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Immunology

M_r 26,000 antigen of *Schistosoma japonicum* recognized by resistant WEHI 129/J mice is a parasite glutathione *S*-transferase

(vaccination/parasite enzyme/genetic resistance)

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ABSTRACT **Mice of the inbred strain 129/J bred at this Institute (WEHI 129/J) are relatively resistant to chronic infection with the parasitic helminth *Schistosoma japonicum*. In contrast to more permissive mouse strains such as BALB/c, the WEHI 129/J mice are high responders to a M_r 26,000 adult worm antigen designated Sj26. Cloned cDNAs corresponding to Sj26 have been identified in a *S. japonicum* phage λgt11 amp3 expression library, and their nucleotide sequences have been deduced. The predicted amino acid sequence of the antigen specified by these cDNAs shows striking homology with class μ isozymes of mammalian glutathione *S*-transferases (RX:glutathione R-transferase, EC 2.5.1.18). Extracts of adult worms contain glutathione *S*-transferase activity, and affinity chromatography of enzyme activity on glutathione columns leads to the purification of a M_r 26,000 molecule that comigrates with Sj26. Although vaccination studies in mice with a β-galactosidase-Sj26 fusion protein from *Escherichia coli* are encouraging, more immunogenic preparations of the antigen are likely to be required to establish the utility of Sj26 as a model vaccine.**

Parasitic helminths of the genus *Schistosoma* are trematode worms that live as permanently coupled egg-laying pairs in the venous system of their mammalian hosts. Schistosomiasis, the disease associated with schistosome infection, is largely a result of the host immune reaction to parasite eggs that lodge in tissues such as the liver, kidney, or bladder and induce severe granulomatous and fibrotic responses. Effective drug treatments for schistosome infection of humans are currently available, but a suitable vaccine that might prevent infection or at least reduce the severity of disease has yet to be developed.

In an attempt to identify parasite molecules that might be capable of inducing host-protective immunity, we have been investigating the genetic resistance of 129/J mice bred at this Institute (designated WEHI 129/J) to infection with *Schistosoma japonicum*. Whereas all mice of a susceptible strain such as BALB/c become infected following challenge with 25 *S. japonicum* cercariae, ≈50% of exposed WEHI 129/J mice are resistant in that they contain no adult worms (1–3). Exposed WEHI 129/J mice possess serum antibody specificities that are different from those found in sera from infected BALB/c mice, and in particular, are high responders to a *S. japonicum* adult worm antigen of M_r 26,000 (Sj26) (2, 3).

We recently identified cloned cDNAs corresponding to a M_r 26,000 adult worm antigen through a novel approach in which a phage λgt11 amp3 expression library of *S. japonicum* adult worm mRNA (4) was screened with affinity-purified antibodies specific for parasite antigens of M_r 20,000 to M_r 30,000 (5). We present here the nucleotide sequence of such cDNAs and report that the predicted amino acid sequence shows close homology with published sequences of mammalian glutathione *S*-transferases (RX:glutathione R-transferase, EC 2.5.1.18).

MATERIALS AND METHODS

Parasites and Sera. Adult worms of *S. japonicum* (Philippines, Mindoro strain) were collected in Manila by portal perfusion of infected rabbits and transported to Melbourne on dry ice. Antibodies specific for the β-galactosidase fusion protein encoded by λ clone 117 (5) were induced in rabbits immunized with 100 μg of purified fusion protein in Freund's complete adjuvant (Difco) and administered boosters 4 and 17 wk later with 50 μg of purified fusion protein alone. Purification of fusion protein was as described (6, 7).

Parasite Extracts and Gel Analyses. Soluble extracts of adult worms (AWE) were prepared and analyzed following immunoprecipitation as described (3). In these experiments, AWE was iodinated by using Iodo-Gen (1,3,4,6-tetrachloro-3α,6α diphenylglycoluril) (Pierce) rather than the Bolton–Hunter reagent. Briefly, 200 μg of AWE was incubated for 10 min on ice in a glass tube coated with 50 μg of Iodo-Gen in the presence of 250 μCi (1 Ci = 37 GBq) of $Na^{125}I$. The reaction was terminated by adding 10 μl of 2-mercaptoethanol and excess bovine serum albumin. Uncoupled iodine was separated from the protein solution by passage over a Sephadex G-25M column (Pharmacia). Two-dimensional gel electrophoresis was performed by the method of O'Farrell (8).

