

THE MAJOR PARASITE SURFACE ANTIGEN ASSOCIATED
WITH HUMAN RESISTANCE TO SCHISTOSOMIASIS IS A
37-KD GLYCERALDEHYDE-3P-DEHYDROGENASE

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Schistosomiasis due to *Schistosoma mansoni* is a snail-transmitted parasitic disease that affects millions of individuals and is a major concern for a number of subtropical countries (1). Control by chemotherapy and mollusciciding is costly, may lead to the emergence of resistant strains of parasites, and requires well-trained personnel who are seldom available in endemic areas (2-6); for these reasons, additional control methods are sought.

Immunological studies in laboratory animals have raised expectations that a vaccine against *S. mansoni* could be produced. However, a major difficulty in developing a vaccine against this parasite is that natural immunization schemes consisting of repeated infections interrupted by chemotherapy fail to protect a sizeable fraction of the population of endemic areas (7-10). The most susceptible individuals often bear heavy infections and are at high risks of developing severe clinical disease (11-13); they are also the principal reservoir of parasites. It is, therefore, most important to identify the causes of the higher susceptibility of these individuals in order to define the properties that a vaccine should have in order to protect them.

Since a number of studies indicated that antischistosomal IgG antibodies were central to animal protection against *S. mansoni* (reviewed in reference 14), we have evaluated the antilarval surface IgG antibody response of subjects with high or low susceptibilities to infection by *S. mansoni* (10). Subjects of both susceptibility had comparable levels of antilarval IgG; IgG from the sera of the majority (50-80%) of subjects with low susceptibility reacted with three major larval surface antigens of *M*, 165, 72, and 37 kD. Sera from 50-80% of the most susceptible individuals also reacted with the 165 and 72 antigens (Ag);¹ most of them, however, failed to react with the 37 Ag (P-37). This indicated that the 37 Ag may represent a marker of resistance to *S. mansoni* and may be a target of protective immunity. We report here on the cloning of the cDNA for this antigen and on the homology it shares with a glycolytic

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¹ *Abbreviations used in this paper:* Ag, antigen; IHS, immune human serum; IPTG, isopropyl β -D-thiogalactopyranosid IRS, immune rabbit serum; MEM, modified Eagle's medium; PS, penicillin and streptomycin.

enzyme that is highly conserved in evolution. The hypothesis is discussed that genetic restriction of the immune response to these proteins might occur in heterogeneous human populations because of the limited number of epitopes borne by these host-like proteins.

Materials and Methods

Chemicals, Enzymes, and Reagents

Modified Eagle's medium (MEM), Hepes, penicillin, and streptomycin were purchased from Gibco Laboratories (Paisley, Scotland); *n*-octylglucoside (1-*O*-*n*-octyl- β -*O*-glucopyranoside), pepstatin, PMSF, α_2 -macroglobulin, leupeptin, dithiothreitol, and cesium chloride were from Boehringer Mannheim Biochemicals (Mannheim, FRG); aprotinin, *N*-lauroylsarcosine, and Triton X-100 were from Sigma Chemical Co. (St. Louis, MO); DMSO was from Merck Biochemica (Darmstadt, FRG). Glyoxal technical was purchased from BDH Chemicals Ltd. (Poole, England) and deionized on analytical grade mixed bed resin AG501-X8 from Bio-Rad Laboratories (Richmond, CA). Protein A-Sepharose CL-4B was from Pharmacia LKB Biotechnology AB (Uppsala, Sweden). SDS was from Fluka Chemie AG (Buchs, Switzerland). CFA and IFA were from Difco Laboratories Inc. (Detroit, MI). Rabbit reticulocyte lysates for in vitro translation, restriction enzymes, and exonuclease III and VII were from BRL Life Technologies, Inc. (Clergy Pontoise, France). DNA sequencing was performed using a sequenase kit (United States Biochemical Corp., Cleveland, OH). Alkaline phosphatase-conjugated anti-IgG antibodies were purchased from Promega Biotec (Madison, WI). FITC-conjugated IgG anti-rabbit or anti-mouse Ig were from Institut Pasteur Production (Marnes-la-Coquette, France). x-ray films were from Fuji Photo Film Co., Ltd. (Japan).

Parasitologic Methods

The Puerto Rican strain of *S. mansoni* used in this study was obtained from the Harvard Medical School colony (Boston, MA), and was maintained by passage through CBA/J mice and *Biomphalaria glabrata* snails. Schistosomula were prepared by the method of Ramalho Pinto, as modified by Lazdins et al. (15), and cultured for 3–4 h at 37°C in MEM supplemented with 10 mM Hepes, 100 IU/ml penicillin, and 100 μ g/ml streptomycin.

Adult schistosomes were recovered from the hepatic portal vein of 6–8-wk-old infected mice by perfusion (16). The collected worms were washed several times in MEM, Hepes, penicillin, and streptomycin medium, and were immediately frozen in liquid nitrogen.

Immunologic and Biochemical Procedures

Tegument Extraction. Schistosomular tegument was extracted by resuspending schistosomula (10^5 /ml) in 1% *n*-octylglucoside containing 10 mM phosphate buffer (pH 7.1), 4 mM $MgCl_2$, 140 mM NaCl, to which the following protease inhibitors were added just before use: 50 μ M PMSF, 50 μ g/ml aprotinin, 1 μ M pepstatin, 20 μ g/ml leupeptin, and 10 μ g/ml α_2 -macroglobulin. After 15 min at 4°C in the extraction solution, schistosomula bodies were pelleted (800 *g*, 3 min, 4°C) and the supernatants were centrifuged at 10^5 *g* for 35 min at 4°C. Then the supernatants of ultracentrifugation were stored at –70°C until use.

