Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 1842–1846, March 1992
Immunology

cDNA cloning and functional expression of the *Schistosoma mansoni* protective antigen triose-phosphate isomerase

(recombinant DNA/glycolysis/vaccine/protective immunity)

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Communicated by Elkan R. Blout, November 12, 1991 (received for review June 27, 1990)

ABSTRACT **M.1 monoclonal antibody has previously been shown to passively transfer partial resistance to schistosome infection within mice and to recognize a 28-kDa antigen that has peptide sequence homology with triose-phosphate isomerase (TPI; D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1). We have now isolated the complete coding DNA for *Schistosoma mansoni* TPI and confirmed that this cDNA encodes the 28-kDa antigen recognized by M.1. The predicted translation product has strong homology with other TPIs, particularly from higher eukaryotes, and the sequence homology is greatest in regions known to form the active site. The complete coding DNA has been expressed within an *Escherichia coli* host to produce high levels of soluble, recombinant *S. mansoni* TPI protein. The product is recognized and purified by the M.1 antibody and is a functional TPI with an intrinsic specific activity comparable to that of rabbit and yeast TPI.**

Schistosomiasis is a chronic, often debilitating, parasitic disease afflicting several hundred million individuals worldwide. In addition, related schistosome parasites that infect livestock cause a serious hardship in many third-world nations. The disease is characterized by the presence of adult worms, or blood flukes, within the portal and mesenteric veins or within the veins of the bladder. The primary pathological consequences of schistosomal infection are caused by the host's granulomatous immune response to the eggs, produced by the male/female worm pairs.

Despite having effective immune avoidance mechanisms, it is the consensus view that schistosomes induce, upon infection of human and rodent hosts, variable levels of resistance to reinfection (1). The partial immunity can be passively transferred, in rodents, by immune sera and by monoclonal antibodies against a variety of different schistosome antigens (see refs. 2 and 3 for reviews). In several cases, the antigens identified by the partially protective monoclonal antibodies have been used as immunogens to induce partial protection to infection. It is hoped that one or several of these antigens, produced through recombinant DNA technology, might form the basis of a schistosomiasis vaccine. A vaccine inducing even partial protection could have a major impact in endemic areas by decreasing the disease pathology and slowing transmission rates.

Monoclonal antibody M.1 recognizes a 28-kDa antigen found in all life cycle stages of schistosomes (4). The antibody imparts a significant, though partial, level of protection against parasite challenge after passive transfer into naive mice (4). Immunization with immune complexes containing the 28-kDa antigen bound to M1 were found to induce a 38% reduction ($P < 0.05$) in parasites after challenge infection (5). Recently, the M.1 antibody was used as an immunoaffinity reagent to purify the 28-kDa antigen (6). Several tryptic peptides of the purified protein were subjected to amino-terminal sequence analysis and their sequence revealed strong homology to mammalian and microbial triose-phosphate isomerase (TPI; D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1). Thus, this antigen is distinct from the protective *Schistosoma mansoni* 28-kDa antigen that has been identified as a glutathione-*S*-transferase (7). In this paper, we report the cloning of a full-length cDNA of *S. mansoni* TPI (STPI) and show it to encode the amino acid sequence found within the 28-kDa antigen recognized by M.1 monoclonal antibody.† The coding DNA has been expressed to high levels within bacteria and the soluble product has been purified by immunoaffinity with the M.1 monoclonal antibody and shown to have TPI enzymatic activity.

MATERIALS AND METHODS

Isolation of STPI cDNA Clones. A 700-base-pair (bp) *Pst* I/*Eco*RI cDNA fragment containing the near complete coding DNA for human TPI (8) was purified from the plasmid PkT217 (kindly provided by L. Maquat, Roswell Park Memorial Institute) by the method of Vogelstein and Gillespie (9). The DNA was radiolabeled by random priming with [α-^{32}P]CTP (10) using the multiprime kit (Amersham) as recommended by the manufacturer.

Approximately 50,000 recombinant phage plaques from an *S. mansoni* cercarial cDNA library (11) were plated onto NZCYM plates (12). Duplicate replica filters of the plates were hybridized overnight with the human TPI probe (10^6 cpm/ml) at 50°C under aqueous conditions and extensively washed in 2× standard saline citrate/0.1% SDS at 50°C. Phage showing homology with the probe were plaque purified, the phage DNA was prepared, and the cDNA inserts were subcloned into an M13 vector for characterization (12).