Detection and Purification of Glutathione *S*-Transferase. Soluble proteins were obtained by homogenization of frozen washed adult worms in 0.05 M Tris·HCl, pH 8/0.15 M NaCl/5 mM EDTA. The extracts were centrifuged at 10,000 × *g* for 30 min, and total glutathione *S*-transferase activity in the supernatant was determined spectrophotometrically at 340 nm (9) by using 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM reduced glutathione as substrates. Worm extracts were subjected to electrophoresis (6 V/cm for 4 hr with cooling) through horizontal 3-mm-thick 1.2% agarose gels containing 1% hydrolyzed starch. The electrode buffer, 0.1 M Tris/0.1 M maleic acid/0.01 M EDTA/0.01 M $MgSO_4$ was adjusted to pH 7.4 with NaOH. The gel buffer was a 1 (buffer) in 4 (total) dilution of the electrode buffer. Glutathione *S*-transferase activity was detected *in situ* by specific histochemical staining (10). Glutathione *S*-transferase was purified from ^{125}I-labeled or unlabeled AWE and from rabbit blood lysates by affinity chromatography on a glutathione-agarose matrix (Sigma) as described (11, 12). The enzyme was eluted from the matrix with 5 mM reduced glutathione/50 mM Tris·HCl, pH 9.6 and then neutralized with 1 M Tris·HCl (pH 7.5).

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Abbreviations: AWE, adult worm extract; WEHI, Walter and Eliza Hall Institute; Sj26, M_r 26,000 antigen of *S. japonicum* adult worms.

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Analysis of column fractions of unlabeled AWE was by dot-blot analysis on nitrocellulose with rabbit anti-β-galactosidase-Sj26 fusion protein followed by ^{125}I-labeled protein A. Control columns were of Sepharose 4B (Pharmacia).

Nucleotide Sequence Analysis. The cDNA insert of λgt11 amp3 clone 117 (5) was isolated as an ≈800-bp *Eco*RI fragment and ligated with *Eco*RI-digested pUR292 (13), generating a plasmid (pSj1) that encodes a β-galactosidase fusion protein of the same size and antigenicity as that specified by phage λ clone 117. The complete sequence of the pSj1 cDNA was deduced by dideoxynucleotide sequencing (14) of *Alu* I, *Hae* III, and *Rsa* I fragments, which were subcloned in phage M13mp18 (15). Southern blots (16) of *S. japonicum* adult worm DNA and rabbit liver DNA [purified as described, (17)] were probed with pSj1 cDNA, which was labeled with ^{32}P by nick-translation (18). Nylon filters (Amersham) were washed in 15 mM NaCl/1.5 mM Na citrate, pH 7.0, at 65°C.

Vaccination Studies in Mice. Female BALB.K or BALB/c mice at age 7–10 wk were injected s.c. and i.p. with purified β-galactosidase-Sj26 fusion protein or a control fusion protein (Ag7.8) derived from a blood-stage antigen of *Plasmodium falciparum* (a gift from R. Anders). Mice received 30–100 μg of protein, which was given over the course of about 6 wk in three equal doses, the first in Freund's complete adjuvant. Other mice were injected with adjuvant alone, with crude lysates of frozen and thawed *Escherichia coli* (19) [clones 106 and 109 (4)] or remained uninjected. For challenge, mice were transported to Manila, where they were infected percutaneously at 10 wk after the first injection with 25 cercariae of *S. japonicum*. Adult worm burdens were determined more than 4 wk later by portal perfusion (1) and are expressed as the arithmetic mean ± SEM.

RESULTS

Nucleotide Sequence of Sj26 cDNA. As a result of screening a λgt11 amp3 expression library of *S. japonicum* adult worm mRNA (4) with antibodies eluted from the M_r 20,000 to M_r 30,000 region of immunoblots of AWE, we identified several clones that correspond to an adult worm antigen of M_r 26,000 (5). Rabbit hyperimmune antibodies affinity-purified on such clones recognized a M_r 26,000 antigen as analyzed by immunoblots of AWE and by immunoprecipitation of iodinated AWE (5). Furthermore, sera from rabbits immunized with the β-galactosidase fusion protein specified by one of these clones (phage λ clone 117) recognizes a single antigen of M_r 26,000 on immunoblots of AWE (data not shown) and immunoprecipitates a M_r 26,000 molecule from ^{125}I-labeled AWE (Fig. 1). This molecule comigrates during both one- and two-dimensional gel electrophoresis with an antigen (Sj26) that is recognized strongly by sera from exposed WEHI 129/J mice but that is recognized only weakly if at all by sera from chronically infected BALB/c mice (Fig. 1).

