

Molecular cloning and expression of a *Schistosoma japonicum* tegumental membrane-associated antigen from Japanese strain [☆]

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Abstract

Diagnosis and vaccine development form the major focus in creating strategies for the control of schistosomiasis. In this study, we established an IgG1 mouse monoclonal antibody (MoAb), SJA111, which strongly reacted with 23–25-kDa *Schistosoma japonicum* tegumental-associated membrane proteins, but not with eight other parasitic antigens. A λ gt 11 cDNA library from the Japanese strain of the *Schistosoma japonicum* adult worm was screened with SJA111 as a probe. A single positive clone was isolated and the nucleotide sequence of the isolated cDNA was determined. The cDNA clone consisted of 844 bp, and the coding region contained 576 bp which was translated to a 22.6-kDa protein. This region showed 99.0% and 99.3% significant homology with those of the Chinese and Philippine strains of *Schistosoma japonicum*, respectively. The deduced amino acid sequence of the protein was identical to that of the Philippine strain and only one residue differed from that of the Chinese strain. The recombinant form of the tegumental protein was expressed in *Escherichia coli* and purified by a combination of ion exchange and affinity chromatography, and the purified protein was found to react with the sera of patients infected with *Schistosoma japonicum*. This result suggests that this antigen may be useful in the immunodiagnosis of schistosomiasis as well as in the development of an effective vaccine. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: *Schistosoma japonicum*; Monoclonal antibody; 22.6 kDa tegumental membrane-associated antigen; Nucleotide sequence; Bacterial expression

Abbreviations: ELISA, enzyme-linked immunosorbent assay; MoAb, monoclonal antibody; ORF, open reading frame; PBS, phosphate-buffered saline; SJWA, adult worm antigen of *S. japonicum*; SEA, soluble egg antigen of *S. japonicum*

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[☆] Nucleotide sequence data reported in this paper are available in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number AB006459.

1. Introduction

Schistosomiasis still remains a major health problem in tropical and subtropical areas of Asia, Africa, and Latin America. It is estimated that more than 200 million people worldwide have been infected with various species of schistosomes. There are three major types infecting humans. One is *Schistosoma japonicum* (*S. japonicum*) which causes a grave, chronic form of intestinal and liver schistosomiasis, which is mainly endemic in China and the Philippines. The second is *Schistosoma mansoni* (*S. mansoni*) which is responsible for intestinal schistosomiasis in Africa, parts of Latin America, and the Caribbean. The third type is *Schistosoma haematobium* which causes urinary schistosomiasis in Africa and the Middle East [1]. To date, the major strategies for control of infection due to schistosomiasis are centered on improving the diagnosis of this disease and establishing community-based chemotherapy with praziquantel.

Improvements in diagnosis are vital if more effective control programs are to be developed. In recent decades, scientists have made great efforts in this area and have established highly sensitive and specific methods for immunodiagnosis. However, most of these are not suitable for field use on a large scale, especially in developing countries. The development of an effective vaccine against schistosomiasis is recognized as a necessary adjunct to reducing the morbidity and the risk of reinfection after drug treatment. There is growing evidence of an acquired immunity to this infection in humans and animal models, which supports the hypothesis that at least partial protection can be achieved by artificial means [2,3]. Advances in molecular biology have made it possible to synthesize, identify, and characterize several recombinant proteins of schistosomes, such as glutathione S-transferase [4–7], fatty acid-binding protein (Sm14) [8,9], and paramyosin [10,11], all of which have been shown to induce statistically significant levels of protection against *S. japonicum* or *S. mansoni* in animal models.

In an investigation, sequences from both nu-

clear and mitochondrial genomes have been selected as markers of evolutionary divergence, random amplified polymorphic DNA analysis of the entire genome showed that sequences of the Chinese and Philippine strains are remarkably conserved at the DNA level [12]. It appears that much of the genome, including many genes of putative vaccine candidates, is almost the same in Chinese and Philippine strains.

We report here the establishment of a monoclonal antibody (MoAb) which specifically recognized tegumental antigens of the Japanese strain of *S. japonicum*, as well as the molecular cloning of an antigen recognized by the established MoAb.

2. Materials and methods

2.1. Animals

BALB/c mice (7 weeks) were purchased from Japan SLC Inc. (Hamamatsu, Japan) and were maintained in the animal center of our university under 12 h light:12 h dark conditions.

