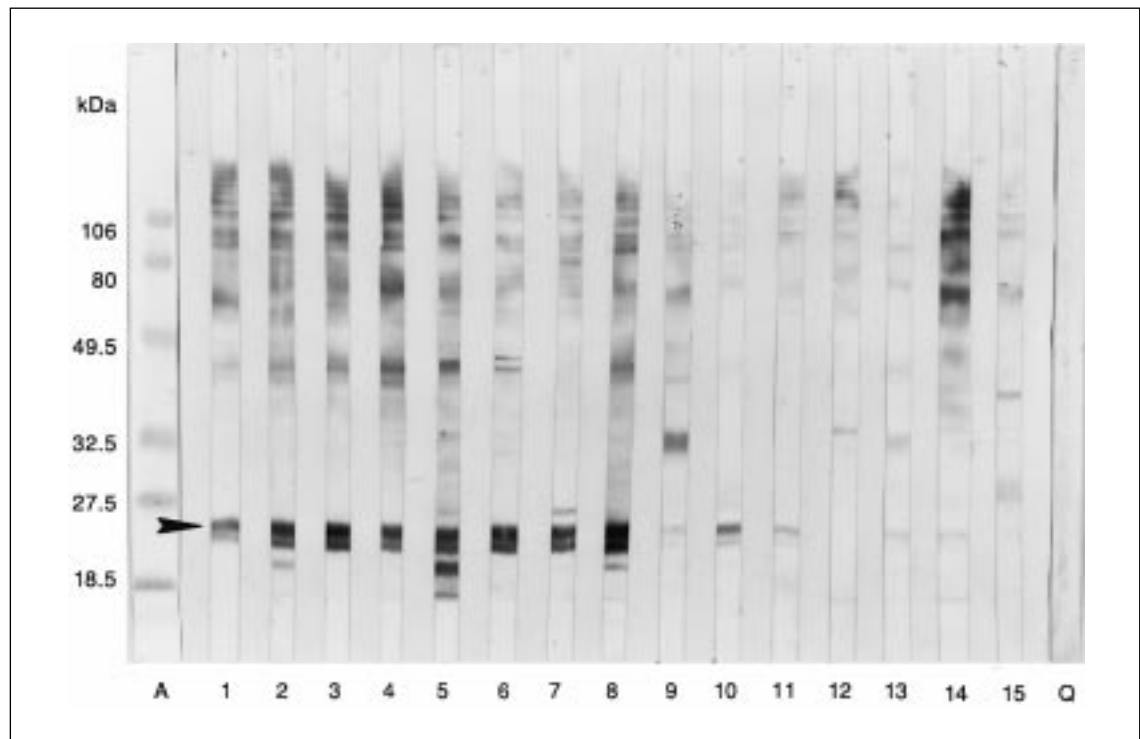


Fig. 1. IgE-specific immunoblot of fifteen high-IgE/SWAP responders against SWAP. High-IgE/SWAP responders were selected from a cohort of 176 individuals [14] who had IgE titers to SWAP above the 85th percentile. IgE antibodies from the majority of individuals also recognized a 22-kDa band, shown here as a doublet (arrow). High-MW antigens of 45, 78 and 85 kDa, however, were also observed. All patients, except patients 12 and 15, recognized the 22-kDa band. Numbers correspond to the patient code. Lane Q represents normal human sera.

Identification of cDNA Clones Recognized by Human IgE

The pooled antisera from the high-IgE/SWAP responders identified thirty cDNA clones in the initial screen. After two subsequent screens, nine clones, designated MJ-1 to MJ-9, were consistently recognized by the antisera. When these nine clones were subscreened with the rabbit anti-Sj22 antisera, a single cDNA clone, MJ-1, was identified.

