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The glutathione transferase activity and tissue distribution of a cloned M_r28K protective antigen of *Schistosoma mansoni*

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A protective M_r28K antigen of *Schistosoma mansoni*, expressed from its cDNA, has been purified in a single step and shown to possess glutathione (GSH) transferase activity as predicted from sequence homologies with two mammalian GSH transferase multigene families. It is notable for its high 1-chloro-2,4-dinitrobenzene GSH transferase and linoleic acid hydroperoxide GSH peroxidase activities. The major GSH transferase of *S.mansoni* has been purified and its subunit is identical to this M_r28K antigen by criteria of M_r, immunochemistry, substrate specificity and peptide sequence analysis. In the parasite, the antigen is present in the tegument, protonephridial cells and subtegumental parenchymal cells. No significant immunological cross-reactivity between the *S.mansoni* and mammalian (human and rat) GSH transferases was observed.

Key words: GSH transferase/protective antigen/*Schistosoma mansoni*/structural relationships/tissue distribution

Introduction

Parasitism by schistosomes results in chronic and debilitating disease (Schistosomiasis, e.g. Bilharzia) which affects 200 million people throughout the world and according to WHO estimation is responsible for 800 000 deaths per year. Immunization of human populations against this common infection would represent a major advance for public health in many developing countries.

Recent and substantial progress has been made in the identification and biosynthesis of protective antigens of schistosomes (Lanar *et al.*, 1986; Smith *et al.*, 1986). The molecular cloning and expression in *Escherichia coli* of the cDNA encoding a M_r28K antigen of *Schistosoma mansoni* has recently been reported and the full sequence of the protein deduced (Balloul *et al.*, 1987a). The recombinant protein has been shown to induce levels of protection similar to that induced by the purified antigen in rats. Significant levels of protection have also been obtained in mice (45%), hamsters (52%) and baboons (up to 80% with a mean of 40%) (Balloul *et al.*, 1987b).

This paper identifies the M_r28K antigen of *S.mansoni* as a subunit of glutathione (GSH) transferase, an enzyme class (EC 2.5.1.18) much studied in mammals. In rat and man a number of dimeric isoenzymes have been identified which fall into at least three non-homologous multigene families comprising classes α, μ and π (Mannervik *et al.*, 1985). In the class α family of the rat three subunits have been characterized by cDNA cloning (Telakowski-Hopkins *et al.*, 1985 and references therein) and at least two others are known to exist; in the class μ family of the rat three subunits (Abramovitz and Listowsky, 1987 and references therein), of at least five, have been cloned; and in the class π group one subunit has been cloned (Pemble *et al.*, 1986 and references therein). Within each family of rat GSH transferases there is amino acid sequence identity of at least 70% and a similar level of conservation of structure occurs between the enzymes of one species and another. For example, comparison of sequences available for human and rat GSH transferases indicate at least 75% homology between human and rat subunits from the same class (Rhoads *et al.*, 1987 and references therein).

In the present paper we show that analysis of the amino acid sequence of the M_r28K antigen, in relation to known sequences of the rat GSH transferases, reveals homologies with both the α and μ families of mammalian GSH transferases. We then demonstrate that the M_r28K antigen produced from cDNA in both *E.coli* and *Saccharomyces cerevisiae* can be purified by GSH Sepharose affinity chromatography and is a GSH transferase which is identical in subunit mol. wt, structure and substrate specificity to the major GSH transferase isoenzyme isolated from homogenates of *S.mansoni*. Immuno-electron microscopical studies which locate the M_r28K antigen in the parasite are described. These results are discussed in relation to the immunochemical basis of antigenicity and structure/function relationships in GSH transferases.