2.2. Preparations of MoAb against tegumental antigens of *S. japonicum*

Parasite antigens: adult worm antigens of the Yamanashi strain (Japan) of *S. japonicum* (SJWA), soluble egg antigen of *S. japonicum* (SEA), *Paragonimus skrjabini* (Ps), *Clonorchis sinensis* (Cs), *Fasciola gigantica* (Fg), *Taenia solium* (Ts), *Diphyllbothrium latum* (Dl), *Ascaris suum* (As), *Dirofilaria immitis* (Di) and *Angiostrongylus cantonensis* (Ac) were prepared by a previously reported method [13].

Hybridoma cell lines: establishment of a hybridoma cell line was carried out according to a method described by Oi and Herzenberg [14]. Briefly, mice were immunized intraperitoneally with 100 µg of SJWA in 0.2 ml phosphate-buffered saline (PBS) which had been emulsified with an equal volume of Freund's complete adjuvant (Difco, Detroit, MI, USA). Two weeks later, mice were immunized with the same antigen mixed with Freund's incomplete adjuvant (Difco).

One week after the second injection, mice were boosted intravenously with the same amount of antigen without adjuvant. Spleen cells of the immunized animals were harvested 3 days after the booster injection and fused with P3-X63Ag8-653 myeloma cells in the presence of 45% polyethylene glycol 4000 (Wako, Osaka, Japan). Screening was carried out using an enzyme-linked immunosorbent assay (ELISA) which was carried out by a previously reported method [13]. The hybridoma clones obtained were recloned four times by limiting dilution. The classes and subclasses of MoAb were determined by double immunodiffusion [15].

2.3. SDS-PAGE and Western blotting

SDS-PAGE and Western blotting were performed according to the methods described by Laemmli [16] and Towbin et al. [17], respectively. SJWA (50 µg/well) or recombinant protein (2 µg/well) were loaded onto the gel.

2.4. Treatment of antigens by periodate or trypsin

To characterize the nature of SJWA components recognized by the established MoAb, trypsin or periodate treatment of the SJWA preparation was carried out [18,19]. A MoAb termed 74D11 which recognizes a carbohydrate moiety of SEA [13] was used in control experiments.

2.5. Immunohistochemistry

Adult worms were fixed with 10% formalin, and embedded in paraffin wax. The embedded worm was sliced at a thickness of 3 µm. After deparaffinization and dehydration, the sections were treated with 1% H₂O₂ in methanol and then with 70% methanol to block the endogenous peroxidase activity. After washing, a 1:10 diluted culture supernatant containing MoAb was applied, and the sections were then incubated with 1:300 diluted horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dako A/S, Glostrup, Denmark). The final step was the exposure to 3,3'-diaminobenzidine tetrahydrochloride

and counterstaining with methyl green. A supernatant of P3-X63Ag8-653 myeloma cells was used as a control.

2.6. Immunoscreening of the cDNA library, DNA sequencing and sequence analysis

We used the cDNA library of the Japanese strain of *S. japonicum* as reported by Nara et al. [20]. The library constructed in λgt 11 expression phage vector was screened with the established MoAb. Briefly, a 20 mM isopropyl-β-D-thiogalactoside (Wako, Osaka, Japan)-soaked nitrocellulose filter (Millipore, Millipore Corp., Bedford, MA, USA) was placed onto a total of 1.5×10^3 recombinant plaques – grown on an agarose plate for 6 h at 37°C. The filter was rinsed with 0.05% Tween 20-PBS and incubated at 37°C for 1 h with a 1:10 diluted culture supernatant containing the MoAb. After washing, the filter was treated at 37°C for 1 h with 1:500 diluted rabbit anti-mouse IgG conjugated to alkaline phosphatase, and finally developed with a substrate consisting of 0.4 mM nitro blue tetrazolium/0.4 mM 5-bromo-4-chloro-3-indolyl-phosphate, *p*-toluidine salt (Wako). The positive plaques were subjected to a re-screening using the same MoAb.

A positive clone was extracted from the purified phage and subcloned into pBluescript SK(–) plasmid vector (Stratagene, La Jolla, CA, USA), then sequenced from both ends using a 373A Applied Biosystems automated DNA sequencer with –21M13 and M13 dye-labeled primers (DNA Sequencing Kit, Perkin Elmer, Foster, CA, USA). Sequence data were analyzed with the GENETYX-MAC program (Version 6.2.0, Software Corp., Ltd., Tokyo, Japan).