Preparation of P-37. Schistosomular tegumental membrane extracts were heated (100°C, 3 min) in sample buffer (1% SDS [wt/vol] 50 mM Tris HCl, pH 8, 5% 2-ME, 1 mM EDTA, 10% [vol/vol] glycerol) and subjected to electrophoresis on a 7.5% SDS polyacrylamide gel. The band of interest was identified as described (10) and cut off the gel, then, P-37 was eluted according to Hunkapiller et al. (17) with few modifications (10). This method allowed the preparation of 0.1–0.3 μ g of P-37 from the membrane extracts of 10^3 schistosomula. The electroeluted Ag yielded one sharp band after rerunning on polyacrylamide gels and staining by the silver stain method (18).

Preparation of the Anti-P37 Rabbit and Mouse Antisera. A rabbit and several BALB/c mice were primed with 500 μ g (rabbit) or 50 μ g (mice) of the electro-eluted P-37 preparation in CFA and boosted twice at 2-mo intervals with the same amount of material in IFA. Animals were bled beginning 3 wk after the last injection. The immune sera reacted with one protein

band (37 kD) on Western blots of schistosomular membrane extracts that was undistinguishable from the P-37 protein band identified by sera from subjects with low susceptibility to infection by *S. mansoni* (data not shown). Control sera were prepared by immunizing animals with the adjuvant only.

Human Sera. The immune human sera (IHS) were from subjects whose susceptibility to infection by *S. mansoni* was determined in a previous study (10).

Immunofluorescence Studies. Fresh schistosomula were washed three times in cold MEM supplemented with 10 mM Hepes, 10 IU/ml penicillin, and 100 UG/ml streptomycin, and were reacted at 4°C for 2 h with a 1:200 (rabbit serum) or 1:50 (mouse serum) dilution of anti-P-37, anti-rP-37 or control antisera. Then, larvae were washed three times in cold MEM/penicillin and streptomycin (PS)/Hepes medium and reacted for a further 1-h incubation period with a 1:50 dilution of FITC-conjugated IgG anti-rabbit or anti-mouse Ig. The larvae were then washed three times in cold MEM/PS/Hepes medium and examined under a fluorescence microscope (E. Leitz, Inc., Wetzlar, FRG) at a magnification of 400.

Immunoprecipitation of In Vitro Translation Products. ³⁵S-labeled in vitro translation products were precleared by incubation for 30 min at 4°C with protein A-coupled Sepharose beads in buffer A (10 mM Na₂PO₄, pH 7, 100 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mg/ml BSA). Then, samples were centrifuged (15 min, 12,000 g) and supernatants reacted for 2 h at room temperature with 10 μl (rabbit) or 5 μl (human) immune sera. Then, 1 vol of protein A-Sepharose 50% (vol/vol) was added and the incubation continued for 1 h at 4°C. Then, beads were washed four times in buffer A and resuspended in sample buffer, heated (5 min, 100°C), centrifuged (3 min, 12,000 g), and the supernatants were analyzed by SDS-PAGE and fluorography.

DNA and RNA Methods

DNA and RNA Isolation. Genomic DNA was isolated from cercarial nuclei (19). 5×10^4 frozen cercariae were homogenized in 4 ml of ice-cold buffer B (10 mM Tris-HCl, pH 7.4, 60 mM NaCl, 0.15 mM spermine, 0.15 mM spermidine, 10 mM EDTA, 0.5% Triton X-100). The mixture was centrifuged 20 s at 1,000 g. This first pellet was discarded and the supernatant centrifuged 7 min at 6,000 g. This second pellet contained the nuclei, it was resuspended in buffer B supplemented with 2% *N*-lauroyl sarcosine, and incubated 15 min at 55°C. Then, 1.273 (wt/wt) ($\rho = 1.7$) of solid cesium chloride was added, followed by ultracentrifugation for 50 h at 45,000 rpm (VTI 65; Beckman Instruments, Inc., Fullerton, CA). The DNA band was recovered and dialyzed against Tris-EDTA, pH 7.4. RNA was isolated from adult worms by the guanidinium isothiocyanate method (20).

cDNA Clones Isolation. The cDNA library was constructed in the bacteriophage λgt11 using poly(A)⁺ RNA isolated from adult worms (21). Recombinants were plated at high density on *Escherichia coli* Y.1090 under isopropyl β-D-thiogalactopyranosid (IPTG) induction conditions and replicates were screened using the rabbit antiserum raised against the 37-kD antigen and either ¹²⁵I-labeled protein A or alkaline phosphatase-conjugated anti-IgG antibodies. Plaques that were clearly positives were transferred to fresh agar plates and screened with the same reagents. This procedure was repeated until 100% of the plaques were found to be positive. The six cDNA clones that were isolated by this procedure also reacted strongly with the anti-P-37 mouse antisera. To further evaluate whether the inserts encoded distinct or related proteins, one cDNA insert was prepared following the procedure of Yamamoto (22), ³²P-labeled by the random priming method (23), and hybridized with replicas of the positive clones. At a stringency of 0.1 × saline sodium citrate (SSC) (1 × SSC is 150 mM NaCl, 15 mM sodium citrate), at 55°C, the probe hybridized with the six cDNA clones while it did not with irrelevant DNA control. Then, two cDNA clones that contained inserts of 0.7 and 1.1 kb were subcloned in the Bluescript vector (Stratagene, San Diego, CA) for the Northern and Southern blotting experiments and for cDNA sequencing.

DNA Sequencing. The 1.1-kb cDNA insert was sequenced using the dideoxy chain termination method (24) with Sequenase. Sequence data were confirmed in both orientations; one strand was sequenced on deleted Exo III-Exo VII Bluescript templates (25), and the opposite strand on restriction fragments subcloned in M13 bacteriophage (26).

