Brief Definitive Report

A MAJOR IMMUNOGEN IN *SCHISTOSOMA MANSONI* INFECTIONS IS HOMOLOGOUS TO THE HEAT-SHOCK PROTEIN Hsp70

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Schistosomes induce an immune response characterized by antibodies that recognize a complex spectrum of parasite antigens, a few being invariably immunogenic in all hosts (1). One of these, an antigen of 70,000 mol wt, is immunodominant both in humans naturally infected with *Schistosoma mansoni* and in other mammals infected under experimental conditions (1, 2). λgt11 expression libraries were screened with various infection sera and several antigen-expressing clones were isolated (1). In this report, clones expressing fusion peptides that specifically adsorbed antibodies capable of immunoprecipitating the 70,000 mol wt antigen from in vitro RNA translation products or from schistosome extracts were used for DNA sequence analysis. The inferred amino acid sequence identified this antigen as a homologue of the heat-shock protein hsp70. Despite striking levels of sequence homology with human and *Drosophila* hsp70, the *S. mansoni* homologue is sufficiently divergent to elicit a strong and noncrossreactive immune response to this antigen. Schistosome development alternates between temperature extremes in its transition between the snail and vertebrate hosts, suggesting a potential regulatory role of this major immunogen in parasite development and pathogenesis.

Materials and Methods

Immunoprecipitation Analysis. Immunoprecipitation of polypeptides translated in vitro from adult worm RNA was performed as described elsewhere (1).

DNA Methods. DNA of λgt11 cDNA clones was prepared from liquid lysates by the standard method for phage (3). Insert cDNA fragments released by Eco RI digestion were cloned in both orientations into M13mp19 (4) and single-stranded templates sequenced by the dideoxynucleotide chain-termination method (5). Genomic DNA, isolated from adult worm homogenates by ultracentrifugation through cesium chloride (6), was digested with restriction endonucleases (5–10 U/μg), resolved by electrophoresis through 1% agarose gel, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled probe as described by Southern (7).

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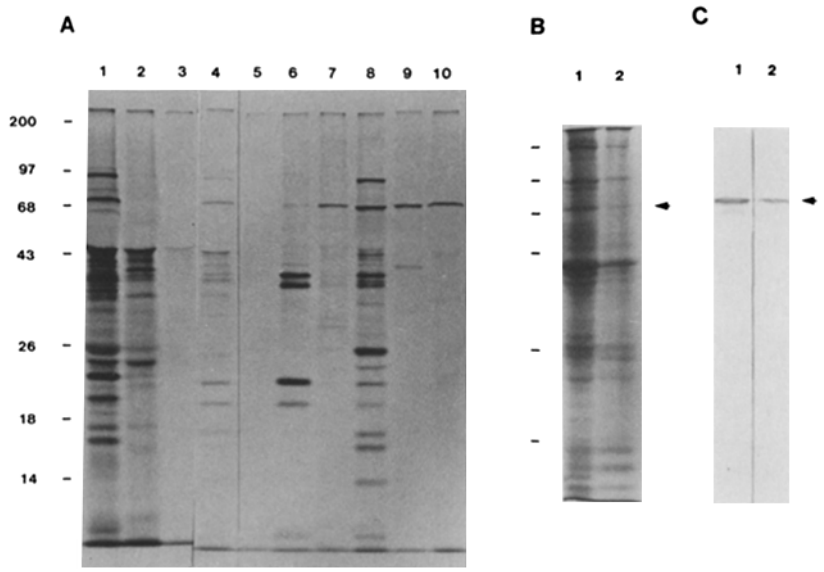