Characterization of cDNA Clones. The M13 subclones of the TPI homologous *S. mansoni* cDNA inserts were characterized by restriction mapping and DNA sequencing. For the complete DNA sequence, replicative form DNA was first digested by a restriction enzyme that cleaved uniquely on one side of the insert and, in a second reaction, with another restriction enzyme that cleaved uniquely on the other side. The two preparations of linear DNA were digested with BAL-31 (New England Biolabs), under conditions recommended by the manufacturer, for various periods of time before quenching. DNAs from the different BAL-31 digestion times were pooled, extracted with phenol/chloroform, ethanol precipitated, and then redigested with the restriction enzyme that cleaves at the unique site on the opposite side of the cDNA insert from the first cleavage site. The DNA from

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Abbreviations: TPI, triose-phosphate isomerase; STPI, *Schistosoma mansoni* TPI.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M83294).

the two deletion series was separated by agarose gel electrophoresis and fragments of size classes differing by ≈200 bp were excised from the gel and purified by the method of Vogelstein and Gillespie (9). Each fraction was individually subcloned into an M13 vector and two recombinant phage plaques from each, showing homology to a probe made from the purified cDNA insert, were sequenced (13). The results were sufficient to generate the complete DNA sequence for both strands of cDNA.

Construction of the Expression Vector for STPI. The vector used, pA/L19 (similar to pA/L181, ATCC 40134), contains the P_L promoter and operator of λ phage immediately followed by the ribosome binding site and initiation codon for the λ *c*II gene product and then a series of unique restriction sites. One site, *Kpn* I, is positioned such that cleavage and treatment with the Klenow fragment of DNA polymerase I (Klenow) results in a flush end immediately following the initiator methionine of the *c*II gene product. We precisely fused the second codon of STPI to the initiator codon of cII at this position. An 18-mer oligonucleotide was prepared that is homologous to the antisense strand of STPI coding DNA and whose 5′ end is the first base of the second codon. This oligonucleotide was used to prime DNA synthesis of an M13 subclone of STPI. The DNA product was treated with S1 nuclease, Klenow, and then a restriction enzyme cleaving downstream of the STPI coding DNA. The DNA fragment containing the complete STPI coding DNA was then ligated into the pA/L19 vector such that the second codon of STPI was blunt-end ligated to the initiator codon of the vector. DNA sequencing was used to determine whether the STPI coding DNA was correctly fused to the cII initiator codon and plasmid QI2-1 was chosen as the expression vector.

Expression, Isolation, and Purification of STPI. The expression vector QI2-1 was introduced, by transformation, into the microbial host GI400. GI400 is an *Escherichia coli* W3110laqI^q derivative that constitutively expresses the *c*I857 (temperature-sensitive repressor of the P_L promoter), *REX*, and *N* gene products of λ (kindly provided by J. McCoy, Genetics Institute, Cambridge, MA). The cells were expanded at 30°C and grown under vigorous agitation until approximately $A_{600} = 1$. At that point, the temperature of the air shaker was adjusted to 42°C and the culture was incubated for an additional 3 hr. Cells were pelleted by centrifugation, resuspended in hypotonic buffer (50 mM phosphate buffer/10 mM 2-mercaptoethanol/10 mM EDTA, pH 7.4), and lysed by extensive sonication. The sonicate was pelleted at 13,000 × *g* for 10 min and the supernatant was obtained. Iodination of the bacterial supernatant proteins was done by the method of Bolton and Hunter (14). STPI protein was purified from the supernatant by affinity to the M.1 monoclonal antibody and will be described elsewhere (6).

TPI Assays. Rabbit muscle TPI and yeast TPI were obtained from Sigma and were desalted on an Excellulose GF-5 column (Pierce). TPI assays were carried out in the forward direction, measuring the conversion of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate, as described by Lambeir *et al.* (15).

RESULTS

Cloning and Characterization of STPI cDNA. M.1 monoclonal antibody recognizes a 28-kDa antigen from *S. mansoni* and imparts a significant, though partial, level of protection against parasite challenge after passive transfer into naive mice (4). Recently, the M.1 antibody was used to purify the 28-kDa antigen, and subsequent amino-terminal sequence analysis of the pure protein revealed strong homology with mammalian and microbial TPI (6). Because TPI is known to be well conserved during evolution, a human TPI cDNA probe was used in an effort to isolate a cDNA clone of STPI.