Northern Blot. RNA samples were treated in 1 M glyoxal, 50% DMSO, 10 mM

Na_2HPO_4 , pH 7, for 1 h at 50°C, and run on a 1.1% agarose gel (27). Then, the gel was blotted against Hybond N (Amersham, les Ulis, France) nylon membrane overnight in 20× SSC. The membranes were then baked 2 h at 80°C to remove glyoxal and were reacted with a ^{32}P -labeled cDNA probe overnight at 37°C in 50% formamide. Washes were proceeded at 55°C to a stringency of 0.5× SSC. Then, membranes were allowed to dry and were autoradiographed at -70°C with an intensifying screen.

Genomic Southern Blot. Genomic DNA (5 µg) was digested with restriction endonucleases as described in Fig. 5 and separated on 1% agarose gel. DNA was then acid depurinated, denaturated, transferred to Nylon membrane (Gene Screen Plus; New England Nuclear, Boston, MA) overnight in denaturing buffer (0.5 M NaOH, 1.5 M NaCl), and neutralized (28). The membrane was then reacted with a ^{32}P -labeled cDNA probe overnight at 65°C in hybridization buffer, and washed at 65°C to a stringency of 0.1× SSC. The membrane, allowed to dry, was then autoradiographed at -70°C with an intensifying screen.

Hybrid Selection. mRNA were selected by hybridization of 10 µg of poly(A)⁺ adult worm RNA to 70 µg of linearized Bluescript vector containing the cloned cDNA and immobilized on cellulose powder. We essentially followed the protocol of Seed (29), except for the use of cellulose powder (Ce 180; Schleicher & Schuell, Inc., Dassel, FRG) instead of Whatman 50 paper. The activated cellulose powder was a generous gift of Dr. Tomaso Meo (Pasteur Institute, Paris). Selected mRNAs were translated in the rabbit reticulocyte lysates and the translation products were analyzed on a 10% SDS/acrylamide gel.

Results

Cloning of P-37 Encoding cDNAs. The anti-serum used for the screening of the cDNA library was prepared by immunizing a rabbit with electroeluted preparations of P-37. This antiserum yielded one 37-kD band on Western blots of schistosomular membrane extracts and it produced a strong membrane fluorescence when reacted with fresh living larvae. This serum, and sera from subjects with low susceptibility to infection, precipitated the same 37-kD polypeptide from the in vitro translated products of schistosome RNA (Fig. 1). The sera also precipitated a 32-kD polypeptide that probably corresponds to an internal initiation of the translation of P-37 RNA. These observations, altogether, indicated that the rabbit antiserum reacted with P-37 denaturated polypeptide chain and was suitable for the screening of an expression library.

With this serum, six independent clones were selected out of 2×10^5 plaques. These clones were also positive with a mouse antiserum raised against P-37. cDNA inserts from the six clones hybridized to each other in high stringency conditions, indicating that they encoded related polypeptides. Two cDNA clones selected for further work encoded a protein that had an apparent M_r of 153 kD (Fig. 2).

Rabbit antibodies, affinity purified on this recombinant protein, reacted with P-37 on blots of schistosomular membrane extracts (data not shown). Then, the cloned cDNA was inserted in the pJF-118EH vector (30) that allowed (in the presence of IPTG) the production of a 37-kD recombinant protein that was selectively recognized by the anti-P37 rabbit serum and by two IHS that had been selected for their high reactivity to P-37 (Fig. 2 A). The immunoreactive recombinant protein was absent from the bacteria that were cultured without IPTG. The reactivity of 18 immune human sera for this recombinant protein was also tested (as illustrated with 10 sera in Fig. 2). 11 of these 18 sera reacted with P-37 (in schistosomular membrane extracts), 6 of these 11 sera reacted with the recombinant protein (IHS 1, 3, 4, 5, 6, 7), and five sera (that exhibited a low reactivity for P-37) did not identify the recombinant protein, probably because the amount of recombinant protein on the

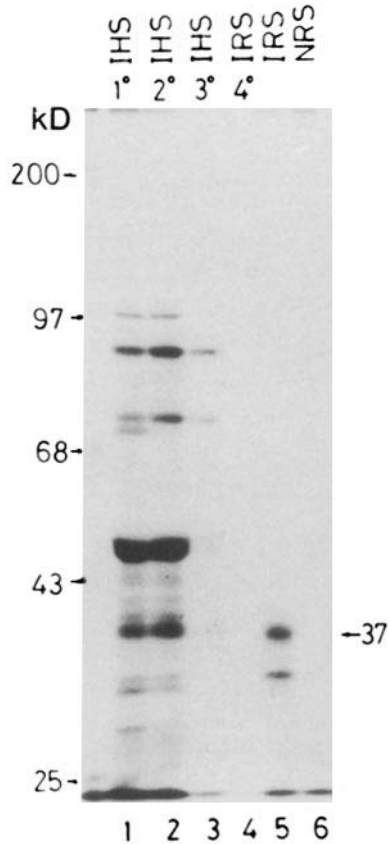


FIGURE 1. Serum from a resistant subject (IHS) and the rabbit anti-P-37 antiserum (immune rabbit serum [IRS]) precipitate the same 37-kD in vitro translated polypeptide of schistosome RNA. (Lanes 1-3) Precipitates from three successive rounds of precipitation with IHS. (Lanes 5 and 6) Polypeptides precipitated by IRS and NRS, respectively. (Lane 4) IRS reacted with the supernatant of the third precipitation of in vitro translation products by IHS.

blot was far lower than that of P-37. Seven sera reacted with neither one (i.e., IHS-2). Finally, the cloned cDNA was coupled to a cellulose matrix and used to purify its transcript from total schistosome mRNA by the method of hybrid selection. The hybrid-selected RNA encoded a 37-kD polypeptide that was selectively precipitated by the rabbit anti-P37 serum and by several IHS containing antibody against P-37 (Fig. 3). Altogether, these observations indicated that the recombinant polypeptide encoded by the cloned cDNA was the P-37 Ag identified in a previous study.