In view of the potential relationship between the antibody response of WEHI 129/J mice to Sj26 and their genetic resistance to *S. japonicum* infection (2, 3), the cDNA present in one of the Sj26 cDNA clones (phage λ clone 117) (5) was subcloned into pUR292 (generating the plasmid pSj1), and its entire nucleotide sequence was deduced (Fig. 2).

The nucleotide sequence contains a series of 58 adenosine residues (not shown in Fig. 2), which presumably derive from the poly(A) tail of the original Sj26 mRNA and define the orientation of the cDNA. A single open reading frame of 236 amino acids extends from the 5′ terminus to 17 nucleotides before the poly(A) region and encodes a polypeptide of predicted M_r 27,800. This open reading frame is in the correct frame to produce a β-galactosidase fusion protein in λgt11 amp3; indeed, a fusion protein of M_r 140,000 (relative to M_r 116,000 for native β-galactosidase) is specified by phage λ clone 117 and pSj1.

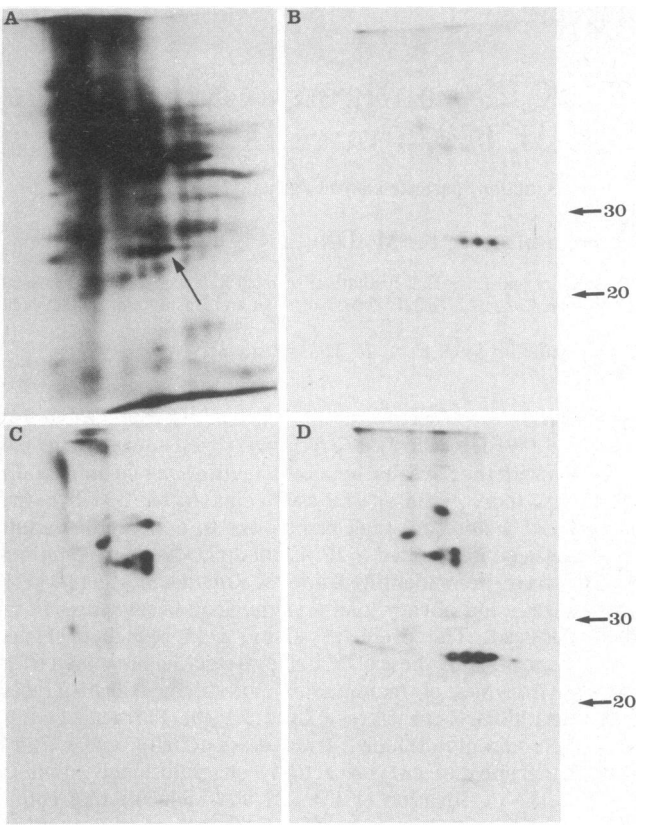

FIG. 1. Two-dimensional PAGE and autoradiography of ^{125}I-labeled AWE. (*A*) Total labeled extract. (*B–D*) Immunoprecipitations of labeled AWE with rabbit anti-β-galactosidase-Sj26 antiserum (*B*), chronically infected BALB/c mouse serum (*C*), or serum from exposed WEHI 129/J mice (*D*). The first dimension was nonequilibrium pH-gradient electrophoresis, and the second dimension involved a 13% $NaDodSO_4$/polyacrylamide slab gel. The positions of M_r 30,000 and 20,000 standards are indicated × 10^{-3}. An arrow in *A* indicates the family of molecules termed Sj26 (2). Control rabbit antisera raised to malaria fusion proteins recognize weakly several high molecular weight proteins (see *B*) but not Sj26. Two-dimensional gel analysis of labeled AWE eluted from glutathione-agarose columns identifies only the characteristic Sj26 molecules (data not shown).