FIGURE 1. (*A*) Detection of serum antibodies to the *S. mansoni* 70,000 mol wt antigen by immunoprecipitation of ^{35}S-labeled polypeptides from *S. mansoni* adult worm RNA in vitro translation. Immunoprecipitates with *S. mansoni*–infected human serum (*1*), *S. japonicum*–infected human serum (*2*), uninfected human serum (*3*), *S. haematobium*–infected baboon serum (*4*), uninfected rat serum (*5*), chronically infected rat serum (*6*), mouse serum 6 wk after infection (25 cercariae) (*7*), chronically infected mouse serum (*8*), serum from mice vaccinated with four exposures of irradiated *S. mansoni* cercariae (*9*), and serum from mice immunized with lysates of *Escherichia coli* lysogenic with λcSMA-F4 (*10*). (*B*) Relative abundance of a 70,000 mol wt protein in total protein extracts of adult worms (*1*) and cercariae (*2*). Proteins extracted with SDS-PAGE sample buffer were electrophoresed on a 12% gel and stained with Coomassie Blue. (*C*) Identification of the 70,000 mol wt antigen in the total proteins extracted from adult worms (*1*) and cercariae (*2*) by immunoblot analysis. Proteins extracted and resolved as above were electrophoretically blotted onto nitrocellulose and probed with a 1:200 dilution of the antiserum described in *A*, lane *10*. Immunoblots were processed as described previously (1). The position of the 70,000 mol wt antigen is indicated by an arrow in each of the right margins.

Results and Discussion

Sera of infected humans, baboons, and mice, permissive hosts for *S. mansoni*, uniformly recognize the 70,000 mol wt antigen (Fig. 1*A*); in the analysis of >100 different serum samples from these hosts, a wide range of antibody activities was catalogued and the 70,000 mol wt antigen was detected by virtually all sera (data not shown). The immune response to the 70,000 mol wt antigen exhibits a degree of species specificity as judged by the lack of crossreactive antibodies to this protein in sera from patients infected with *Schistosoma japonicum* (Fig. 1*A*, lane *2*). Noncrossreactive antisera from *S. japonicum*–infected mice display a similar species-specific recognition of the 70,000 mol wt antigen, further demonstrating the specificity of the response (data not shown). Crossreactivity between *Schistosoma haematobium* infected baboon (Fig. 1*A*, lane *4*) and human sera predicts the existence of an analogous antigen in this close relative of *S. mansoni*. In *S. mansoni*–infected mice, 70,000 mol wt antigen antibody titers arose early in the course of infection and appeared to be the first strongly induced response (Fig. 1*A*, lane *7*). This response seems to persist as chronically infected mice

continue to elicit high levels of antibodies to this antigen (Fig. 1 *A*, lane *8*). Mice vaccinated with irradiated cercariae produce antisera that can confer passive protection to cercarial challenge by as much as 47% (8); these sera recognize two immunodominant species among the in vitro translation products of adult worm mRNA; one of these is the 70,000 mol wt antigen (Fig. 1 *A*, lane *9*). Sera from infected rats, which rapidly resolve infections (9), display only a weak antibody response to the 70,000 mol wt protein. Whether this is due to the dynamics of the infection in rats (nonpermissive hosts) or a difference in the binding affinity of protein A for the rat antibody has not been determined. The immunodominant nature of the 70,000 mol wt antigen may be a reflection of the relatively high abundance of this protein as judged by SDS-PAGE (Fig. 1 *B*) and immunoblot (Fig. 1 *C*) analyses with adult worm– and cercariae-extracted proteins. The consistently immunodominant character of this antigen, its recognition by protective antisera, and its early detection by the host immune system commended it, from our large repertoire of antigen expressing clones, for further characterization.

The clone designated λcSMA-F4 (F4) has a cDNA insert size of 516 nucleotides and contains the major immunodominant epitopes of the 70,000 mol wt antigen, as judged by its ability to remove essentially all of the 70,000 mol wt protein precipitating activity from infection sera (data not shown). The F4 clone was used to select a nearly full-length clone, designated λcSMA-F7 (F7), that was shown to express the 70,000 mol wt antigen. The primary DNA and deduced amino acid sequences of the cDNA insert from clone F7 are shown in Fig. 2; the F7 clone encodes a polypeptide with striking sequence homology to the major heat-shock protein, hsp70 (Fig. 2 *B*). Sequence comparisons of this cDNA indicate 80% identity at the amino acid level with human hsp70 and 71% with *Drosophila*. Regions of sequence divergence between the polypeptides occur predominately near the 3′ terminus of the molecule; it seems likely that regions of immunological divergence between species and the immunodominant epitopes of the F4 fusion peptide reside in this portion of the protein.