Approximately 50,000 recombinant phage plaques from an *S. mansoni* cercarial cDNA library (11) were screened with a human TPI cDNA probe (8) at low stringency. Four weakly positive plaques were identified from this library screen. The cDNA inserts from the purified recombinant phage were prepared and subcloned into M13 vectors for characterization.

The *S. mansoni* cDNAs, each ≈1.1 kilobases, were sequenced from both ends and one was sequenced in its entirety. A poly(A) sequence is located in the same position at one end of each clone and presumably identifies the 3′ end of STPI mRNA. The largest cDNA (STPI2) is 1082 bp and contains an open reading frame encoding 253 amino acids (Fig. 1). The amino acid sequences obtained from tryptic peptides of the purified, native 28-kDa antigen (6) are found within the predicted translation product (underlined in Fig. 1), confirming that the STPI cDNA clone derives from the mRNA encoding this antigen.

The amino acid sequence of STPI displays strong homology to TPI from organisms spanning the broad evolutionary range from microbes to mammals (Fig. 2). Of the known sequences, TPI from higher eukaryotes such as human (8), rabbit (20), chicken (16), maize (21), and coelocanth (22) are most homologous to STPI, each with ≈60% identity. Alignment of STPI and mammalian TPI requires only a single amino acid gap to obtain maximal homology (STPI amino

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TAATAATCATATAGATTCCGTTCACCAAGGAAATGTCTGGATCTCGCAAATTTTTTGTTG      60
                          M  S  G  S  R  K  F  F  V                 9

GGGGGAACTGGAAAATGAACGGAAGCAGAGATGACAACGATAAGTTACTTAAGTTACTCT     120
G  G  N  W  K  M  N  G  S  R  D  D  N  D  K  L  L  K  L  L       29

CAGAAGCTCATTTTGATGATAACACGGAAGTTTTAATTGCTCCACCTTCAGTCTTTTTGC     180
S  E  A  H  F  D  D  N  T  E  V  L  I  A  P  P  S  V  F  L       49

ACGAAATTCGAAAAAGTTTGAAGAAAGAAATACACGTGGCCGCTCAAAATTGCTATAAAG     240
H  E  I  R  K  S  L  K  K  E  I  H  V  A  A  Q  N  C  Y  K       69

TATCAAAGGGTGCATTCACCGGAGAAATCAGCCCAGCAATGATAAGAGATATTGGTTGTG     300
V  S  K  G  A  F  T  G  E  I  S  P  A  M  I  R  D  I  G  C       89

ATTGGGTCATACTTGGACATTCTGAGCGTAGAAACATTTTTGGTGAATCTGATGAACTTA     360
D  W  V  I  L  G  H  S  E  R  R  N  I  F  G  E  S  D  E  L      109

TTGCTGAAAAAGTTCAACATGCACTTGCTGAAGGTCTAAGCGTTATTGCATGTATTGGTG     420
I  A  E  K  V  Q  H  A  L  A  E  G  L  S  V  I  A  C  I  G      129

AAACATTATCAGAGCGTGAATCTAATAAAACAGAGGAAGTATGCGTTAGACAGTTAAAAG     480
E  T  L  S  E  R  E  S  N  K  T  E  E  V  C  V  R  Q  L  K      149

CTATTGCAAATAAGATTAAATCAGCTGATGAATGGAAACGAGTAGTCGTAGCATATGAAC     540
A  I  A  N  K  I  K  S  A  D  E  W  K  R  V  V  V  A  Y  E      169

CAGTTTGGGCTATTGGAACAGGTAAAGTTGCTACACCACAACAAGCTCAAGAAGTTCATA     600
P  V  W  A  I  G  T  G  K  V  A  T  P  Q  Q  A  Q  E  V  H      189

ATTTCCTTCGTAAATGGTTTAAAACGAATGCACCAAATGGAGTTGATGAAAAAATACGTA     660
N  F  L  R  K  W  F  K  T  N  A  P  N  G  V  D  E  K  I  R      209

TTATCTATGGTGGATCTGTAACTGCTGCCAATTGTAAAGAATTAGCTCAACAACATGATG     720
I  I  Y  G  G  S  V  T  A  A  N  C  K  E  L  A  Q  Q  H  D      229

TCGATGGATTTTTGGTTGGTGGAGCTTCATTAAAACCGGAATTCACTGAGATATGTAAAG     780
V  D  G  F  L  V  G  G  A  S  L  K  P  E  F  T  E  I  C  K      249

