

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 lambda 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 $\pm$ SE)	% Reduction
1	Control (9) ^b MAb A6 (11)	29.9 $\pm$ 1.7 21.5 $\pm$ 2.1 ^c	28
2	Control (12) MAb A6 (11)	35.9 $\pm$ 2.0 19.2 $\pm$ 2.1 ^d	47
3	Control (10) MAb A6 (10)	32.2 $\pm$ 2.0 22.4 $\pm$ 1.5 ^d	30
4	Control (11) MAb A6 (11)	27.8 $\pm$ 1.7 20.7 $\pm$ 2.3 ^e	26

^a Mice were injected intraperitoneally with 165 μ g of MAb A6 prepared from hybridoma culture medium (experiment 1), 200 μ g 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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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).

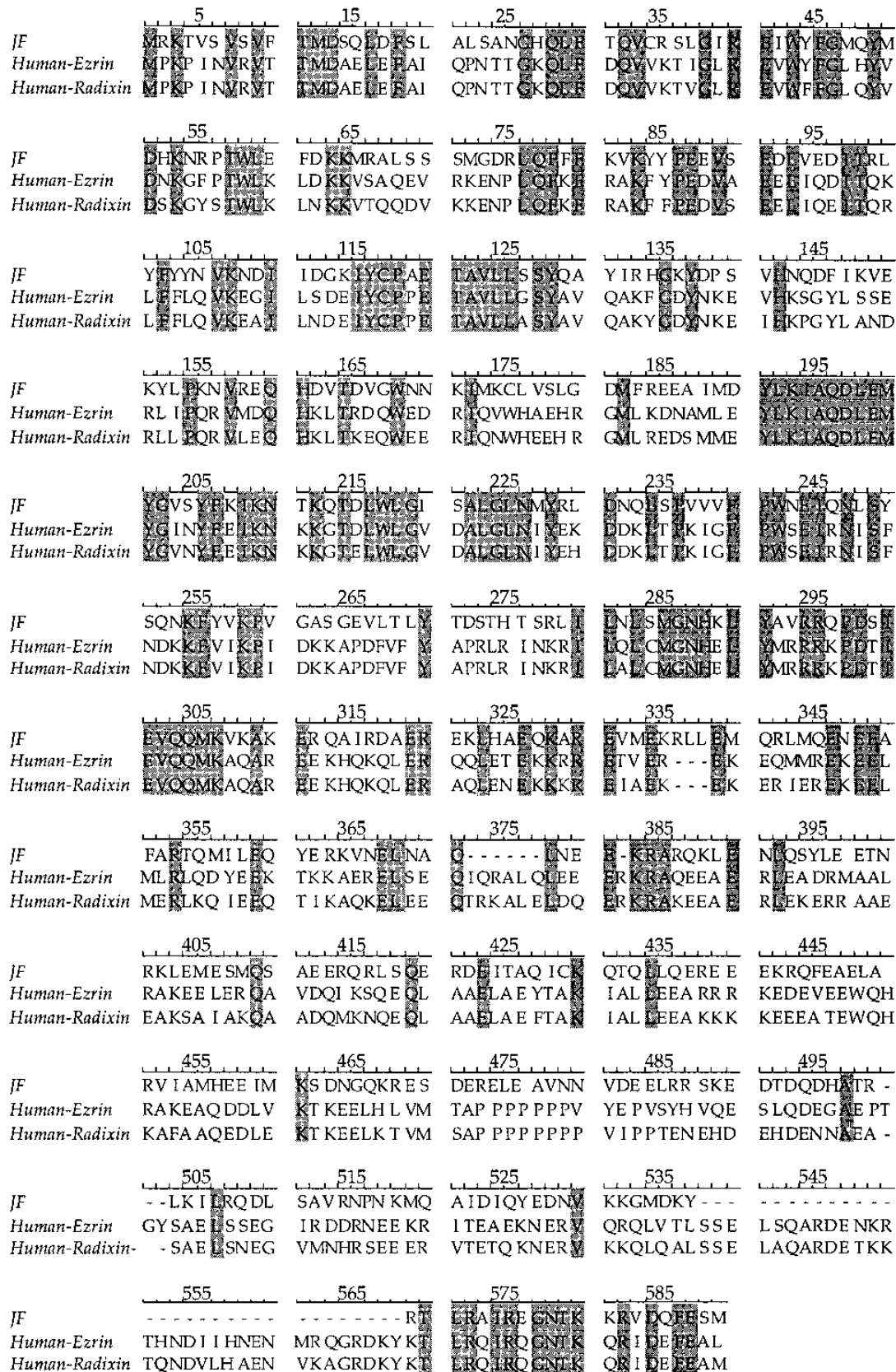


FIG. 2. Alignment of JF gene product with human ezrin and radixin. Amino acid sequences were aligned with GeneWorks software. Gaps introduced to maximize the alignment are indicated with a dash. Identical residues are shaded.

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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