Gene and mRNA Encoding P-37. The inserts hybridized with a single 1.2-kb species in Northern blots of schistosome RNA (Fig. 4). Probing of genomic Southern blots of restricted cercarial DNA with the cDNA insert yielded single bands that, depending on the enzyme, varied in size from 3 to 11 kb (Fig. 5). Thus, the results of Northern and Southern blots are consistent with a single copy gene encoding a single mRNA species.

Nucleotide Sequence of Cloned cDNA. Nucleotide sequencing of an 1,103-bp cDNA insert revealed a 1,017-bp open reading frame that terminates with three stop codons and is flanked by 5' and 3' untranslated regions of 13 and 73 bp, respectively, and contains an AATAAA poly(A) addition signal in position 1070 (Fig. 6). The 338-amino

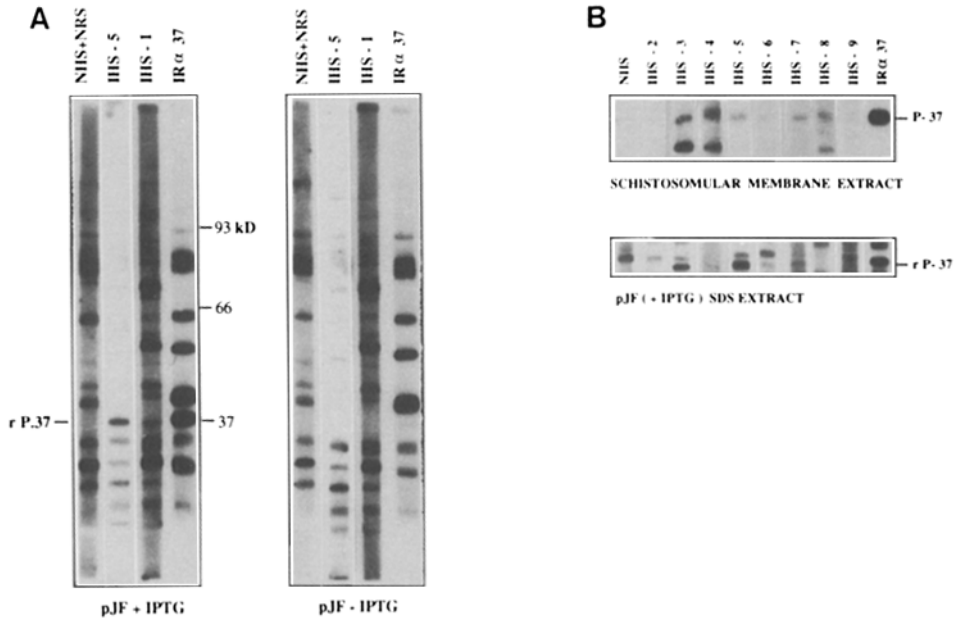


FIGURE 2. Reactivity of the anti-P37 IHS and IRS for the recombinant protein produced in pJF vector. (A) Reactivity of two IHS and of the anti-P-37 rabbit sera for detergent extracts of pJF-transformed *E. coli* that were cultured with (*left*) or without (*right*) IPTG. (B) Reactivity of eight IHS for P-37 (in schistosomular membrane extracts) and for the recombinant protein (in pJF extracts). The reactivity of the sera (dilution 1:50 to 1:200) for the recombinant protein and for P-37 was tested by immunoblotting using the equivalent of 200 μ l of bacterial culture or of 5-10 $\times 10^3$ schistosomula per lanes.

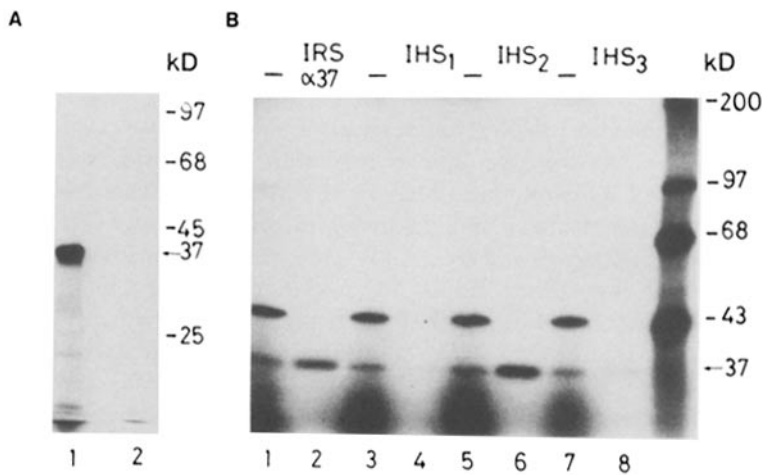


FIGURE 3. The cloned cDNA hybridizes with a worm mRNA that encodes a 37-kD polypeptide precipitable by sera from resistant humans. (A) Cell-free translation of mRNA hybrid selected by the cDNA subcloned in Bluescript (lane 1), or endogenous background in the absence of added RNA (lane 2). (B) Immunoprecipitation of cell-free translation products of the hybrid-selected mRNA (lanes 1, 3, 5, and 7) by the anti-37-kD IRS (lane 2) and by three sera from resistant humans (lanes 4, 6, and 8). The 45-kD band is also observed when the reticulocyte lysate is incubated with 35 S-methionine in the absence of added RNA.