The hsp70 gene in many other eukaryotic organisms has been shown to exist in multiple copies that are usually dispersed in the genome; some members of the multigene family have alternate functions evidenced by differential patterns of expression in normal and heat-shocked cells (reviewed in reference 10). To determine the number and genomic organization of the *S. mansoni* hsp70 homologue, Southern hybridizations were performed using the F7 clone and various restriction endonuclease digestions of adult worm DNA. As shown in Fig. 3, F7 hybridized with a single large restriction fragment when DNA was digested with any of several enzymes. This unusual pattern suggests that the *S. mansoni* gene is arranged in the genome as tandemly repeated copies of identical or closely related genes.

The relationship of hsp70 to schistosome infections is a curious one. While schistosomes clearly experience a heat shock in their transition from the invertebrate snail to the vertebrate host, our studies indicate that the production of the hsp70 antigen is more likely to be constitutive rather than heat induced. Additionally, immunofluorescence studies in other organisms suggest that hsp70 activity is intracellular (11–13), whereas the universal response of infected hosts

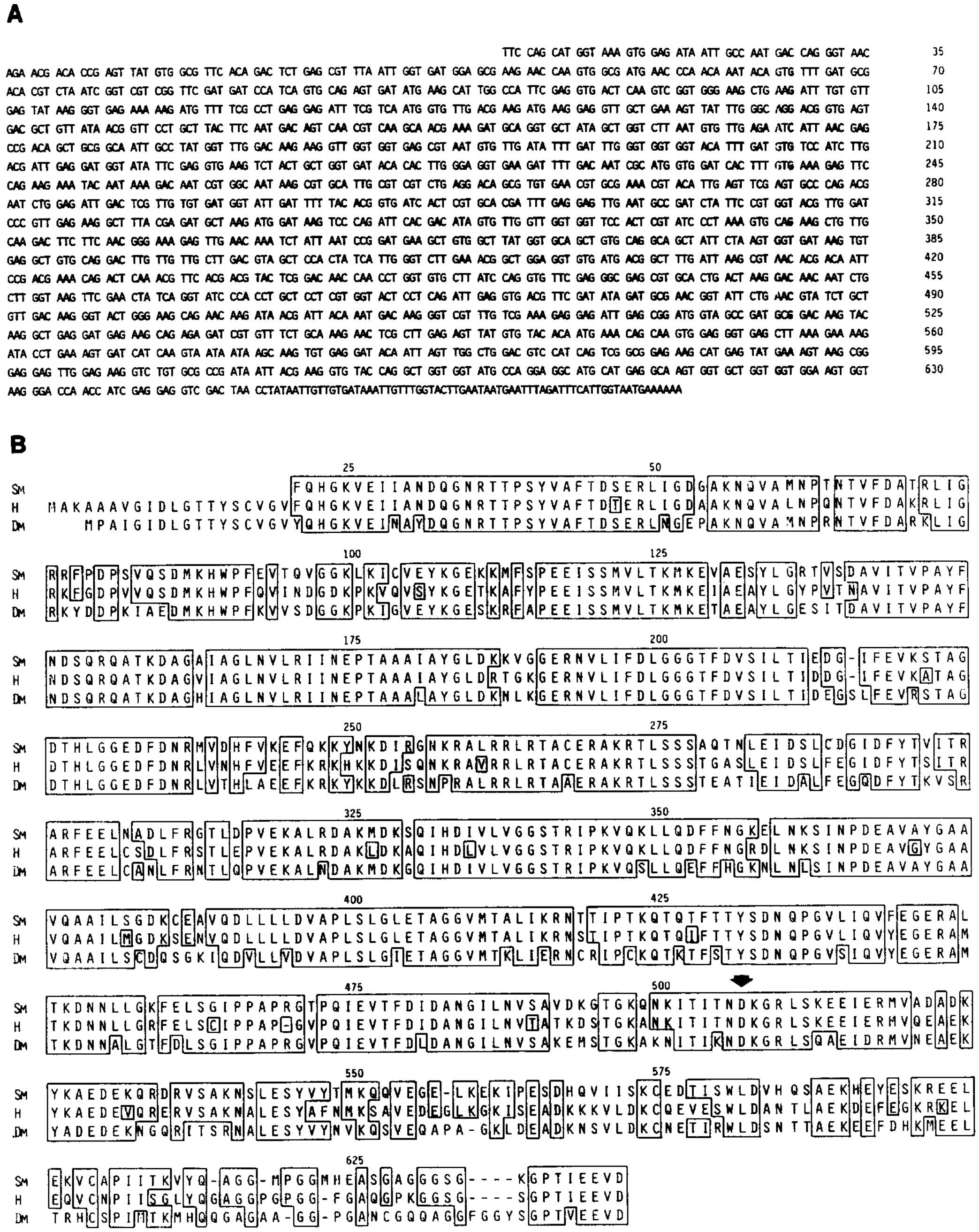

FIGURE 2. (*A*) Nucleotide sequence of the cDNA insert in λcSMA-F7 (F7) and (*B*) amino acid homologies between the *S. mansoni* 70,000 mol wt peptide sequence (*Sm*) and the corresponding region of the hsp70 protein sequence from human (*H*), and *Drosophila* (*Dm*). The amino acid sequence for the *S. mansoni* peptide was derived from the cDNA sequence of clone F7, and the human and *Drosophila* sequences were from Hunt and Morimoto (17). Regions of identity are enclosed and numbers refer to the amino acid residues of the human sequence. The F4 clone encodes the COOH-terminal region beginning with the aspartic acid residue at position 507 (*arrow*).

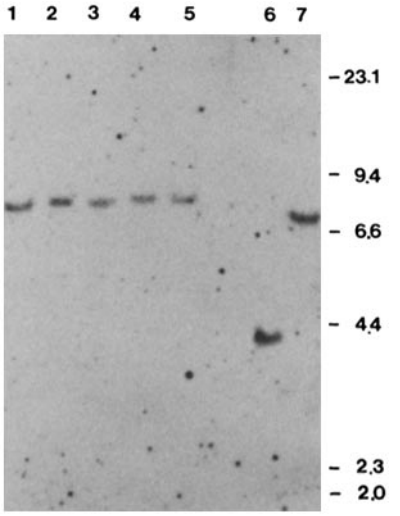