To confirm that the MJ-1 gene product corresponds to the Sj22 antigen identified in the IgE blots, expression lysates from this clone (pBluescript/MJ-1 construct) and a control (pBluescript only) were probed using the rabbit and mouse anti-Sj22 polyclonal antisera and a high-titer IgE human antisera (patient 1). A band of approximately 28 kDa was uniquely observed by all three antibody probes in the MJ-1 expression lysate (fig. 4, lanes R1, E1, M1) but not in the pBluescript lysate (lanes R2, E2, M2). These data suggest that the MJ-1 gene product (which is fused with the 4-kDa fragment of β -galactosidase) corresponds to the native 22-kDa antigen recognized by the high-IgE/SWAP responders in figure 1 and recombinant Sj22.6 expressed the relevant IgE-binding epitopes.

Molecular Sequence of the MJ-1 cDNA Clone

The identified clone, MJ-1, contained a 546-bp ORF beginning with the first nucleotide of the cDNA. Based on homology to Sm22.6, MJ-1 was missing approximately 27 nucleotides at the 5' coding region [22]. The complete coding sequence was determined by 5'-RACE, the full-length gene having a 41-bp 5' UTR region, followed by a 576-bp ORF, a 197-bp 3' UTR and a 17-bp poly-A tail. The complete nucleotide sequence exhibited significant identity to Sm22.6 (79.7%), and almost complete identity to similarly cloned Sj22.6 antigens (98.8%) from China and Sorsogon province, Philippines [13, 23]. Differences between Sj22.6 sequences from the two Philippine isolates include five silent substitutions and a conservative nonsilent substitution at codon 88 (AGA $\rightarrow$ AAA leading to an R $\rightarrow$ K change).

The deduced amino acid sequence showed significant identity to several schistosome tegumental antigens which contain an EF-hand motif (aa 56–68), including Sm21.7 [24], Sm20.8 [25] and Sm16 [26]. Similar to earlier reports [12, 22], Sj22.6 also exhibited similarity to calmodulins, myosin regulatory light chains and other calcium-binding

