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# Identification and Molecular Cloning of a 67-Kilodalton Protein in *Schistosoma japonicum* Homologous to a Family of Actin-Binding Proteins

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**A monoclonal antibody to *Schistosoma japonicum* which conferred significant protection against cercarial challenge in mice was produced. The predicted translation product of the cDNA corresponding to the antigen recognized by this antibody was homologous to a newly identified family of actin-binding proteins. The expressed protein bound polymerized actin and was recognized by serum from patients infected with *S. japonicum*.**

Population-based chemotherapy significantly reduces the prevalence, incidence, intensity, and morbidity of schistosomiasis japonica in China and the Philippines, but despite continued treatment, transmission persists (18, 23, 26). Thus, alternative approaches such as a vaccine will be needed to control *Schistosoma japonicum* in countries where it is endemic (3). One strategy to identify candidate vaccine antigens is to develop monoclonal antibodies (MAbs) against *S. japonicum*, an approach used successfully for identification of vaccine antigens for *Schistosoma mansoni* (5, 10, 11, 27). We report the production of a MAb, A6, that conferred significant protection against *S. japonicum* infection upon passive transfer into mice. The adult worm cDNA translation product recognized by MAb A6 is homologous to a family of actin-binding proteins.

The Philippine strain of *S. japonicum* was maintained in BALB/c mice (The Jackson Laboratory, Bar Harbor, Maine) and *Oncomelania quadrasi* snails (19). (The procedures used in this research were approved by the animal care committee at The Miriam Hospital and Brown University.) Production and screening of MAbs and preparation of schistosomula and adult worm antigen as well as indirect immunofluorescence were performed as previously described (25). In passive transfer experiments, ICR mice (Shizuoka Experimental Animal Farms, Hamamatsu, Japan) were injected with hybridoma culture supernatant or ascitic fluid while controls received mouse immunoglobulin. On day 0, mice were infected percutaneously with 50 *S. japonicum* Philippine cercariae, and adult worm recovery was determined 6 weeks later (19, 27). Data were compared by Student's *t* test.

An *S. japonicum* cDNA library was constructed in lamba ZAP XR with adult *S. japonicum* Philippine RNA (15) (Stratagene, La Jolla, Calif.). The cDNA library was screened with MAb A6, and reactive clones were plaque purified and sequenced (6, 15). 5′ rapid amplification of cDNA ends (5′-RACE) protocol and Northern analysis were performed as described previously (15). Recombinant proteins were expressed by using the pRSET expression system (6, 15) (Invitrogen, San Diego, Calif.).

To identify candidate vaccine antigens against *S. japonicum*, MAbs from mice immunized with 3-h-old schistosomula were produced. One clone, MAb A6, an immunoglobulin M, reacted strongly with adult worm extract by enzyme-linked immunosorbent assay (ELISA) and recognized a 67-kDa antigen, designated Sj67, by immunoblot analysis. The antibody reacted with the surface of 3-h-old schistosomula as determined by indirect immunofluorescence (10). Passive immunization experiments demonstrated that the mean adult worm burden was significantly ($P < 0.05$ to 0.001) reduced by 26 to 47% in mice injected with MAb A6 compared to controls (Table 1).

To obtain a cDNA clone corresponding to Sj67, a lambda

TABLE 1. Protection against *S. japonicum* cercarial challenge conferred by MAb A6 following passive transfer in mice

| Expt[a] | Treatment group | No. of adult worms (mean ± SE) | % Reduction |
|---|---|---|---|
| 1 | Control (9)[b] | 29.9 ± 1.7 | 28 |
| | MAb A6 (11) | 21.5 ± 2.1[c] | |
| 2 | Control (12) | 35.9 ± 2.0 | 47 |
| | MAb A6 (11) | 19.2 ± 2.1[d] | |
| 3 | Control (10) | 32.2 ± 2.0 | 30 |
| | MAb A6 (10) | 22.4 ± 1.5[d] | |
| 4 | Control (11) | 27.8 ± 1.7 | 26 |
| | MAb A6 (11) | 20.7 ± 2.3[e] | |

[a] Mice were injected intraperitoneally with 165 μg of MAb A6 prepared from hybridoma culture medium (experiment 1), 200 μg of ascitic fluid (experiments 2 to 4), or an equivalent concentration of normal mouse immunoglobulin (Control). Animals were challenged with 50 cercariae, and adult worm burden was determined on day 42 postinfection (19).
[b] The number in parentheses represents the number of mice per group.
[c] $P \leq 0.005$.
[d] $P \leq 0.001$.
[e] $P \leq 0.05$.