2.7. Production and purification of recombinant protein

For production of recombinant protein, we used the pET expression system (Novagen, Madison, WI, USA) as described previously [21]. The cDNA insert was first subcloned into vector pET 23b. *Escherichia coli* (*E. coli*), BL21(DE3) competent

cells were then transformed with the pET23b-insert. After expression, cultures were centrifuged and the cells were suspended in a solution consisting of 0.125 M Tris-HCl (pH 6.8), 4% SDS, 0.01% bromophenol blue, 14% glycerol, and 4% 2-mercaptoethanol, and were then boiled and subjected to SDS-PAGE.

For purification of recombinant protein, the harvested cells were sonicated in PBS (pH 7.5) containing 1% Triton-100 and 1 mM phenylmethylsulfonyl fluoride. After centrifugation, the supernatant and pellet were subjected separately to SDS-PAGE for detecting the solubility of the expressed protein. For solubilization, we followed the method described by Lin and Cheng [22] with slight modifications. After solubilization, the protein was dialysed against a buffer containing 20 mM Tris-HCl/pH 8.0 and 25 mM NaCl, and centrifuged. The supernatant was collected and applied to a DEAE-Sepharose CL-6B column (1 × 30 cm, Pharmacia, Uppsala, Sweden) equilibrated with the dialysis buffer. The absorbed proteins were eluted with a linear NaCl gradient. The fractions containing the recombinant protein were pooled and further purified using a T7 Tag Affinity Purification kit (Novagen) according to the manufacturer's instructions with NaSCN (6.0 M guanidine thiocyanate and 0.05 M Hepes/NaOH, pH 7.2) [23] as the elution buffer.

3. Results

3.1. Establishment of a MoAb-producing hybridoma and characterization of the MoAb

We established a hybridoma cell line producing a MoAb designated SJA111 (IgG1) which was reactive with SJWA from spleen cells of BALB/c mice immunized with this antigen preparation. As shown in Table 1, the reactivity of SJA111 against SJWA, SEA and eight different heterologous antigens was examined by ELISA. SJA111 was strongly reactive with SJWA, but did not react with any of the other antigens tested.

An attempt was then made to determine the molecular masses of the SJWA components recognized by SJA111. Western blotting analysis revealed three bands of 23, 24, and 25 kDa in the

antigen components of both male and female worms (Fig. 1, lanes C and D). Furthermore, SJWA components reactive with serum from a BALB/c mouse infected with *S. japonicum* had molecular masses ranging from 20 to 94 kDa. Similar bands appeared having the same molecular masses which reacted with SJA111 (Fig. 1, lanes E and F). No bands were obtained with ascites from a BALB/c mouse inoculated with the parental myeloma (data not shown).

3.2. Effects of trypsin or periodate treatment of antigens reactive with SJA111

In order to characterize the antigens recognized by SJA111, SJWA was treated with trypsin and periodate. As shown in Fig. 2, the antigenic determinants which reacted with the MoAb were denatured by a very low concentration (10 µg/ml) of trypsin but were unaffected by periodate, even at the highest concentration (40 mM) used (Fig. 3). The SEA recognized by MoAb 74D11 used as control was sensitive to periodate oxidation and resistant to trypsin treatment, as previously described [13]. These results suggest that the epitope of SJWA recognized by SJA111 may be proteinaceous in nature.

3.3. Localization of antigens in *S. japonicum* recognized by SJA111

As shown by the arrows in Fig. 4A, the adult tegument of both male and female adults was stained, especially that of the male adult worm. The control experiment using the supernatant of P3-X63Ag8-653 myeloma cells did not show any positive signal (Fig. 4B).