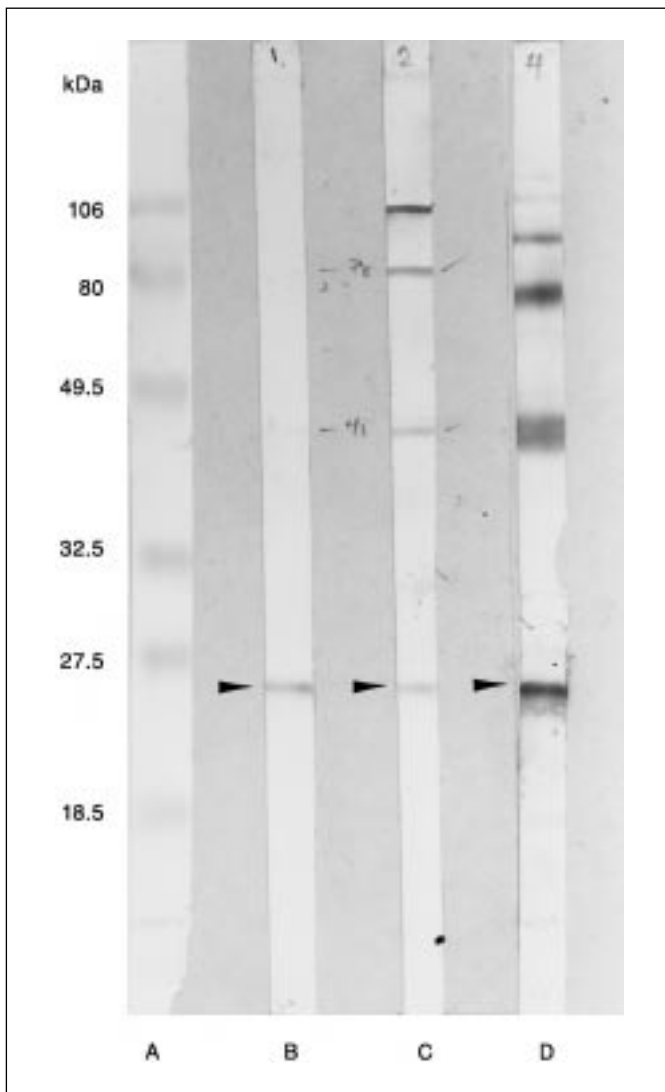


Fig. 2. IgE blot of pooled antisera against various worm preparations. A = MW marker; B = AWT; C = DWA; D = SWAP. Pooled antisera from high-IgE/SWAP responders except patients 12 and 15 were utilized. Immunodominant bands in DWA correspond to antigens of 22, 45, 78 and 97 kDa, but only the 22-kDa band was present in AWT (arrows). The 78- and 97-kDa bands appear to be degraded in SWAP.

proteins. Interestingly, the N-terminal half of the molecule also exhibited similarity to the C-terminal half of parvalbumins, with 57.3% homology in a 68-aa stretch with respect to the Baltic cod allergen Gad c 1 (fig. 5A). Although the regions of similarity were most significant in the parvalbumin EF helix-loop-helix, some upstream residues were also conserved, particularly with respect to the CD helix-loop-helix domain. The N-terminal half of Sj22.6 also exhibited sig-

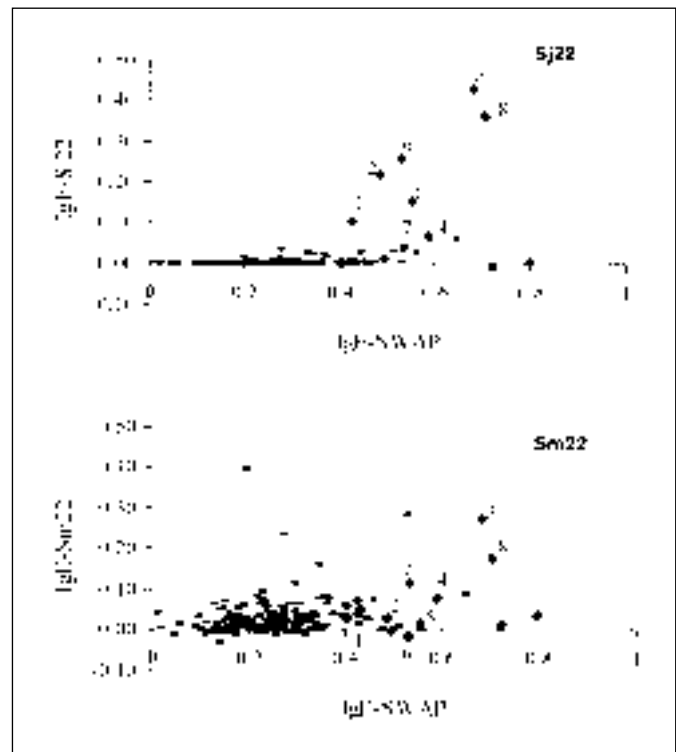


Fig. 3. IgE cross reactivity between Sj22 and Sm22. IgE responses of 136 *S. japonicum*-infected Filipinos against native Sj22 and Sm22 eluted from polyacrylamide gels were compared in an IgE ELISA. Diamonds correspond to codes of high-IgE/SWAP responders, with numbers corresponding to the patient code specified in figure 1. Only those individuals with the most intense anti-Sj22 patterns in IgE blots (patients 1–8) were labeled. Dashed vertical lines refer to an arbitrary cutoff IgE titer ($A_{260 \text{ nm}} = \text{SWAP}$) [14].

nificant similarity to plant calcium-dependent protein kinases and α -lactalbumin (data not shown). The C-terminal half, on the other hand, exhibited similarity to plant phosphoproteins and a dynein light chain from yeast with 47% homology in a 68-aa stretch (fig. 5A).