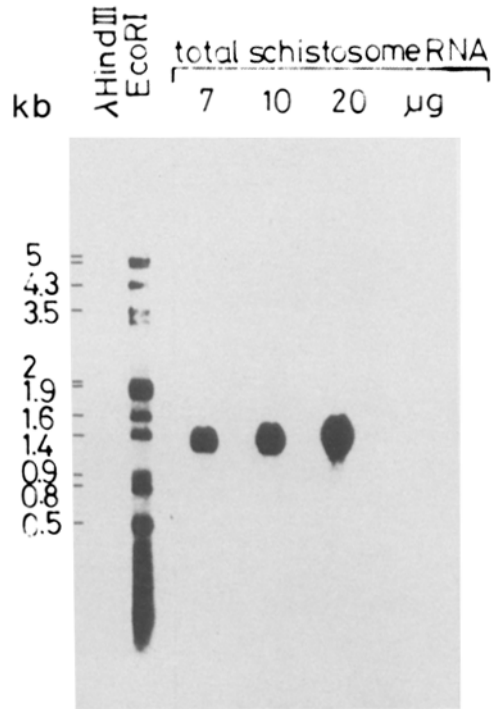


FIGURE 4. Northern blot analysis of total schistosome RNA probed with P-37 cDNA.

acid-encoded polypeptide has a predicted molecular mass of 36.4 kD and contains one potential *N*-glycosylation site (Asn-Ala-Ser) in position 150–152. Codon usage is nonrandom, A and T being more frequently used, except for lysine and asparagine, as previously reported for other schistosome proteins (31). Consequently, the cDNA has a 57.3% adenosine/thymidine content. The predicted amino acid sequence contains two clusters of hydrophobic residues (Leu-Val-Leu and Ala-Ala-Phe-Leu) near the NH₂ terminus that may represent a signal peptide for the translocation of the protein through the membrane. Similar signal peptides have been reported for human preprolactin and *E. coli* prepenicillinase (32).

P-37 Shows High Homology with the Glyceraldehyde-3-P-dehydrogenase (3-GAPDH). Comparison of the amino acid sequence of the recombinant protein (rP-37) with the sequence of other proteins compiled in the Microgenie program revealed 75.3 and 72.5% positional identity with chicken and human 3-GAPDH, respectively (Fig. 6), and 85 and 83.2% when accounting for conservative substitutions. If P-37 was a functional enzyme, the amino acids involved in the interactions between GAPDH, GAP (substrate) and NAD (coenzyme), would be conserved. These amino acids have been identified on a *Bacillus stearothermophilus* enzyme using mutants and crystallography (33, 34). 12 were shown to play a critical role in the catalytic reaction, including an oxido reduction that yields an acyl-enzyme and NADH, followed by a phosphorylation in the presence of NAD that leads to 1,3 diphosphoglycerate. These amino acids were involved either in charge transfer, in the binding of NAD, of the inorganic phosphate or of GAP, or contributed to the specificity of the enzyme for

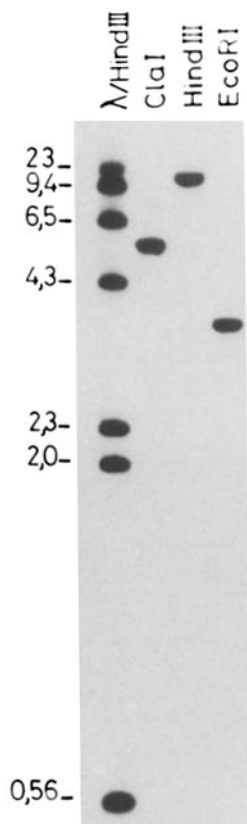


FIGURE 5. Southern blot analysis of cercarial genomic DNA digested with restriction enzymes as indicated, and probed with P-37 cDNA.

the D orientation of GAP (Table I). All these amino acids are conserved on P-37 (after proper alignment of the sequences); three amino acids, underscored in Table I, were shifted by one residue as compared with their positions in the bacterial enzyme. This indicates that P-37 probably has a functional catalytic site.

Antibodies to the Recombinant Protein Identify P-37 on the Larvae. To further confirm the presence of P-37 on the parasite, CBA/J mice were immunized with the recombinant protein in adjuvant and their sera were tested by immunofluorescence on intact living larvae. A strong homogeneous fluorescence associated with the surface of the parasite was observed (Fig. 7). Control sera from mice immunized with the adjuvant only or sera containing antibodies directed against the β -galactosidase were negative in this assay. Since schistosomes were prepared by mechanical methods in protein-free chemically defined medium, the protein identified by the antibodies was from parasite origin and could not have been acquired from a host.

Discussion

In a previous study (10), we have shown that susceptibility to infection by *S. mansoni* varies markedly among residents of endemic area; certain subjects resist infections or maintain low levels of infections for long periods of time, while others are reinfected shortly after parasitological cure and develop heavy infections. This latter

SCHISTOSOME Sm37 : 1
 CHICKEN 3-GAPDH : 2
 HUMAN 3-GAPDH : 3

13
CGTGACTTCGCTG

1 ATGTCGAGAGCAAGGTTGGTATTACGGTTTTGGTCGTATCGGGAGACTTGTACTTCGTGCAGCTTTCCTCAAG
 2 MetSerArgAlaLysValGlyIleAsnGlyPheGlyArgIleGlyArgLeuValLeuArgAlaAlaPheLeuLys
 3 MetValLysValGlyValAsnGlyPheGlyArgIleGlyArgLeuValThrArgAlaAlaValLeuSer
 MetGlyLysValLysValGlyValAsnGlyPheGlyArgIleGlyArgLeuValThrArgAlaAlaPheAsnSer

125 AACACCGTGGATGAGTTTCGGTGAATGACCCCTTCATAGATTTAGAGTATAGGTGTACATGATAAAACGGTAT
 AsnThrValAspValValSerValAsnAspProPheIleAspLeuGluTyrMetValTyrMetIleLysArgAsp
 GlyLysValGlnValValAlaIleAsnAspProPheIleAspLeuAsnTyrMetValTyrMetPheLysGlyAsp
 GlyLysValAspIleValAlaIleAsnAspProPheIleAspLeuAsnTyrMetValTyrMetPheGlnTyrAsp