3.4. DNA sequencing and protein structure analysis of the target protein

We used SJA111 to screen the cDNA library of *S. japonicum* and obtained one positive clone which we designated SJJ22.6. This was subcloned into pBluescript SK(–) plasmid vector and sequenced from both ends, and the resulting partial DNA sequence was used to search the GenBank database for homology. The sequences obtained

Table 1
Reactivity of SJA111 against various parasite antigens

Antibodies	Antigens									
	SJWA	SEA	Ps	Cs	Fg	Ts	DI	As	Di	Ac
SJA111 ^a	1.14	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Immune sera ^b	1.27	0.33	0.11	0.21	0.27	0.37	0.14	0.06	0.33	0.25
Infected sera ^c	1.35	1.61	0.23	0.36	0.45	0.34	0.13	0.46	0.40	0.27

ELISA titers are expressed as the mean net OD₄₉₂ of three separate experiments (OD₄₉₂ titers of SJA111 were obtained from SJA111 ascites titers minus ascites control titers; OD₄₉₂ titers of immune and infected sera were obtained from immune and infected sera titers minus serum controls). Ascites controls were prepared from naive BALB/c mice i.p. injected with P3-X63-Ag8-653 myeloma cells, and normal serum controls were prepared from untreated BALB/c mice. All antibodies were used at a 1:100 dilution. Preparation of antigens and the ELISA assay are described in Section 2.

^aSJA111 from ascites.

^bSera from BALB/c mice immunized with SJWA.

^cSera from BALB/c mice collected 8 weeks after animals were infected with *S. japonicum*.

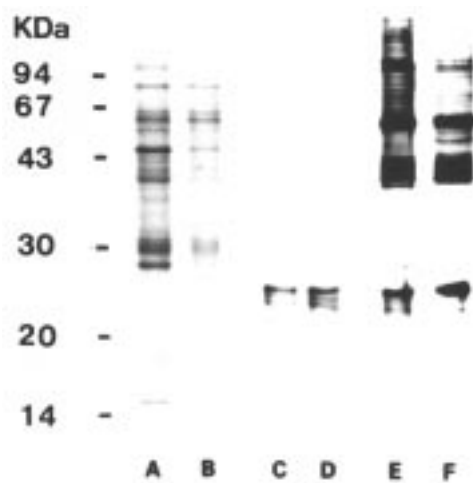


Fig. 1. Western blotting analysis of SJWA using SJA111. Lanes A, C, E are male SJWA and B, D, F are female SJWA. Lanes A and B were stained with CBB. Lanes C and D were treated with SJA111 from ascites (1:200 dilution), and lanes E and F were treated with serum from a BALB/c mouse infected with *S. japonicum* (1:200 dilution).

showed significant homology to the gene (SJC22.6) encoding a 22.6-kDa surface antigen of the Chinese strain of *S. japonicum* [24].

The full nucleotide sequence of SJJ22.6 and its deduced amino acid sequence are shown in Fig. 5. The 844-bp cDNA of SJJ22.6 includes a 576-bp nucleotide open reading frame (ORF), which encodes 191 amino acids with a deduced molecular

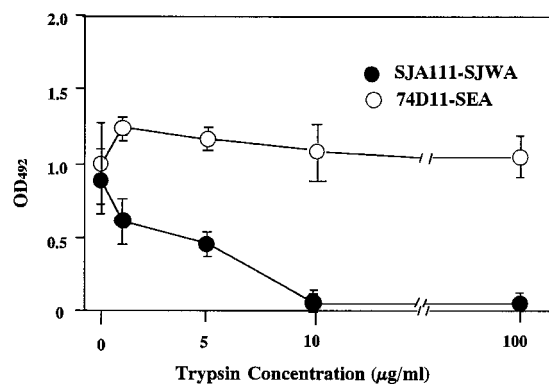


Fig. 2. Trypsin treatment of antigens recognized by SJA111. SJWA (1 μ g/well) was coated on a microtiter plate, and trypsin was added at concentrations of 0 to 100 μ g/ml. After washing and blocking, an ELISA was performed as described in Section 2. The MoAb 74D11 and SEA were used as the control. Results are expressed as means $\pm$ S.D. of quadruplicate experiments.

mass of 22.6 kDa, beginning with an initiation codon ATG at position 7 and ending with a termination codon TAA at position 580, followed by a presumptive polyadenylation signal (AATAAA) at position 622, and a 61-bp poly (A) tail.