The first in-frame methionine codon does not occur until nucleotide 210 of the cDNA, and if this codon were utilized for the initiation of protein synthesis, the Sj26 cDNA could only encode a M_r 19,400 polypeptide. The discrepancy between this molecular weight estimation and the observed molecular weight of Sj26 and the strong homology between the predicted amino acid sequence 5′ to this methionine codon and the sequence of mammalian glutathione *S*-transferases (see below) suggest that the Sj26 cDNA present in pSj1 lacks the authentic initiation codon. Indeed, nucleotide sequence analysis of a number of independent Sj26 cDNA clones suggests that the 5′ end of the Sj26 cDNA is unstable in *E. coli* and that the proline codon at nucleotides 12–14 may actually be preceded by two codons specifying Met-Ser. Analysis of genomic clones or direct sequencing of mRNA will be required in order to resolve this uncertainty.

The cloned Sj26 cDNA is derived from a parasite-specific mRNA rather than from a rabbit mRNA, which might have contaminated the rabbit-derived adult worms used to create the *S. japonicum* expression library (4), because Southern-blot analysis of *S. japonicum* adult worm DNA with ^{32}P-labeled pSj1 cDNA as probe detected *Eco*RI fragments of 8.4

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   Thr Lys Leu Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro Thr Arg Leu Leu Leu Glu Tyr Leu Glu
TG ACC AAG TTA CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC ACT CGA CTT CTT TTG GAA TAT CTT GAA
          10          20          30          40          50          60          70          80

 Glu Lys Tyr Glu Glu His Leu Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu Gly Leu Glu Phe
 GAA AAA TAT GAA GAG CAT TTG TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG GGT TTG GAG TTT
           90          100         110         120         130         140         150         160

 Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His
 CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC
          171         181         191         201         211         221         231         241

 Asn Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu Gly Ala Val Leu Asp Ile Arg Tyr Gly Val
 AAC ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT
          252         262         272         282         292         302         312         322

 Ser Arg Ile Ala Tyr Ser Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu Met Leu Lys Met Phe
 TCG AGA ATT GCA TAT AGT AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA ATG CTG AAA ATG TTC
          333         343         353         363         373         383         393         403

 Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
 GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT
          414         424         434         444         454         464         474         484

 Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro
 GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA
          495         505         515         525         535         545         555         565

 Gln Ile Asp Lys Tyr Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala Arg Leu Val Val Ala Thr
 CAA ATT GAT AAG TAC TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCA CGT TTG GTG GTG GCG ACC
          576         586         596         606         616         626         636         646

 Ile Leu Gln Asn Lys Leu Arg Met Ile Val Leu Val Asn Ile Ile Tyr His Leu Gln Leu Asn ***
 ATC CTC CAA AAT AAA TTA AGA ATG ATT GTT TTA GTA AAC ATT ATT TAT CAC TTA CAA TTA AAC TAA ATATAAATGTCGACA
          657         667         677         687         697         707         717         727
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FIG. 2. Nucleotide sequence and predicted coding capacity of a Sj26 cDNA. The Sj26 cDNA present in phage λgt11 amp3 clone 117 was first subcloned into pUR292 (generating the plasmid pSj1), and its nucleotide sequence was deduced by dideoxynucleotide sequencing of phage M13 mp18 subclones. The first 11 nucleotides may be cloning artefacts (see *Results*).

and 7.1 kilobases with no hybridization to rabbit liver DNA after high-stringency washing.

Homology Between Sj26 and Glutathione *S*-Transferases. The National Biomedical Research Foundation Protein Sequence Database (Washington, DC) was screened for sequences related to the predicted amino acid sequence of Sj26 by using the computer program of Dayhoff *et al.* (20). A low level of homology was detected in three separate regions with a rat glutathione *S*-transferase isoenzyme (21, 22). Mammalian glutathione *S*-transferases comprise a family of isozymes that can be grouped together into four distinct classes on the basis of their enzyme activity, antigenicity, and structure (23). Comparison of the Sj26 sequence with that of representatives of each of these classes (23, 24) led to the discovery of strong homology (up to 60%) between the N-terminal region of Sj26 and the N-terminal amino acid sequence of glutathione *S*-transferase class μ isozymes. The complete sequence of a rat class μ glutathione *S*-transferase cDNA has become available recently (25), and comparison of the predicted amino acid sequence of this isozyme with that of Sj26 reveals several homologous regions (Fig. 3) with an overall identity of 42%. Further evidence of a relationship between Sj26 and mammalian glutathione *S*-transferases comes from the similar molecular weights of Sj26 and that of the monomeric forms of mammalian glutathione *S*-transferase isozymes (M_r 23,000–28,000) (26).