200 TCCACTCATGGGCATTTCCAGGCCAGGTTTCGACTGAAAACGGAAAGTTAAAAGTCAATGGGAAGTTAATATCA
 SerThrHisGlyThrPheProGlyGluValBerThrGluAsnGlyLysLeuLysValAsnGlyLysLeuIleBer
 SerThrHisGlyHisPheLysGlyThrValLysAlaGluAsnGlyLysLeuValIleAsnGlyHisAlaIleThr
 SerThrHisGlyLysPheHisGlyThrValLysAlaGluAsnGlyLysLeuValIleAsnGlyAsnProIleThr

275 GTTCATTGTGAAGAGATCCAGCAACATCCCGTGGGATAAGGACGGGGCTGAATATGTCGTTGAGCTACTGGA
 ValHisCysGluArgAspProLeuAsnIleProThrAspLysAspGlyAlaGluTyrValValGluSerThrGly
 IlePheGlnGluArgAspProSerAsnIleLysThrAlaAspAlaGlyAlaGluTyrValValGluSerThrGly
 IlePheGlnGluArgAspProSerLysIleLysThrGlyAspAlaGlyAlaGluTyrValValGluSerThrGly

350 GTCTTTACGACCATTGATAAAGCTCAGGCTCATATTAATAAATCGAGCTAAAAGGTCATATACTCGACCT
 ValPheThrThrIleAspLysAlaGlnAlaHisIleLysAsnAsnArgAlaLysLysValIleIleSerAlaPro
 ValPheThrThrMetGluLysAlaGlyAlaHisLeuLysGlyLysAlaLysArgValIleIleSerAlaPro
 ValPheThrThrMetGluLysAlaGlyAlaHisLeuGlnGlyLysAlaLysArgValIleIleSerAlaPro

425 TCTGCAATGACCCCATGTTGTTGGTGAATGAAAATTCGTATGAAAAGTCTATGTCCTGGTTTCTAAG
 SerAlaAspAlaProMetPheValValGlyValAsnGluAsnSerTyrGluLysSerMetSerValSerAsn
 SerAlaAspAlaProMetPheValMetGlyValAsnHisGluLysTyrAspLysSerLeuLysIleValSerAsn
 SerAlaAspAlaProMetPheValMetGlyValAsnHisGluLysTyrAspSerLeuLysIleIleSerAsn

500 GCATCATGTACAACTAATTTGTTAGCACCTTCGGCTAAGTCAATCATGATAGTTTGAATAGTTGAGGCGTTA
 AlaSerCysThrThrAsnCysLeuAlaProLeuAlaLysValIleHisAspLysPheGluIleValGluGlyLeu
 AlaSerCysThrThrAsnCysLeuAlaProLeuAlaLysValIleHisAspLysPheGlyIleValGluGlyLeu
 AlaSerCysThrThrAsnCysLeuAlaProLeuAlaLysValIleHisAspLysPheGlyIleValGluGlyLeu

575 ATGCACTGTGCACTCATTACCGCTACACAAAAGGTTGTAGATGGACCCTCCTCAAAATATGGCGAGATGGC
 MetThrThrValHisSerPheThrAlaThrGlnLysValValAspGlyProSerBerLysLeuTrpArgAspGly
 MetThrThrValHisAlaIleThrAlaThrGlnLysThrValAspGlyProSerGlyLysLeuTrpArgAspGly
 MetThrThrValHisAlaIleThrAlaThrGlnLysThrValAspGlyProSerGlyLysLeuTrpArgAspGly

650 CGTGGACGATGCAAAATATTTCAGCCTTCAGCTGGTCTGCCAAGCTGTGGGAAAAGTCAATCCAGCACTA
 ArgGlyAlaMetGlnAsnIleIleProAlaSerThrGlyAlaAlaLysAlaValGlyLysValIleProAlaLeu
 ArgGlyAlaAlaGlnAsnIleIleProAlaSerThrGlyAlaAlaLysAlaValGlyLysValIleProAlaLeu
 ArgGlyAlaAlaGlnAsnIleIleProAlaSerThrGlyAlaAlaLysAlaValGlyLysValIleProAlaLeu

725 AACGGAAAATTAACGGGAATGGCTTCCCGCTCCCAACCCAGACGTTTCAGTCGTTGATCTGACATGATGGTTA
 AsnGlyLysLeuThrGlyMetAlaPheArgValProThrProAspValSerValValAspLeuThrCysArgLeu
 AsnGlyLysLeuThrGlyMetAlaPheArgValProThrProAsnValSerValValAspLeuThrCysArgLeu
 AsnGlyLysLeuThrGlyMetAlaPheArgValProThrProAsnValSerValValAspLeuThrCysArgLeu

800 GGGAAAGGAGCTTCCTATGAGGAAATCAAGGCTCAGTTAAGACAGCTGCATCTGGACCATTGAAGGGGATTTTG
 GlyLysGlnAlaSerTyrGluGluIleLysLaaAlaValLysAlaAlaAlaBerGlyProLeuLysGlyIleLeu
 GlyLysProAlaLysTyrAspIleLysArgValValLysAlaAlaAlaAspGlyProLeuLysGlyIleLeu
 GlyLysProAlaLysTyrAspIleLysLysValValLysGlnAlaSerGluGlyProLeuLysGlyIleLeu

875 GAATACACTGAAGATGAGTTGTGAGTTTCAGCTTCAGACTTTGTTGGAAGTACAGTTTCATCTATCTTTGATGCGAAGGCT
 GluTyrThrGluAspGlnValValSerSerAspPheValGlyPheThrSerSerIlePheAspAlaLysAla
 GlyTyrThrGluAspGlnValValSerSerAspPheAsnGlyAspSerHisSerSerThrPheAspAlaGlyAla
 GlyTyrThrGluHisGlnValValSerSerAspPheAsnSerAspThrHisSerSerThrPheAspAlaGlyAla