Mapping of Sj22.6 Epitopes Recognized by Human IgE

In order to further define the binding of human IgE to Sj22.6 (Philippine strain), overlapping 15-aa peptides (peptides 1–23) were synthesized from the deduced amino acid sequence of MJ-1. Four linear antigenic determinants were recognized by the pooled high-titer IgE antisera (fig. 5B, 6). These include peptide 7 (epitope 1): aa 56–70 (DTDKDGK-VSLEEFGR), peptide 11 (epitope 2): aa 85–99 (LKKDK-EGKVSTLPLD), peptide 14 (epitope 3): aa 108–122 (MSK-AKQYNICCKFKE) and peptide 23 (epitope 4): aa 175–189 (FKYSNYVCLLWRTPS). In all cases, faint signals were

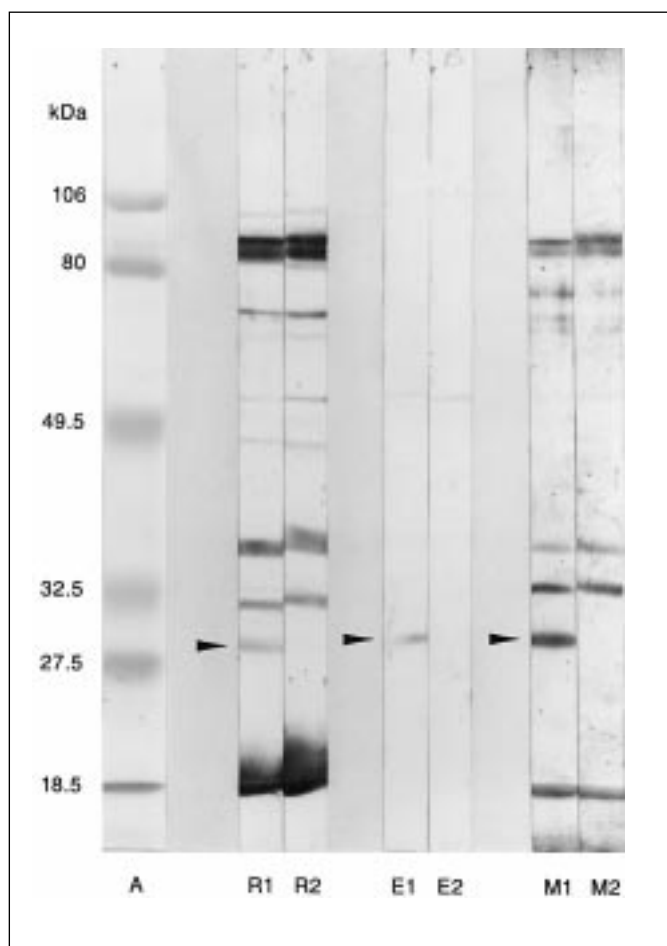


Fig. 4. Recognition of the bacterial expression lysates of the cDNA clone MJ-1 by three different antibody probes. R = Rabbit polyclonal anti-Sj22 antibody; E = IgE antibodies from a high-IgE responder (patient 1), M = mouse polyclonal anti-Sj22 antibody. These antibodies were used to probe two sets of expression lysates of XL-1 Blue bacteria: bacteria transformed with the MJ-1 cDNA clone (1) and bacterial lysate with pBluescript only (2). Several bacterial antigens reacted to the antisera in both preparations, but a 22-kDa band was uniquely identified in expression lysates with the MJ-1 construct as shown by the arrows.

also observed in adjacent peptides (fig. 6). Hence, the minimal epitopes are likely to be present in these underlined residues. When compared to Sm22.6, the four epitopes had several substitutions (fig. 5B), ranging from two substitutions (epitope 4), three substitutions (epitopes 1 and 3) to eight substitutions (epitope 2).