FIGURE 3. Genomic Southern hybridization of adult worm DNA with the *S. mansoni* hsp70 cDNA insert probe from clone F7. DNA (5 μg) was digested with Ava I (*1*), Bgl I (*2*), Hind III (*3*), Xho I (*4*), Bam HI (*5*), Eco RI (*6*), and Eco RV (*7*). A 16-h autoradiographic exposure is shown. Kilobases shown at right.

to the schistosome hsp70 antigen indicates a more ready access of the molecule to the host immune system. Heat shock or stress-induced synthesis of hsp70 is a response seen in all organisms, however, members of the hsp70 gene family have also been found that are constitutively expressed. An emerging but speculative view, based on the identification of constitutively expressed hsp70-related proteins, is that hsp70s have multiple in vivo functions in addition to the heat-shock response (14–16); hsp70 is thought to be generally involved in ATP-dependent disruption of protein aggregates formed in response to heat shock.

With the definition of the functional roles of heat-shock proteins and their constitutive analogues it will be possible to assess the role of the *S. mansoni* hsp70 homologue and to integrate our understanding of this protein in the developmental biology and biochemistry of these parasitic helminths. The abundance and restricted but dominant immunogenicity of the COOH-terminus of this *S. mansoni* hsp70 and the universality of the mammalian response to this antigen suggest an important role for the hsp70 gene family in schistosomes.

Summary

A 70,000 mol wt protein of *Schistosoma mansoni* was shown to be a major immunogen that invariably elicited an antibody response in infected humans. The universality of the response to this abundant antigen was confirmed in experimental animals and included the antibody response associated with the protective irradiated cercarial vaccine. We identified the 70,000 mol wt antigen as an *S. mansoni* homologue of the major eukaryotic heat-shock protein hsp70 by DNA sequence analysis of a cDNA insert from a λgt11 clone expressing the antigen and located the immunodominant epitope near the COOH-terminus of the molecule. The antigenic relationship of hsp70 to schistosome infections suggested an important role for this protein in parasite development and pathogenesis.

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