950 GGGATATCTCTTAACAACACACTTCGTGAAATTAGTTCATGGTATGATGATGAGTTTGGATACAGTTGCCGCGTA
 GlyIleSerLeuAsnAsnAsnPheValLysLeuValSerTrpTyrAspAsnGluPheGlyTyrSerTyrArgVal
 GlyIleAlaLeuAsnAspHisPheValLysLeuValSerTrpTyrAspAsnGluPheGlyTyrSerAsnArgVal
 GlyIleAlaLeuAsnAspHisPheValLysLeuIleSerTrpTyrAspAsnGluPheGlyTyrSerAsnArgVal

1025 GTTGATCTCATACGCACATGCATAAAGTCGACCATGCATAATGAAGTAGTTTCAACTGTAGCCGACTAAGTTT
 ValAspLeuIleThrHisMetHisLysValAspHisAla***
 ValAspLeuMetValHisMetAlaSerLysGlu***
 ValAspLeuMetAlaHisMetAlaSerLysGlu***

1090 TGTCAATCGATAGATAACGGAATAAGCGTTAATCGCCTA

FIGURE 6. Nucleotide and predicted amino acid sequence of P-37 cDNA compared with chicken and human 3-GAPDH amino acid sequences. The nucleotides are numbered in the 5' to 3' direction, starting with the first nucleotide in the codon corresponding to the putative NH₂-terminal residue in the protein. Gaps were inserted in sequences to achieve maximum alignment. Identical amino acids are boxed. These sequence data have been submitted to the EMBL/GenBank Data Libraries.

group runs a high risk of severe disease (11-15) and is the principal reservoir of parasites in the endemic area. We (10) have attempted to determine the causes of their higher susceptibility. Analysis of their antibody response to *S. mansoni* revealed that their sera failed to react with a 37-kD larval surface protein that is a major antigen for antibodies in the sera of the most resistant subjects (10). The cDNA encoding

TABLE I
*Amino Acid Residues Essential to GAPDH Enzymatic Activity:
 Comparison of P-37 with B. stearothermophilus GAPDH*

	Amino acid	<i>B. stearothermophilus</i> (3-GAPDH)	<i>S. mansoni</i> (P-37)
Charge transfer	cys	149	153
	tyr	317	321
Nucleophilic functions	cys	149	153
	his	176	180
NAD binding site	asn	313	317
	asp	32	35
Inorganic phosphate binding site	ser	148	152
	thr	150	154
	arg	195	198
	thr	208	212
GAP phosphate binding site	thr	179	183
	arg	231	236
D specificity of GAPDH in C2 position	ser	148	152

this antigen has now been cloned; its amino acid sequence and its nature have been determined.

A number of observations confirmed that the cloned cDNA encodes P-37: first, sera from the rabbit and mice immunized with P-37 reacted with the recombinant protein on immunoblots while control sera failed to do so; conversely rabbit antibody, immunopurified on the fusion protein, selectively bound to P-37 on blots of larval membrane extracts. Second, all six cDNA clones identified by the rabbit and mouse immune sera crosshybridized, indicating that they encoded related proteins; two of these cDNA clones were shown, by hybrid selection and *in vitro* translation, to encode a 37-kD polypeptide that was precipitable by anti-P-37 rabbit serum and by sera from several resistant subjects. Third, the 37-kD recombinant protein produced in the pJF vector was identified by all six human immune sera that reacted strongly with P-37 on immunoblots; conversely, all seven immune human sera that did not react with P-37 failed to react with the recombinant protein. Fourth, antibodies raised in mice against the recombinant protein, reacted with the surface of the parasite, as did anti-P-37 antibodies.

The observation that certain immune human sera reacting with P-37 failed to identify the recombinant protein can not be taken as an indication for more than one P-37 protein or for the absence of certain antigenic determinants on the recombinant product, because the amount of recombinant protein on the blot was probably too low (at least 10-fold lower than P-37) to allow the detection of the protein by these sera that exhibited a low reactivity toward P-37.

The amino acid sequence of P-37 does not indicate how this protein associates with the parasite tegument because the sequence of the recombinant polypeptide contains neither a typical transmembrane domain nor the terminal hydrophobic tail

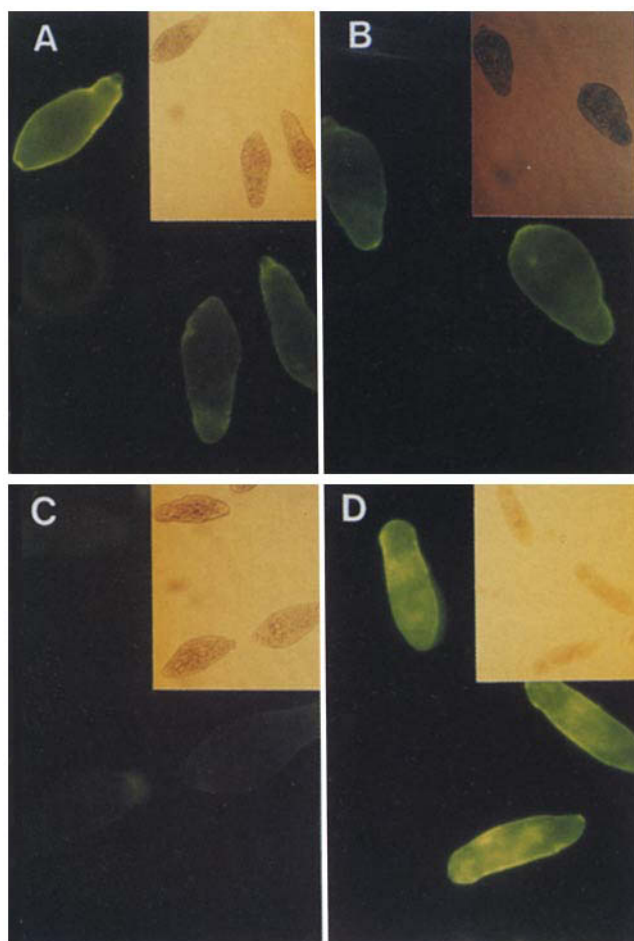


FIGURE 7. Antibodies to the recombinant protein locate P-37 on the larvae. Fresh intact larvae were reacted either with the rabbit sera used for the cloning or with sera from mice immunized with the recombinant protein in CFA. Surface-bound antibodies were revealed with FITC-conjugated rabbit anti-mouse or goat anti-rabbit IgG. (A) Anti-P-37 rabbit serum; (B) anti-recombinant protein mouse serum 1; (C) control mouse serum; (D) anti-recombinant protein mouse serum 2.