Discussion

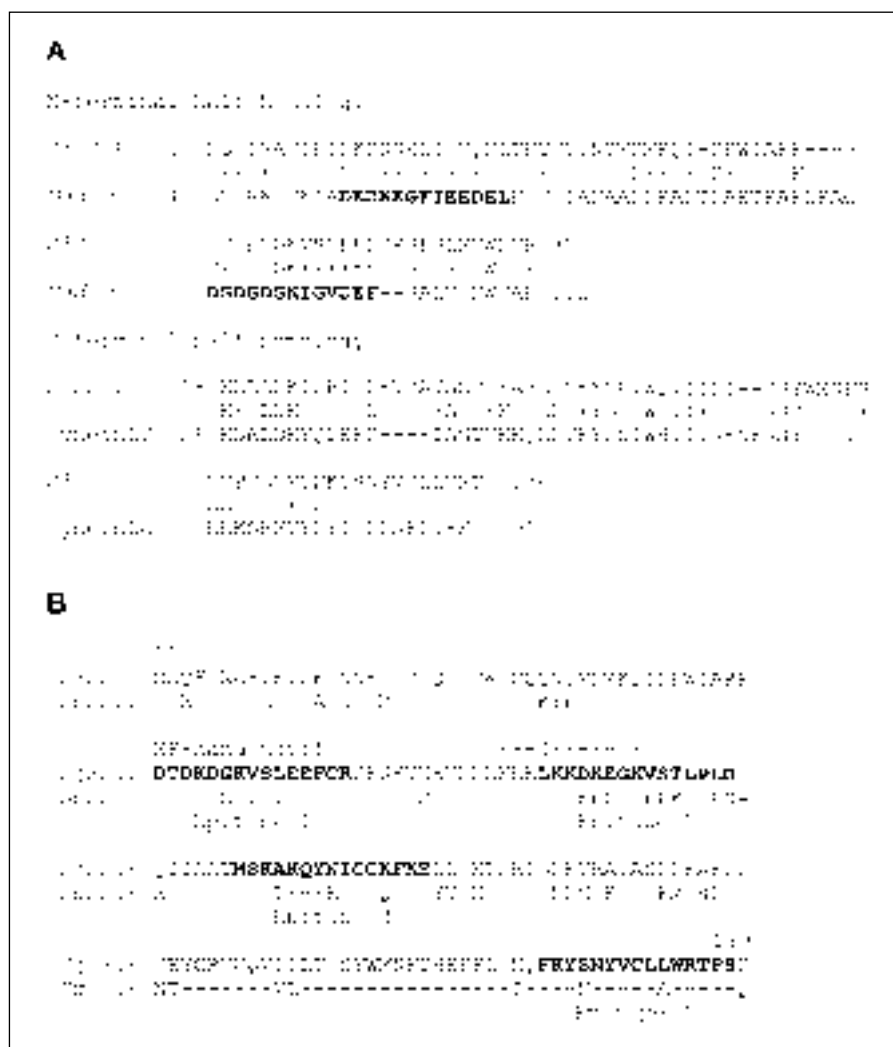
For many years, vaccine development approaches against human *S. japonicum* infection relied on testing *S. japonicum* homologues of *S. mansoni* vaccine candidates in the mouse model [16, 27, 28]. This approach has the advantage of rapid testing of antigens, however (1) some candidate vaccine antigens may be species-specific [17, 29] and (2) the concordance between the murine and human immune response against candidate schistosome vaccines is still unclear [30, 31].

Recent findings on the potential role of human IgE in protective immunity therefore provide an alternative strategy for identifying *S. japonicum* vaccine candidates. In this study, IgE antibodies from older individuals with high IgE titers (high-IgE/SWAP responders) were utilized as probes to screen an adult *S. japonicum* expression library. The IgE-screening approach is widely used in the molecular cloning of allergens, but, to our knowledge, has not yet been used in the identification of potential antihelminth vaccines. Using this approach, nine unique cDNA clones were identified, but only one matched the immunodominant 22-kDa band observed in IgE blots. The antigen was homologous to the 22.6-kDa tegumental antigen in *S. mansoni*, also reported as a major target of the human IgE response for this infection [12].