Results

Sequence comparisons of the S.mansoni M_r28K antigen and subunits of rat GSH transferases

The *S.mansoni* M_r28K antigen, over much of its length, displays a low level of homology to rat GSH transferases of class α (subunits 1,2) and class μ (subunits 3,4,6) but not to class π (subunit 7). However, high homology to class α or class μ subunits occurs in short regions of the amino acid sequence (Figure 1). Thus the *S.mansoni* antigen, at its amino-terminal, shows substantial homology to the rat GSH transferase class α family whilst further into the protein occur two regions of high homology to the rat GSH transferase class μ family and finally there is a return to homology to the class α family. It is noteworthy that these homologous residues occur at similar positions along the sequences of the mammalian GSH transferase subunits and the M_r28K antigen.

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Figure 1. Rat GSH transferase subunits vs *Schistosoma mansoni* antigen

	residue no.		
S. mansoni	9	Y F D G R G R A E S I R M T L V A A G V	
subunit 2	8	Y F D G R G R M E P I R W L L A A A G V	(75%)
S. mansoni	70	S L A I A R Y M A K K H H M M G E T D E E	
subunit 3	73	S N A I M R Y L A R K H H L C G E T E E E	(67%)
S. mansoni	152	G D K V T L A D	
subunit 3	149	G D K V T Y V D	(75%)
S. mansoni	158	A D L V L I A V I D H V T D L D K G F L	
subunit 2	155	A D V Y L V Q V L Y H V E E L D P S A L	(45%)

Fig. 1. Regions of high homology between GSH transferase subunits and the M_r28K antigen of *S.mansoni*. Residue no. relates the first amino acid in a homologous region to its position in the protein. Sequence from Balloul *et al.* (1987a); Telakowski-Hopkins *et al.* (1985); Ding *et al.* (1985).

M_r28K antigen expressed in E.coli and S.cerevisiae: purification and characterization

The M_r28K antigen produced from cDNA in *E.coli* and *S.cerevisiae* (G.Loison *et al.*, to be published) was purified in one step on a GSH affinity column. Of the total GSH transferase activity, 98% was recovered in the GSH eluate and gave a single band of M_r28K on SDS−PAGE (Figure 2). The mol. wt of the recombinant enzyme (~50K) was determined by gel filtration on Sephacryl S-200 and is consistent with a dimeric structure. The substrate specificity of the enzyme is shown in Table I. It is high in 1-chloro-2,4-dinitrobenzene (CDNB) GSH transferase and linoleic acid hydroperoxide GSH peroxidase activities. Despite the presence of sequences with high homology to rat GSH transferase subunits 3 and 4 the antigen possesses negligible 1,2-dichloro-4-nitrobenzene and *trans*-4-phenyl-3-buten-2-one GSH transferase activities even though these compounds are substrates for subunits 3 and 4 respectively (see Beale *et al.*, 1983). Likewise the antigen is notable in that it cannot use as substrates either androst-5-en-3,17-dione which is associated specifically with subunit 1 or cumene hydroperoxide which is associated with both subunits 1 and 2 (see Beale *et al.*, 1983). Among known rat GSH transferases its activity most resembles GSH transferase 7-7 (see Meyer *et al.*, 1985) although it bears no apparent sequence homology to this isoenzyme.

When expressed from its cDNA in *E.coli*, 60% of material of M_r28K did not bind to the affinity column, had no GSH transferase activity (see Figure 2, lane 5), and was assumed to be GSH transferase in an inappropriate conformation. In contrast, from the *S.cerevisiae* expression system, 95% of the recombinant product, which comprised 30% of the yeast protein, was purified by GSH affinity chromatography and was an active enzyme.