CCAGACAACGTTGAACGTTTTGTTGGTCTTCTTCTTCTTACGCTTCTAAATTTTGTCTCT     840
A  R  Q  R  -                                                   252

TCTCATGTCAGTTACTTGCTTTTTTTGTCTTGTTTTTTTGTTTTGACAACCAAAGCATAT     900

TATAATTAGTAGTATCTTAATGTCTAATGTAAAAAAAAATATCACCACTAGTTCGAACTA     960

TTTAATTACTATCAGTCAAAAAAATAATATTTGTCAATTTAGTTCATTGTTTCATTTATC    1020

GTCCCTTCTATTTAAATTTCTTATGGCCTAAATATACCTTGAATTAATAAAAAAAAAAAA    1080
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FIG. 1. DNA sequence and deduced amino acid sequence of STPI cDNA. Numbering for the DNA begins at the first base of the longest cDNA isolate. Numbering for the amino acid sequence begins at the methionine encoded by the first initiation codon and ends at the first termination codon. Underlined amino acids represent sequences previously identified by microsequencing of tryptic peptides from the 28-kDa antigen recognized by the M.1 monoclonal antibody.

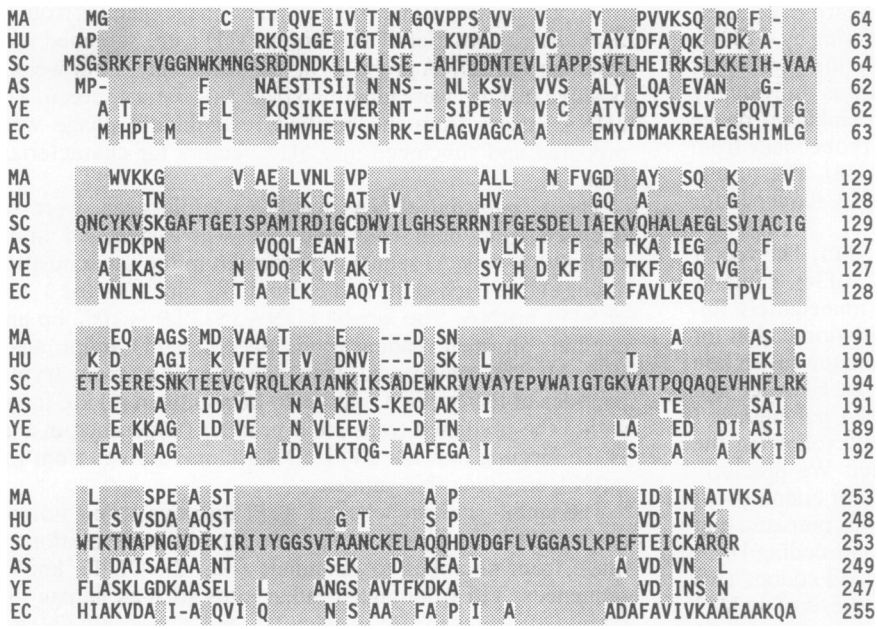

FIG. 2. Homology between STPI and TPI from various other organisms. The six TPI sequences compared are maize (MA) (16), human (HU) (8), *S. mansoni* (SC), *A. nidulans* (AS) (17), *Saccharomyces cerevisiae* (YE) (18), and *E. coli* (EC) (19). Amino acid identity is shown as a shaded area. Positions in which a gap was introduced to maximize homology are shown as dashes.

acid 157). TPI from prokaryotes such as *E. coli* (19) and *Bacillus stearothermophilus* (23) or from unicellular eukaryotes such as yeast (18) and trypanosomes (24) exhibit ≈45% identity with STPI. The slime mold *Aspergillus nidulans* TPI (17) has 54% homology to STPI. Not surprisingly, the sequence homology is greatest in those regions of the protein that are known to be part of the active site from crystallographic data (schistosome residues 12–14, 73–76, 96–98, 101–102, 127, 129–131, 167–171, 174, 213–214, 216, and 234–239; ref. 25) and poorest in the hydrophilic, surface-exposed regions.