Sj22.6 was already cloned twice from two strains of *S. japonicum* but was considered as a vaccine candidate mainly because of its homology to Sm22.6 [13, 23]. A 22-kDa antigen(s) was also identified as a target of human IgE in our recent study in the Philippines [14], but the antigen responsible cannot directly be construed as Sj22.6, since there are many antigens which comigrate in this kilodalton range [11, 12]. Based on human studies in Sm22.6, recombinant Sj22.6 has also been shown to elicit IgE antibodies in mice [32], but this animal model may have several important limitations in studying IgE-mediated immunity to parasites [33]. Confirmation that the Sj22 antigen recognized by human IgE is the Sj22.6 molecule is therefore an important gap in considering the latter as a vaccine candidate against *S. japonicum* infection. This study provides the first direct evidence that the immunodominant 22-kDa antigen (Sj22) recognized by IgE is the Sj22.6 molecule.

The positive correlation between Filipino IgE responses to Sj22 and Sm22 suggests that both molecules share similar IgE-binding epitopes. In an epitope mapping experiment, we identified four linear IgE-binding epitopes in Sj22.6, three of which had minor substitutions with respect to Sm22.6. These relatively conserved epitopes may therefore be responsible for the observed IgE cross-reactivity be-

Fig. 5. Molecular comparisons with the Sj22.6 amino acid sequence. **A** Similarity of the N-terminal half of Sj22.6 to the Gad c 1 allergen [35]. Residues in boldface correspond to the upstream CD and downstream EF helix-loop-helix domains of parvalbumin beta. Homology with the C-terminal half to a 92-aa yeast dynein light chain is also shown (PID g2494224). Alignment was done using the Lipman-Pearson algorithm. **B** Epitope map of Sj22.6. Amino acid residues in boldface-type correspond to IgE-binding epitopes identified using the overlapping peptide method. The first nine N-terminal amino acids were not included since these were not in the MJ-1 cDNA clone identified in the IgE immunoscreen. The epitopes vary with respect to Sm22.6 from 2 substitutions (Epitope 4) to 8 substitutions (epitope 2).



tween Sj22 and Sm22. The cross-reactivity is not universal, however, since some patient antisera who had high IgE titers to Sj22 had relatively lower IgE titers to Sm22. This suggests that amino acid differences in the linear epitopes (e.g., epitope 2) may be significant, or that Sj22.6-specific discontinuous epitopes exist. Given the inherent limitation of the approach to map only linear epitopes, a quantitative assessment of the relative contributions of each IgE epitope in protective immunity is merited. We have reason to believe, however, that the EF-hand motif may be a major cross-reactive epitope (see below).

Epitopes 1 and 2, the putative calcium-binding domain and the charge-dense region [13], were recognized, in agreement with a recent Sj22.6 epitope map based on mouse IgG antibodies [34]. However, the other two IgE epitopes we identified (epitopes 3 and 4) were not recognized

by any of the mouse strains immunized with recombinant Sj22.6. These epitopes resided in the C-terminal half, predicted to have low antigenicity in hydrophobicity and secondary structure analyses [34]. The rationale for this discrepancy between IgE and IgG epitopes is not understood. However, it is interesting to note that being homologous to dynein light chain, the C-terminal half may be critical for Sj22.6 activity. Similarly, a conserved glycine residue in epitope 1 suggests that it may functionally bind calcium, in contrast to a similar region in Sm21.7 [24]. Hence, IgE antibodies may have been generated to bind to these critical domains to disrupt Sj22.6 activity. Based on studies on Sm20.8 [25] and its homology to calmodulin, parvalbumin and myosin and dynein regulatory light chains, Sj22.6 may play an important role in the regulation of muscle relaxation near the tegument through cytoskeletal interactions.