that is a common feature of most proteins anchored on a phosphatidylinositol. Nor have we found evidence for distinct mRNAs encoding a putative cytosolic protein and the membrane antigen. Thus, we have no evidence indicating that P-37 is inserted in or covalently linked to the lipid bilayer of the parasite tegument.

Since most amino acid residues involved in the enzymatic activity are conserved in the cloned protein, P-37 is likely to possess an intact enzymatic site; indeed, when the substrates of the enzymatic reaction were provided, GAPDH activity was detected associated with living and healthy schistosomula, while no such activity was detected in parasite supernatant fluids, indicating that the detected activity was probably not released by damaged organisms (A. Bourgois, unpublished data). Thus, two glycolytic enzymes have been located on the surface of schistosomula since Harn and collaborators (34a) have observed that the 28-kD target of their protective mouse mAb is a surface-located triose-phosphate isomerase that is located on the larvae.

Schistosome ingest large quantities of glucose, as much as 26% of their dry body weight per hour, that they ferment into lactic acid (35–37). Since they depend al-

most exclusively on glycolysis for their energy, GAPDH is certainly critical to parasite survival. The function of the surface-located enzyme is, however, not clear. So far there is no evidence indicating that it plays a metabolic role at the surface since it would have to be associated with other glycolytic enzymes to perform its catalytic activity.

The observation that P-37 is homologous to a protein that is highly conserved in evolution adds to the reports of others that several major targets of immunity on schistosomes are proteins sharing large regions of homology with the corresponding host proteins, such as the heat shock proteins (38, 39), glutathione-S-transferase (40, 41), myosin (42), and a serine protease (43). It is not unexpected to find host-like proteins on the surface of sophisticated parasites since it has been hypothesized for a long time that such determinants would allow parasites to escape recognition by the host immune system (43-45). The experimental facts, however, do not fulfill the predictions of the mimicry theory since most of these proteins are immuno-dominant antigens on the parasite and are major targets of immunity (10, 39-46). On the other hand, the T cell response to these antigens is probably directed to a very low number of epitopes: first, because only a small number of epitopes are recognized by T cells on any protein (47-49); and second, because the large homologies between these proteins and host molecules reduce even further the number of possible T cell epitopes. Consequently, the human T cell response against these proteins is likely to be very sensitive to genetic restrictions, increasing the probability for the parasite to infect nonresponder individuals. Such genetic restriction of the immune response to these host-like parasite antigens may account for the high susceptibility of certain subjects and may allow parasite transmission in a population that, for the most part, has developed efficient although incomplete immunity. One may speculate that this process fits well with the needs of parasites for survival since the parasites would be maintained mostly by passage through the most susceptible subjects without markedly affecting the remaining population. The most susceptible group, an important parasite reservoir, would not be rendered extinct by the disease if heterozygotes carrying the allele(s) for susceptibility did not develop a severe disease. Since P-37 has been cloned and can be produced in large quantity, it is now possible to determine whether genetic restriction affects its immunogenicity in human subjects. Our recent report on a major gene controlling human susceptibility to infection by *S. mansoni* lays the ground for such studies (Abel, L., and A. Dessein, unpublished results).

Finally, these findings also raise the possibility that these antigens may, in certain subjects, stimulate the production of autoantibodies (50) if parasite-specific regions on those antigens could play the role of carriers for an antibody response toward the conserved regions.

Summary

Schistosomiasis, due to *Schistosoma mansoni*, is a major health problem in many subtropical countries, and major efforts are being made to define a vaccine. In this regard, we have reported that sera from subjects with low susceptibility to infection by *S. mansoni* react with a major larval surface antigen (P-37), having an apparent molecular mass of 37 kD, against which sera of susceptible individuals show little reactivity.

We have now cloned the cDNA for this antigen by screening a schistosome cDNA expression library with antibodies against the purified protein. The selected cDNAs encode a protein that is specifically identified by immune human sera containing antibodies against P-37, while sera exhibiting low or no reactivity toward P-37 fail to recognize the recombinant protein. The cloned cDNAs hybridize with a 1.2-kb RNA that is the transcript of a single copy gene. This RNA directs the synthesis of a 36.5-kD polypeptide that is precipitated by sera from the most resistant subjects. The amino acid sequence of the encoded polypeptide shows homology with the glycolytic enzyme Glyceraldehyde-3P-dehydrogenase (72.5% of positional identity with human Glyceraldehyde-3P-dehydrogenase). Antibodies against the recombinant protein identified P-37 on the larva.

These findings, together with other reports, indicate that a number of conserved proteins may be major targets of host-protective immunity against *S. mansoni*. The hypothesis is discussed that genetic restriction of the immune response to these antigens may occur in heterogeneous human populations because of the limited number of T cell epitopes carried by these host-like proteins. Such genetic effects might allow parasite transmission through nonresponder (susceptible) individuals. This hypothesis and the protective properties of P-37 can now be tested using the recombinant protein and synthetic peptides derived from selected regions of the polypeptide chain.

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