The nucleotide sequence of SJJ22.6 is almost the same as that of SJC22.6 (Chinese strain) [24] and SJP22.6 (Philippine strain) [25]. An alignment of nucleotides of SJJ22.6, SJC22.6 and SJP22.6 is shown in Fig. 6. The ORF of SJJ22.6 shares

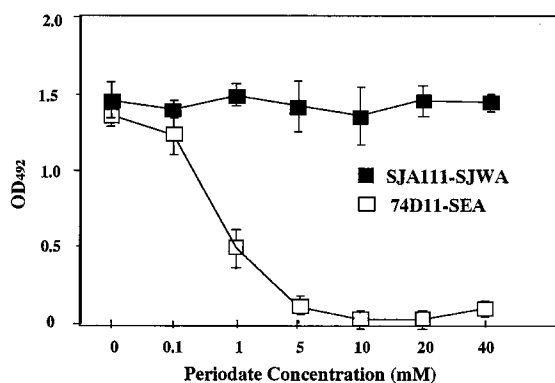


Fig. 3. Periodate treatment of antigens recognized by SJA111. SJWA (1 μ g/well) was coated on a microtiter plate, and periodate was added at concentrations of 0 to 40 mM. After washing and blocking, an ELISA was performed as described in Section 2. The MoAb 74D11 and SEA were used as the control. Results are expressed as means $\pm$ S.D. of quadruplicate experiments.

99.0% and 99.3% identity with those of SJC22.6 and SJP22.6, respectively. The ORF region of SJJ22.6 has six nucleotides which differ from those of the SJC22.6 ORF region, however, only one of the nucleotides at position 415 results in a different amino acid in the two clones. Four nucleotides in the ORF region of SJJ22.6 are different from those in the SJP22.6 ORF region, but all of them are silent mutations. The nucleotide sequences of both SJJ22.6 and SJP22.6 encode identical amino acid residues (Fig. 7).

3.5. Expression and purification of the recombinant protein

The recombinant protein was expressed in BL21(DE3) expression *E. coli* after induction with isopropyl- β -D-thiogalactoside and was detected in bacterial lysates at a concentration of $\sim$ 100 mg/l of bacterial culture, indicating a high level of expression using this expression system. The time course and overexpression of the recombinant protein are shown in Fig. 8 (lanes A–G). Expression reached the highest level between 4–6 h after the addition of IPTG. The apparent molecular mass of the protein was about 25 kDa. This protein was not present in non-recombinant

BL21(DE3) induced under the same conditions (Fig. 8, lane H).

After expression of the protein, cell cultures were harvested and sonicated, and both supernatant and pellet were subjected to SDS-PAGE electrophoresis. We found that most of the expressed protein was in the insoluble fraction (data not shown) and used guanidium chloride to solubilize the precipitate. The recombinant protein was subsequently purified by a combination of DEAE ion exchange chromatography and a T7 Tag Affinity Purification kit (Fig. 9, lane C). The recombinant protein was identified as the pass-through fractions after DEAE ion exchange chromatography. The antigen-positive fractions were pooled and subjected to T7 Tag affinity chromatography as described in Section 2.

3.6. Reactivities of the recombinant protein with SJA111 and sera of patients

Lane B shown in Fig. 9 reveals the staining patterns of antigens transblotted from SDS-PAGE to a polyvinylidene difluoride membrane. The epitope found in this material was associated with molecular masses of $\sim$ 25 kDa and 21 kDa which were visualized by Western blotting. The lower band may have been a prematurely terminated translation or breakdown product of the 25-kDa recombinant protein. The 25-kDa molecule was the major recombinant protein product. After purification, this protein also reacted with SJA111 (Fig. 9, lane D). In addition, the purified recombinant protein was reactive with 12 sera tested of patients infected with the Chinese strain of *S. japonicum* (a representative result shown in lane E in Fig. 9) although the degree of recognition was variable, but not with normal human serum or rabbit anti-human immunoglobulins (Fig. 9, lanes F and G). The above results showed that the recombinant protein was efficiently recognized by SJA111 as well as by the sera of *S. japonicum*-infected patients.

4. Discussion

In the present study, we established a MoAb against a tegumental antigen of the Japanese

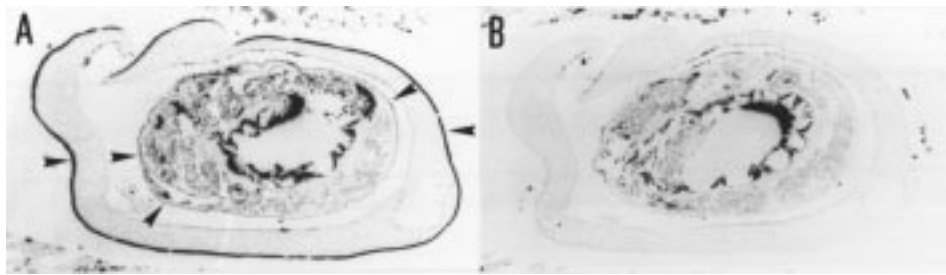


Fig. 4. Immunostained antigens in a section of the *S. japonicum* adult worm recognized by SJA111. (A) A section which reacted with SJA111, arrows indicate the tegument which reacted with SJA111. (B) A section which reacted with the supernatant of P3-X63Ag8653 myeloma cells (control).