Glutathione *S*-Transferase Activity in *S. japonicum* Adult Worms. If Sj26 is a functional glutathione *S*-transferase, then it should be possible to detect this activity in extracts of adult worms. Spectrophotometric measurement of glutathione *S*-transferase activity in homogenates of adult worms revealed that the parasite possesses ≈40% of the enzyme activity displayed by an equivalent amount of rabbit liver extract. Parasite enzyme activity was further characterized by electrophoresis on agarose-starch gels, followed by *in situ* detection of glutathione *S*-transferase activity. Enzyme activity was localized in two distinct regions of the gel (Fig. 4), the more anodal of which was recognized on immunoblots by rabbit serum prepared against the β-galactosidase-Sj26 fusion protein. We have yet to identify a cDNA corresponding to the second apparent isozyme.

Because it seemed possible that glutathione *S*-transferase activity in parasite extracts might reflect the acquisition of host-derived molecules, we also assayed extracts of rabbit tissues for enzyme activity. The glutathione *S*-transferase isozymes of rabbit liver and lung all had electrophoretic properties that indicated that their pI was much higher than that of the parasite isozymes. No enzyme activity could be detected after electrophoresis of crude extracts of whole rabbit blood. After purification of enzyme activity from 60 ml of rabbit blood on immobilized glutathione, weak enzyme activity could be detected that migrated slightly in advance of

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                10          20          30          40          50
pSj1      TKLPILGYWK IKGLVQPTRL LLEYLEEKYE EHLYERDEGD K.....WRNK
  Common       *****    **  * ** ****    ** *  *             * *
RatYb1    .MPMILGYWN VRGLTHPIRL LLEYTDSSYE EKRYAMGDAP DYDRSQWLNE

                60          70          80          90          100
pSj1      KFELGLEFPN LPYYIDGDVK LTQSMAIIRY IADKHNMLGG CPKERAEISM
  Common  ** *** *** *** ***  *  *** ** **  * **   *     **
RatYb1    KFKLGLDFPN LPYLIDGSRK ITQSNAIMRY LARKHHLCGE TEEERIRADI

                110         120         130         140         150
pSj1      LEGAVLDIRY GVSRIAYSKD FETLKVDFLS KLPEMLKMFE DRLCHKTYLN
  Common   *  * * *         * * **  *  **    **  *       *
RatYb1    VENQVMDNRM QLIMLCYNPD FEKQKPEFLK TIPEKMKLYS EFLGKRPWFA

                160         170         180         190         200
pSj1      GDHVTHPDFM LYDALDVVLY MDPMCLDAFP KLVCFKKRIE AIPQIDKYLK
  Common  ** **  **   ** **       * ******  *  *  * *     *  *
RatYb1    GDKVTYVDFL AYDILDQYHI FEPKCLDAFP NLKDFLARFE GLKKISAYMN

                210         220         230         240         250
pSj1      SSKYIAWPLQ GWQARLVVAT ILQNKLRMIV LVNIIYHLQL N
  Common   * *   *     *
RatYb1    CSRYLSTPIF SKLAQWSNK. ..... ..... .
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FIG. 3. Amino acid (in standard one-letter code) sequence homology between Sj26 and rat Yb_1 glutathione *S*-transferase. The amino acid sequence of Sj26 predicted from the nucleotide sequence of the cDNA present in pSj1 (Fig. 2) was aligned to give a best match with the predicted amino acid sequence of rat Yb_1 glutathione *S*-transferase (25). Identities between the two sequences are indicated by asterisks.

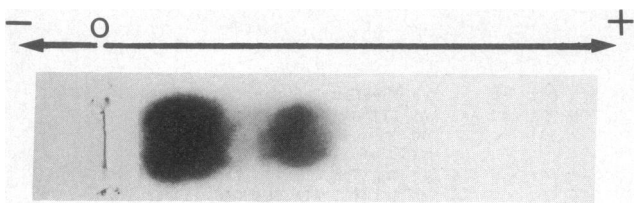

FIG. 4. The glutathione *S*-transferase isozymes of *S. japonicum*. *S. japonicum* AWE was subjected to electrophoresis through an agarose-starch gel, and enzyme activity was detected *in situ* (10). Two isozymes can be distinguished in AWE, both of which migrate toward the anode. O, origin.

the isozymes present in parasite extracts. These isozymes were not recognized on immunoblots by rabbit antibodies specific for Sj26. Hence, the great majority of glutathione *S*-transferase activity present in AWE appears to be parasite-encoded.