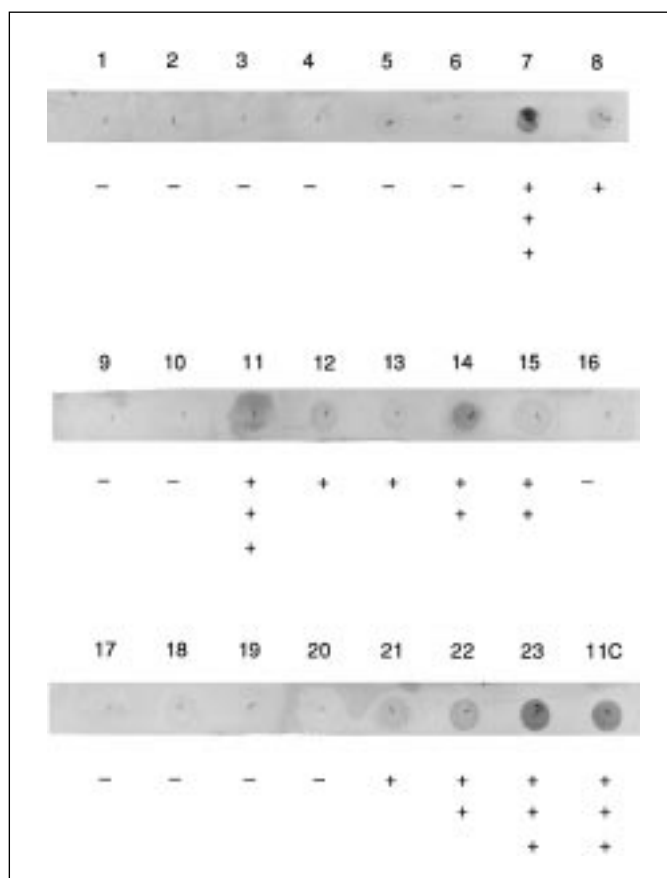


Fig. 6. Determination of IgE-binding epitopes. Dots 1–23 (peptides 1–23) show positions of specific 15-aa peptides covalently bound to a hydrophilic membrane and overlapping at 7–8 residues as deduced from the MJ-1 cDNA clone. Pooled high-titer IgE antisera were used as primary antibody. Peptide 11C correspond to the alternate form of peptide 11 in Sj22.6 (Chinese strain, R→K). Immunoreactive peptides were marked by a +++ sign. Weakly reacting adjacent peptides were marked by either a ++ or + sign. Based on this analysis, four IgE-binding epitopes in Sj22.6 were identified: peptides 7, 11, 14 and 23. These were subsequently referred to as epitopes 1–4, respectively.

An unexpected finding in this study is the similarity of two of these epitopes to known IgE-binding epitopes from allergenic proteins (table 1, epitopes 1 and 4). Epitope 1 was similar to the IgE-binding epitope Gad c 1 (aa 88–103) in the EF helix-loop-helix region from a major fish allergen, Allergen M (parvalbumin), in the Baltic cod *Gadus callarias* [35]. Interestingly, this epitope functioned as a monovalent hapten which blocked rather than elicited an allergic reaction [35]. Epitope 1 also exhibited similarity to the second calcium-binding domain of the rape allergen, Bra n 1 [36].

A recurring theme in continuing efforts for allergen identification is that proteins with EF-hands may constitute a major group of cross-reactive plat allergens [37], exemplified by allergens from Bermuda grass (Cyn d 7), birch pollen (Bet v 3 and Bet v 4), weeds (Bra r 2 and Bra n 1) and trees (*Olea*). Moreover, parvalbumins from different fish species were recently shown to exist as cross-reactive allergens [38]. It is therefore intriguing when expressed sequence tag analysis of the remaining nine cDNA clones we have identified yielded four as putative calcium-binding proteins. These recent findings suggest that the EF-hand motif may be a major cross-reactive IgE-binding epitope, contributing significantly to the protective effect of anti-schistosome IgE antibodies in humans. It would therefore be important to determine the extent of IgE cross-reactivity between these EF-hand-containing molecules, and if significant, to assess whether IgE antibodies generated against these schistosome antigens can elicit hypersensitivity and vice versa, and whether IgE antibodies directed against these calcium-binding allergens will have a protective effect against schistosome infection.