strain of *S. japonicum*, and using this MoAb as a probe, obtained a cDNA clone which showed a high homology to sequences of Chinese [24] and Philippine strains [25]. It further supports the previous finding that this molecule is highly conserved among all *S. japonicum* strains.

Cruise and Rogers reported that a MoAb termed I-134 was reactive with a 23-kDa molecule (Sj23) of *S. japonicum* adult worms, which is an integral membrane protein [26,27], however, after comparison of Sj23 cDNA [28] and SJJ22.6 cDNA sequences, we found these two clones to be different (data not shown). Thus, I-134 and SJA111 recognize different surface epitopes of the *S. japonicum* adult worm.

Both Stein et al. [29] and Jeffs and David [30] have cloned a gene encoding a 22.6 kDa (sm22.6) antigen from a *S. mansoni* cDNA library. Their results also showed that their 22.6-kDa protein is a tegumental antigen, and that it is probably a major tegumental membrane-associated protein since its mRNA forms a moderately abundant portion of the total adult RNA [29] and since sm22.6 is recognized by IgG antibodies of mice vaccinated with purified adult *S. mansoni* tegumental membrane as well as by sera from *S. mansoni*-infected Brazilians. It is present in all post-snail stages of the life cycle (except in eggs) in cercariae, schistosomula, lungworms and adult worms, is not sex-specific, and is common to nine species of schistosome, but is undetectable in other helminth antigens [30]. The inferred amino acid sequence of sm22.6 which has one amino acid less than SJC22.6 shows 71% identity to

SJC22.6, which rises to 84% identity when conservative substitutions are taken into account [24]. This is also the case in comparisons with SJP22.6 and SJJ22.6 because of the high homology among the different strains of *S. japonicum*. The biological significance of this 22.6-kDa protein is currently unknown, but according to Waine et al. [24], the protein contains a sequence motif characteristic of a family of proteins such as calmodulin, parvalbumin, and troponin C which have a Ca^{2+} binding domain. The protein may play some role in binding and regulating the movement of Ca^{2+} across the tegumental surface membrane. Alternatively, the protein may have another biological role, but needs the coordination of Ca^{2+} for activation and/or normal functioning, and since the tegumental membrane molecule of schistosomes are expressed at the parasite–host interface, it receives much attention from the developers of immunodiagnosis and vaccine. We speculate that the molecule is not only likely to have essential protective and maintenance functions for the parasite but they are also subject to immune attack by the host.

The following observations argue for this protein's consideration as a vaccine candidate against schistosomes based on another report [30] and our results: (1) the special localization of the antigen in adult worms; (2) its recognition by antibodies of mice vaccinated with purified adult *S. mansoni* tegumental membrane, sera from *S. mansoni* and *S. japonicum*-infected patients; (3) lack of cross-reaction with the eggs; and (4) schistosome-specific expression. In view of concerns

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1      GTTATTATATGGCAACCACTGAGTACAGATTAAGTTTAAATGGAACAATTTATTAGAGCATTG
1      M A T T E Y R L S L M E Q F I R A F