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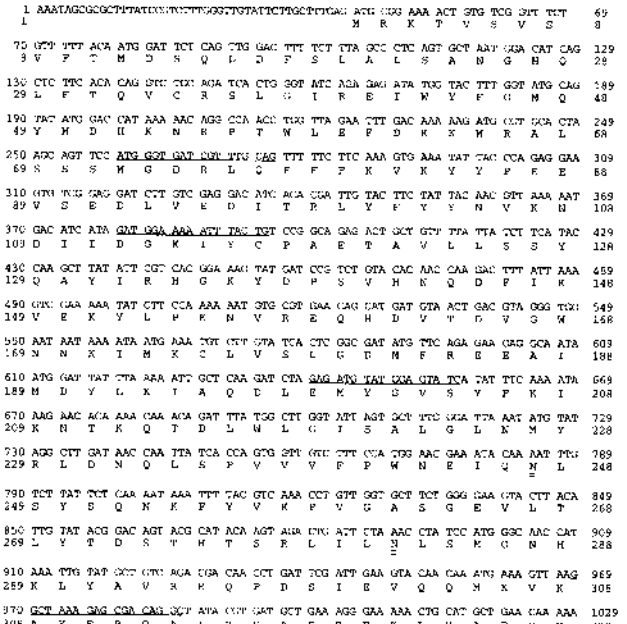

FIG. 1. Sequence of JF cDNA. The amino acid translation of the 548-codon ORF is indicated in one-letter code below the corresponding DNA sequence. The reverse complements of oligonucleotide primers used in RACE reactions are underlined. The potential N-linked glycosylation sites at asparagines 247 and 282 are double underlined. The stop codon is indicated by an asterisk.

ZAP XR adult *S. japonicum* Philippine cDNA library was immunoscreened with MAb A6. Six reactive clones were plaque purified, and clone JF-2, containing a 2.0-kb cDNA insert, was sequenced. JF-2 contained an open reading frame (ORF) of 519 codons; however, the ORF began with the first nucleotide of the cDNA insert, suggesting a partial cDNA sequence. To obtain a full-length cDNA, 5′-RACE reactions were performed on adult worm (A+) RNA with JF gene-specific primers (Fig. 1). A single cDNA fragment (JF-5) was cloned, and sequence analysis revealed 146 bp of identity with JF-2 at its 3′ terminus with an additional 130 bp at the 5′ terminus of JF-5. The hybrid JF cDNA contained 2,128 bp with 45 bp of 5′ untranslated region, an ORF of 548 codons followed by a TAG stop codon, a 412-nucleotide 3′ untranslated region, and a 24-nucleotide poly(A) tail (Fig. 1). Northern analysis of *S. japonicum* Philippine adult worm poly(A)$^+$ RNA probed with a JF gene-specific probe revealed a single message of 2,370 bp with no detectable hybridization of probe to rat liver poly(A)$^+$ RNA, suggesting that the clone is of *S. japonicum* worm origin.

TABLE 2. Homology and identify of the JF gene product with members of the band 4.1 gene family[a]

| Protein | % Homology | % Identity |
|---|---|---|
| *E. multilocularis* antigen II | 71 | 43 |
| Human ezrin | 70 | 38 |
| Bovine ezrin | 70 | 39 |
| Human radixin | 69 | 38 |
| Mouse radixin | 68 | 38 |
| Human moesin | 69 | 38 |
| Pig moesin | 69 | 38 |
| Human band 4.1 | 54 | 22 |

[a] Homology and identify were determined with the Align program with a BLOSUM50 matrix.

The deduced 548-amino-acid translation product of the JF ORF displayed up to 71% homology and 43% identity with members of the band 4.1 family of actin-binding proteins (1, 2, 4, 7, 8, 13, 17, 20, 21) (Table 2). Homology was greatest for the amino-terminal half of the JF gene product with one short highly homologous domain at the carboxy terminus (Fig. 2). Chou-Fasman secondary structure analysis predicted a 200-amino-acid-long alpha helix at the carboxy terminus of the JF gene product (4). These structural features are similar to those of other members of the band 4.1 gene family (14).

The clone JF-2 was expressed with a six-histidine tag, and the expressed protein (rSj67) was purified by affinity chromatography using nickel–nitrosilo-triacetic acid (6). MAb A6 recognized rSj67 by immunoblot analysis. Polymerized actin bound to microtiter plates coated with rSj67, as determined by ELISA, while no significant binding was observed with unpolymerized actin or wells not coated with rSj67 (21). These data suggest that rSj67 is indeed an actin-binding protein. Furthermore, serum from 87% (52 of 60) of patients from the island of Leyte in the Philippines infected with *S. japonicum* recognized rSj67 by ELISA, while serum from only 32% (15 of 47) of *S. japonicum*-infected patients from Jishan Island, Jiangxi Province, China, recognized this protein (12, 18, 26).

In summary, we produced an immunoglobulin M MAb which recognized a 67-kDa protein in adult worm extract and, upon passive transfer into mice, conferred significant protection against cercarial challenge. The level of protection was similar to that observed with MAbs against *S. mansoni*. The worm cDNA translation product displayed significant homology to the band 4.1 family of actin-binding proteins, suggesting that we have identified and cloned a unique antigen in *S. japonicum.*

The family of actin-binding proteins includes band 4.1 ezrin, radixin, and moesin, as well as *Echinococcus multilocularis* antigen II (1, 2, 4, 7, 8, 13, 17, 20, 21). One potential functional role for these proteins is to link surface membrane proteins with cytoskeletal proteins, such as actin. The striking structural homology between Sj67 and the actin-binding proteins as well as in vitro binding of rSj67 to polymerized actin suggests that this protein may have a similar function in *S. japonicum.*

Immunofluorescent studies suggest that Sj67 is exposed on the surface of schistosomula. This may reflect rapid membrane turnover, the unusual structure of the schistosome heptalaminate membrane, or the dual localization to both the internal and external surface membranes, as has been observed for moesin (7, 16, 24).