Expression of Recombinant STPI. The coding DNA of STPI was precisely inserted into the microbial expression vector pA/L19 such that the primary structure of the recombinant translation product, expressed within *E. coli*, should be identical to native STPI (see *Materials and Methods*). This expression vector utilizes the powerful λ P_L promoter for transcription of the inserted coding DNA. The resulting expression plasmid, QI2-1, was introduced into the *E. coli* host cell GI400, which constitutively expresses the temperature-sensitive repressor of λ P_L promoter (cI857). Transformed GI400 cells harboring QI2-1 and grown at 32°C have little or no visible 28-kDa protein. When the growth temperature was raised from 32°C to 42°C to inactivate cI857, the cells began to produce large quantities of a 28-kDa protein (Fig. 3*A*). A large portion of the induced 28-kDa protein remains in the supernatant when the cells are lysed and centrifuged at 13,000 × *g* (Fig. 3*A*). Most of the 28-kDa protein in the pellet can be solubilized by resuspending the pellet in lysis buffer and recentrifuging (data not shown).

Purification and Characterization of Recombinant STPI. M.1 monoclonal antibody is highly specific for schistosome TPI and does not recognize TPI from yeast, rabbit, or dog (6). We tested the ability of M.1 to recognize and purify recombinant STPI from the bacterial extraction supernatant. M.1 antibody, crosslinked to protein A Sepharose, was incubated with the bacterial extract, pelleted, and extensively washed; the bound protein was eluted at pH 12. As shown by SDS/PAGE analysis (27) in Fig. 3*A*, the temperature-induced 28-kDa protein is purified to near homogeneity by absorption to the M.1 immunoaffinity resin. The band of ≈60 kDa within the purified fraction is also induced by the temperature shift and presumably is a dimer form of the 28-kDa product (the 70-kDa band is a silver stain artifact).

To approximate the proportion of the soluble 28-kDa protein that is recognized by the M.1 resin, the proteins in the crude bacterial extract were labeled with iodine and the immunoaffinity purification was performed by using resin containing a molar excess of antibody. SDS/PAGE analysis, followed by autoradiography, reveals that the majority of the soluble 28-kDa protein is specifically bound and eluted from the M.1 resin. Thus, it appears that recombinant STPI is

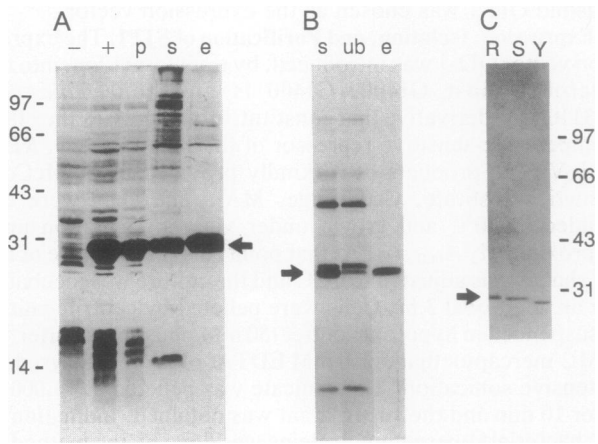

FIG. 3. Characterization of recombinant STPI expression and properties by SDS/PAGE analysis. (*A*) Expression and purification of recombinant STPI. GI400 cells harboring the QI2-1 plasmid were grown at 32°C to A_{260} = 1 and harvested before (lane –) or after (lane +) a 3-hr incubation at 42°C. The temperature-induced cells were concentrated, lysed by extensive sonication, and centrifuged. The pellet (lane p) was removed and the supernatant (lane s) was absorbed to M.1 immunoaffinity resin. The resin was washed and the high pH eluate (lane e) was obtained. Equivalent aliquots of each fraction were analyzed on a 12.5% gel and stained with silver (26). (*B*) Analysis of the M.1 antibody-binding fraction of soluble, recombinant STPI. The supernatant fraction (lane s) was radioiodinated and a small aliquot was absorbed to immunoaffinity resin containing a molar excess of crosslinked M.1 antibody. The unbound protein (lane ub) was removed, the resin was washed, and the high pH eluate (lane e) was obtained. Equivalent aliquots of each fraction were analyzed on a 12.5% gel, and the gel was dried and subjected to autoradiography. (*C*) Comparison of the specific activity of purified recombinant STPI (lane S) to that of TPI from rabbit and yeast. Rabbit muscle TPI (lane R) (Sigma) and yeast TPI (lane Y) (Sigma) were desalted and assayed for TPI activity. Purified recombinant STPI was also assayed for TPI activity. Volumes equivalent to 1.5 units of TPI activity were analyzed on a 10% gel and stained with Coomassie brilliant blue R-250 (GIBCO/BRL). Arrows indicate 28-kDa protein.

efficiently expressed in soluble form by bacteria carrying the QI2-1 plasmid and that most of the product is recognized by the M.1 monoclonal antibody. Rabbit polyclonal antisera against native schistosome 28-kDa antigen also recognizes the recombinant STPI (data not shown).