Epitope 4, on the other hand, exhibited similarity to a segment of lobster crustacyanin Cl, a member of the β -lactoglobulin superfamily [39], which includes allergens such as cow's milk, a cockroach calycin, and rat and mouse urinary proteins [40]. In molecular modeling studies, these low-similarity antigens were shown to harbor three structurally conserved regions (SCRs) which upon alignment contained several conserved amino acids [40]. Sj22.6/epitope 4 shared the Y-x-[N]-Y motif in SCR2, identified as the major allergenic epitope in cow's milk [41]. We did not identify allergenic epitopes similar to epitopes 2 and 3; however, top-scoring peptides in the search corresponded to proteins from organisms known to cause allergy such as yeast and castor bean (table 1). The allergenic potential of these peptides remains to be determined.

In summary, we have shown that Sj22.6 is a major antigen recognized by high-IgE/SWAP responders in a relatively well-characterized population of individuals chronically infected with *S. japonicum*. This finding is analogous to that reported for *S. mansoni*, despite the limited cross-reactivity between Sm22 and Sj22 against antisera from *S. japonicum*-infected individuals. This argues that similar IgE-mediated protective immune responses may be operating against both parasites, and strengthens the potential of Sj22.6 as a vaccine candidate against human schistosomiasis japonicum.

Finally, the results of our peptide similarity studies provide additional insights into the evolution of human IgE. It has been postulated that allergies result from a homeostatic perturbation due to the loss of a protective function for IgE

Table 1. Sj22.6 IgE epitope similarities.

	Peptide sequence	Homology	PID No.
Sj22.6 epitope 1	<u>DTDKDGKVSLEEFGR</u>		
Cow neurocalcin	DTNRDGLSLEEFIR	14/15	266611
Rat/mouse calcineurin	DKDGDGRISFEFC	11/14	286206
Rice calcium-Dept kinase	DRDNDGRINYEEFC	10/14	589498
Baltic cod parvalbumin	DSDGDGKIGVDEF	11/13	131112
Bra n I allergen	DTDGDGNISFQEF	9/13	2129799
Sj22.6 epitope 2	<u>LKKDKEGKVSTLPLD</u>		
Yeast Chr Seg' gn protein	LKKQRAGTASFIPLD	10/15	417778
Maize H+ ATPase	LEQNKDESIASLPID	13/15	533775
Sj22.5 epitope 3	<u>MSKAKQYNICCKFKE</u>		
Castor bean desaturase	SKSYNLCSSFK	08/11	414732
Yeast TTP1 protein	STAKQYNEACLLK	08/13	586137
Cod somatolactin precursor	RDYNVLCCKFKK	08/11	482585
Sj22.6 epitope 4	<u>FKYSNYVCLLWRTPS</u>		
Lobster crustacyanin A1	TDYSNYACL	06/07	117423
<i>B. germanica</i> calycin	TDYENYAIV		
Mouse urinary protein	TDYDNFLMA		
Rat α_{2u} -globulin	TDYDRYVMF		
Cow β -lactoglobulin	TDYKKYLLF		

Shown are peptides from specific proteins in the list of top-scoring peptides obtained (BCM Search Launcher, Tex.) in similarity searches. Not all similarities were shown. Fractional values correspond to similarity values which took into account conservative amino acid substitutions. PID numbers were specified for the peptide matches shown. For the epitope 4/crustacyanin similarity, SCR2 sequences of related allergens were also listed [40].

against helminth infection [42]. In this study, we show that specific domains recognized by human IgE in Sj22.6 are similar to IgE-binding epitopes from known allergens. It is therefore tempting to speculate that IgE responses may have evolved to counteract the activity of certain helminth proteins, such as tegument-associated calcium-binding proteins, by binding to critical molecular domains. Effective eradication of helminth infection, however, may have diverted this response to structurally similar epitopes in food and environmental proteins. Based on this hypothesis, it will be important to determine the extent of IgE cross-reactivity between EF-hand-containing schistosome vaccine candidates such as Sj22.6 and allergens such as parvalbumins and certain pollen proteins. These considerations may eventually lead to a deeper understanding of the specific antigen-induced mechanisms which determine the protective and pathological consequences of IgE in the human host.

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