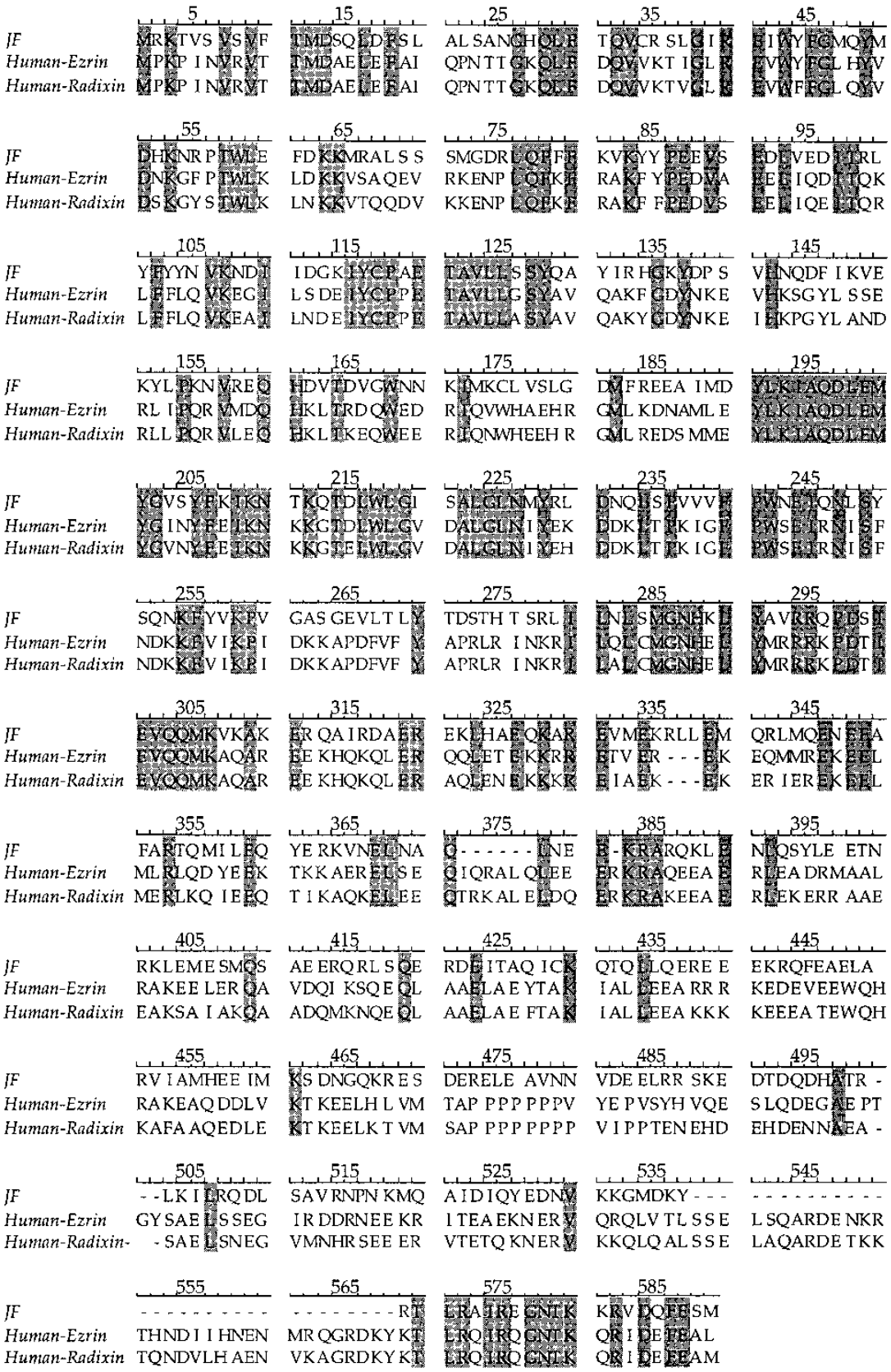


FIG. 2. Alignment of JF gene product with human ezrin and radixin. Amino acid sequences were aligned with GeneWorks software. Gaps introduced to maximize the alignment are indicated with a dash. Identical residues are shaded.

Since MAb A6 confers significant protection against cercarial challenge and is recognized by patients infected with *S. japonicum* from the Philippines, these data suggest that Sj67 may represent a candidate vaccine antigen. Protection studies are ongoing to determine if this antigen induces significant protection upon active immunization in mice.

(The sequence reported in this paper has been deposited in the GenBank database [accession no. U13986].)

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