61     ATAGAAATAGATAAAGATAAATGAATTGATTGATAAACAAGAACTAACGAAATATTGT
19     I E I D K D N N E L I D K Q E L T K Y C

121    CAACAGAACCAATGGATATGAAACAAATAGATCCATGGATTGCAAGGTTTGATACTGAT
39     Q Q N Q M D M K Q I D P W I A R F D T D

181    AAAGATGGTAAAGTCAGTTTAGAGGAGTTTGTCTGGATTCTGCTAAAGGTTTGGGAA
59     K D G K V S L E E F C R G F G L K V W E

241    GTCCGCCGTGAAAAGGAAGAATTAAAGAGAGACAAGGAAGGCAAAGTATCCACACTTCCA
79     V R R E K E E L K R D K E G K V S T L P

301    CTGGATATTCAAATTATCGCGCAACCATGTCAAAAGCTAAGCAATATAACATATGTTGT
99     L D I Q I I A A T M S K A K Q Y N I C C

361    AAATTTAAAGAACTCCTTGATAAACTAGTCGAACTGGTGATGAAGTAAGAGCGGTGGCA
119    K F K E L L D K T S R T G D E V R A V A

421    AATGACTTAAAGCATTTTGGATTCTGAATATGGTCGTGTATGGCAAGTGATTATACTA
139    N D L K A F L D S E Y G R V W Q V I I L

481    ACTGGTTCATATTGGATGAATTTTTCACATGAACCATTTTATCAATGCAGTTTAAAGTAC
159    T G S Y W M N F S H E P F L S M Q F K Y

541    AGTAATTATGTTTGTCTATTGTGGCGAACACCGTCTTCGTAAAAGTTTAAATGAATAGTACA
179    S N Y V C L L W R T P S S

601    AATGAATATACATTATACACAATAAAGCGACAGTATGCTAATCTACTATTCACTAAGTGC
661    AATAATAATGACAATTTTACATAACTTTGAATTTGTTCAATGATTAAATTACTAGTGCTT

721    TAACTAACTCAAATTCCTAATCATTTTGTATGAATGACAAAACAGTACATTATTAAATGA

781    ATCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

841    AAAA

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Fig. 5. The nucleotide sequence of the *S. japonicum* clone SJJ22.6 (upper line) and its deduced amino acid sequence (lower line). The initiation codon (ATG) and termination codon (TAA) are underlined. The presumptive polyadenylation signals (AATAAA) are underlined in bold.

related to safety and production, recombinant forms are preferable to native antigens.

In conclusion, SJA111, a MoAb against *S.*

japonicum tegumental membrane protein antigens, was established and one of the recombinant forms of these target proteins was expressed and

SJJ22.6 1:-----GTTATTGGCAACCACTGAGTACAGATTAAGT
 SJC22.6 1:-----CTT.....T.....
 SJP22.6 1:CATTTAATCAATAATTAGTATTAA.....T.....

 SJJ22.6 34:TTAATGGAACAATTTATTAGAGCATTTCATAGAAATAGATAAAGATAAATAATGAATTGATT
 SJC22.6 37:.....
 SJP22.6 61:.....

 SJJ22.6 94:GATAAACAAGAACTAACGAAATATTGTCAACAGAACCAATGGATATGAAACAAATAGAT
 SJC22.6 97:.....T.....
 SJP22.6 121:.....T.....

 SJJ22.6 154:CCATGGATTGCAAGGTTTGATACTGATAAAGATGGTAAAGTCAGTTTIGAGGAGTTTGT
 SJC22.6 157:.....
 SJP22.6 181:.....

 SJJ22.6 214:CGTGGATTTCGGTCTAAAGGTTTGGGAAGTCCGCCGTGAAAAGGAAGAATTAAAGAGAGAC
 SJC22.6 217:.....T.....
 SJP22.6 241:.....T.....

 SJJ22.6 274:AAGGAAGGCAAAGTATCCACACTTCCACTGGATATTCAAATTATCGCGGCAACCATGTCA
 SJC22.6 277:.....
 SJP22.6 301:.....

 SJJ22.6 334:AAAGCTAAGCAATATAACATATGTTGTAAATTTAAAGAACTCCTTGATAAACTAGTCGA
 SJC22.6 337:.....T.....
 SJP22.6 361:.....T.....

 SJJ22.6 394:ACTGGTGATGAAGTAAGAGCGGTGGCAAATGACTTAAAGCATTTTTGGATTCTGAATAT
 SJC22.6 397:.....T.....
 SJP22.6 421:.....

 SJJ22.6 454:GGTCGTGTATGGCAAGTGATTATACTAAGTTCATATTGGATGAATTTTCACATGAA
 SJC22.6 457:..C.....
 SJP22.6 481:.....

 SJJ22.6 514:CCATTTTATCAATGCAGTTTAAGTACAGTAATTATGTTGTCTATTGTGGCGAACACCG
 SJC22.6 517:.....
 SJP22.6 541:.....

 SJJ22.6 574:TCTTCGTAAAGTTTAAATGAATAGTACAAATGAATATACATTCATACACAATAAAGCGACA
 SJC22.6 577:.....
 SJP22.6 601:.....A.....