Purified recombinant STPI was next assayed for TPI activity. The purified preparations displayed an unambiguous ability to catalyze the conversion of glyceraldehyde 3-phosphate to dihydroxyacetone phosphate. Demonstration of enzymatic activity of the recombinant protein implies that at least some portion of the soluble, recombinant STPI retains a native conformation and forms the functionally active homodimeric structure (25). To estimate the active fraction, the specific activity of purified STPI was compared to that of rabbit muscle and yeast TPI by SDS/PAGE analysis. Equivalent units of the three TPI samples were loaded onto the gel and the results are shown in Fig. 3*C*. Only small differences in the quantity of the 26- to 28-kDa TPI bands are indicated, suggesting that recombinant STPI, produced in *E. coli*, has a specific activity that is similar to that from its distant evolutionary relatives.

DISCUSSION

During the past 10 years, much of the effort toward developing a schistosomiasis vaccine has focused on the use of monoclonal antibodies to identify schistosome antigens capable of inducing protection against cercarial infection. Several laboratories have produced monoclonal antibodies against schistosome antigens that are capable of transferring partial protection against cercarial challenge in mice by passive transfer (reviewed in ref. 2). One such antigen is a 28-kDa protein found in all stages of the parasite and recognized by the M.1 protective monoclonal antibody (4). Recently, we have found that this antigen contains considerable amino acid sequence homology to TPI. In this report, we have isolated the complete coding DNA for STPI and confirmed its identity to the 28-kDa antigen. We have also shown that microbially expressed STPI is enzymatically active and recognized by the M.1 protective monoclonal antibody.

The amino acid sequence homology between STPI and TPI from other sources spanning a wide evolutionary range did not produce any major surprises. Sequences within the known active site are very well conserved and there are no new sites that require insertion or deletion of amino acids to retain the close homology. Because STPI is frequently recognized by antisera and T cells from infected humans and mice (D.H., unpublished data), it must be a good immunogen as presented by schistosomes. We are, thus, particularly interested in identifying those regions of STPI with the weakest homology to their mammalian counterparts as they represent the best candidates for the immunogenic epitope(s). By this criteria, the most apparent candidate site is the highly charged region between STPI amino acids 18 and 61 as the sequence identity within this region is only ≈28% between STPI and human TPI. Other candidates include the regions between amino acids 190 and 208 and amino acids 137 and 163, both of which are hydrophilic, with the latter including a three-amino acid insertion.

The ability of the anti-STPI M.1 monoclonal antibody, and the natural 28-kDa STPI antigen, to impart some protection against schistosome infection in mice raises the distinct likelihood that STPI will be capable of inducing a similar or improved level of protection upon appropriate immunization. With the availability of large quantities of full-sized, functionally active, and easily purified STPI made in *E. coli*, we have now begun to test this possibility and the preliminary results are promising (G. Wei and D.H., unpublished data). We can also utilize the cloned STPI coding DNA to express recombinant STPI within well-characterized vaccination systems such as vaccinia virus (28) or bacillus Calmette–Guérin mycobacteria (29). Even if partial levels of protection can be achieved with a STPI vaccine, this would reduce the worm burden of infected individuals and potentially provide a significant reduction in disease-related pathology. The levels of protection might be improved further by immunizing with other recombinant antigens, such as paramyosin (30) and/or glutathione-*S*-transferase (7, 31), that have also been shown to induce partial protection after immunization.

We are grateful to Dr. Lynne E. Maquat for providing the PkT217 plasmid containing human TPI cDNA and to Dr. John McCoy for providing the pA/L19 plasmid and the GI400 host strain. We thank Tomas Sanchez and Mitermeyer Reis for their help with DNA sequencing and Sandy Reynolds for supplying TPI preparations and running protein gels. These studies were funded by National Institutes of Health Grants 5POI AI22794, AI24557, and AI24570, and by grants from the Edna McConnell Clark Foundation, the United Nations Development Programme/World Bank Program for Research and Training in Tropical Diseases, the MacArthur Foundation, and the TDR/Rockefeller Research Strengthening Program.

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