Attempts were made to purify glutathione *S*-transferase isozymes from parasite extracts by affinity chromatography of AWE on glutathione columns. Whereas glutathione *S*-transferase activity failed to bind to a control column, such activity was retained by a column of glutathione-agarose and could be specifically eluted with free glutathione (Fig. 5). Only those column fractions that contained enzyme activity were specifically recognized by rabbit antibodies raised against the β-galactosidase-Sj26 fusion protein. Furthermore, the only labeled molecule in ^{125}I-labeled AWE that bound specifically to immobilized glutathione had a M_r of 26,000 and comigrated on two-dimensional gels with Sj26, the antigen preferentially recognized by exposed WEHI 129/J mice (Fig. 1). These data indicate that Sj26 is a functional glutathione *S*-transferase that is encoded by *S. japonicum*.

Vaccination of Mice. Since the antibody response of exposed WEHI 129/J mice to Sj26 could be either a cause or an effect of their resistance to infection with *S. japonicum* (1–3), attempts have been made to protect susceptible mice against infection by vaccination with the cloned pSj1 gene product. Young mice were injected with purified β-galactosidase-Sj26 fusion protein in adjuvant and challenged with 25 cercariae. As determined by radioimmunoassay, the fusion protein only induced a low-titered antibody response to Sj26 (data not shown), and overall resistance in the Sj26-immune mice was low, namely 30% [11.4 ± 1.5 adult worms compared with mean worm burdens of 15.5 ± 1.1 (17 mice), 16.8 ± 0.8 (13 mice), and 16.5 ± 0.9 (12 mice) in three control groups; $P < 0.025$, Mann–Whitney *U* test]. Nevertheless, 50% of the 18 Sj26-vaccinated mice contained ≤10 worms, this proportion being 7% for the 42 control mice. No hint of resistance has been obtained in numerous mouse vaccination trials using other *S. japonicum* antigens produced in *E. coli* and described previously (4).

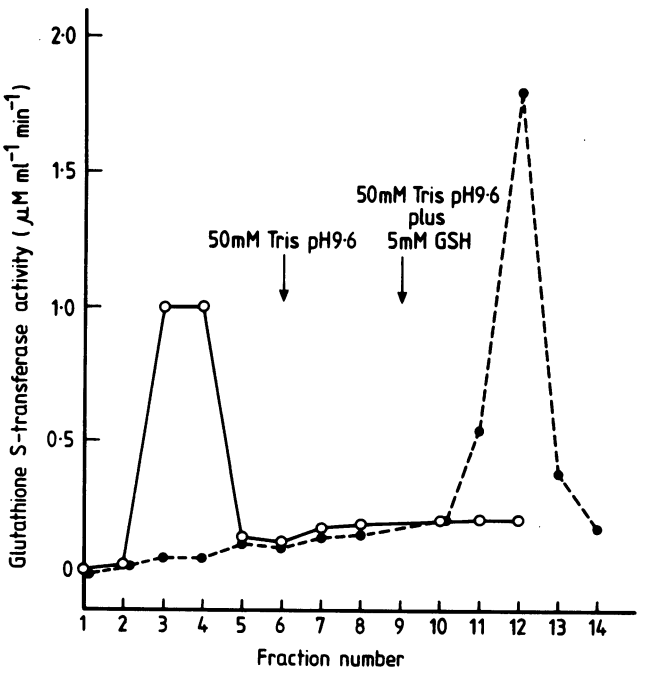

FIG. 5. Fractionation of glutathione *S*-transferase from *S. japonicum* AWE on columns of control agarose (○) and glutathione-agarose (●). After dialysis against 20 mM sodium phosphate buffer (pH 7.2), AWE was applied to each column (2 ml), and the columns were washed with 50 mM Tris·HCl (pH 9.6) with and without 5 mM reduced glutathione (GSH) as indicated. Glutathione *S*-transferase activity in column fractions was determined spectrophotometrically, the activity of the original AWE being 1.05 μM·ml^{-1}·min^{-1}.

DISCUSSION

In this paper, evidence is presented that a M_r 26,000 antigen of *S. japonicum* adult worms (Sj26) is a functional glutathione *S*-transferase. Adult worms of *S. japonicum* contain abundant glutathione *S*-transferase activity, and a M_r 26,000 molecule can be purified from iodinated AWE by affinity chromatography on columns of immobilized glutathione. There are several indications that Sj26 is encoded by the parasite rather than being a host-derived (rabbit) enzyme, which might contaminate parasite extracts or be acquired during parasite maturation. Firstly, we have identified clones in an *S. japonicum* cDNA expression library that encode polypeptides related to class μ isozymes of mammalian glutathione *S*-transferase and that are copies of *S. japonicum*-specific mRNAs as judged by Southern blot analysis of parasite and host DNA. Secondly, antibodies directed against the β-galactosidase fusion protein specified by these clones can be produced in rabbits, and such antibodies recognize Sj26 (Fig. 1) but give negative results when used to probe immunoblots of rabbit lung, liver, or whole-blood extracts. Finally, the glutathione *S*-transferase isozymes of rabbit liver and lung have distinct electrophoretic properties on agarose-starch gels from the parasite enzymes. The isozymes of rabbit blood migrate somewhat similarly to the parasite enzymes but are much less abundant.

Since purified *S. japonicum* glutathione *S*-transferase comigrates with Sj26, the antigen better recognized by sera from exposed WEHI 129/J mice than by sera from chronically infected BALB/c mice, the data raise the interesting possibility that the genetic resistance of WEHI 129/J mice is a consequence of their increased immune response to glutathione *S*-transferase. Genetically susceptible mice are significantly protected against infection by *S. japonicum* after vaccination with a β-galactosidase-Sj26 fusion protein. However, the level of protection obtained to date is unsatisfactory, and experiments are needed to determine whether vaccination efficacy can be increased by using both of the isozymes (Fig. 4) purified from worms and conjugated to immunogenic carrier molecules, rather than a denatured fusion protein.

Glutathione *S*-transferases have been most widely studied in mammalian liver, kidney, and erythrocytes but have been detected also in extracts of certain parasites, such as the cestode *Moniezia expansa* and the nematodes *Ascaris suum* and *Haemonchus contortus* (27, 28). In the case of *A. suum*, enzyme activity has been localized to the intestinal epithelium (27). The glutathione *S*-transferases comprise a family of isoenzymes that are capable of detoxifying a variety of xenobiotics through the conjugation of reduced glutathione to electrophilic centers in such molecules (26). In addition, these enzymes bind with high affinity to various endogenous

molecules such as bilirubin and heme and thereby solubilize them. If Sj26 has similar detoxifying activities, then one obvious target is the hematin that accumulates as insoluble pigment in the lumen of the parasite gut (29). Hematin is the reduced form of the heme prosthetic group and is produced during the metabolism of host erythrocyte hemoglobin at a rate of >1 μg of hematin per worm pair per day, most of which is excreted from the parasite and becomes lodged in the host liver (29). Sj26 might contribute to this process by binding to hematin and inhibiting the formation of large hematin crystals, which might otherwise block the evacuation of the parasite gut. Thus, antibodies directed against Sj26 could inhibit the binding of Sj26 to hematin and produce lethal constipation of the parasite. Such a model would imply that parasite attrition in exposed WEHI 129/J mice should occur after the parasites begin to ingest blood at about day 5 after cercarial transformation (30, 31). Some evidence for this has already been obtained (1).

Alternatively, the *S. japonicum* glutathione *S*-transferase might be capable of protecting the parasite against the free radicals of oxygen metabolism produced by immune-effector cells. Detoxification could be mediated by the peroxidase activity that is displayed by some mammalian glutathione *S*-transferase isozymes or by the conjugation of glutathione to the toxic products of peroxide attack on lipids (26). An important issue is the accessibility of Sj26 to antibodies and immune-effector cells, since glutathione *S*-transferase isozymes are generally cytosolic (26).

If the glutathione *S*-transferase in adult worms proves to be a target of host-protective immunity, then therapeutic as well as prophylactic vaccination becomes a possibility, thereby greatly simplifying the assessment of vaccine efficacy in humans. Such an effect also would provide support for a hypothesis formulated some 50 years ago that enzymes fulfilling some essential function in complex worms are attractive candidates for an anti-helminth vaccine (32).

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