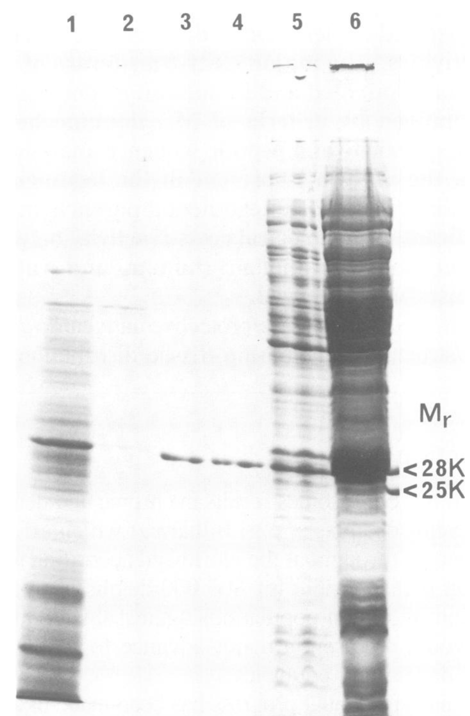

Fig. 2. Purification by affinity chromatography of recombinant M_r28K antigen. SDS−PAGE of proteins from *S.cerevisiae* (**lanes 1,2,3**) and *E.coli* (**lanes 4,5,6**). Lanes 1 and 6, total soluble fractions from culture lysates; Lanes 2 and 5, flow through; Lanes 3 and 5, GSH-eluates.

M_r28K antigen expressed in E.coli and S.cerevisiae: amino acid sequence

The proteins purified from *E.coli* and *S.cerevisiae* each gave

Table I. Enzymic characterization of natural and recombinant GSH transferase of *S.mansoni*

Substrate	Activity (μmol/min/mg protein)		
	Source of enzyme		
	S.mansoni	*E.coli*	*S.cerevisiae*
1-Chloro-2,4-dinitrobenzene	124	120	130
Linoleate hydroperoxide	5.5	6.3	6.3
Ethacrynic acid	0.8	1.2	1.4
Cumene hydroperoxide	0.4	0.5	0.5
trans-4-Phenyl-3-buten-2-one	0.4	0.3	0.3
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	<0.5	0.1	<0.5
1,2-Dichloro-4-nitrobenzene	<0.1	0.02	0.02
Androst-5-en-3,17-dione	–	<0.001	–
Inhibitor	I_{50}[a] (μM)		
Haematin	0.35	0.31	–
Lithocholate-3-sulphate	9.5	9.0	–

[a]I_{50}s were determined from inhibition curves with 1-chloro-2,4-dinitrobenzene as second substrate.

a single peak on RP-HPLC, with a retention time of 19.9 min. Approximately 30 μg of each preparation was used for sequence analysis which, in both cases, showed an amino terminal residue of alanine and subsequent amino acid sequence consistent with that of the amino terminal of the M_r28K antigen as deduced from its cDNA (Balloul *et al.*, 1987a). Although the presence of a proportion of N-terminally blocked protein cannot be excluded, the results show that cleavage of the initiating methionine by aminopeptidase had occurred in both expression systems.

The major GSH transferase of S.mansoni: purification and characterization

The major GSH transferase of *S.mansoni* has been purified in a two-step process consisting of affinity chromatography on a GSH affinity column followed by anion exchange FPLC (Figure 3a). The purification was monitored by GSH transferase activity towards CDNB. The subunits of this enzyme have a similar mobility to rat GSH transferase subunit 2 (M_r28K, Beale *et al.*, 1982) on SDS–PAGE (Figure 3a, inset) and the substrate specificity of the enzyme was similar to that of the M_r28K antigen recombinant protein (see Table I).

Approximately 10% of the total GSH-CDNB conjugating activity of the soluble extract was not adsorbed by the GSH affinity column. This was due to the presence of an isoenzyme which differs from the major GSH transferase in possessing activity towards 1,2-epoxy-3(*p*-nitrophenoxy) propane.

The major GSH transferase of S.mansoni: amino acid sequence

The major GSH transferase, on RP-HPLC, gave a single symmetrical peak with the same retention time as the recombinant M_r28K antigen (19.9 min) and 30 μg of protein from a micro-preparative run was applied to the protein sequencer. No sequence could be assigned but no overall increase in background signal was observed with successive cycles, indicative of a protein with a blocked N-terminal. To obtain sequence information, fragments were produced by partial

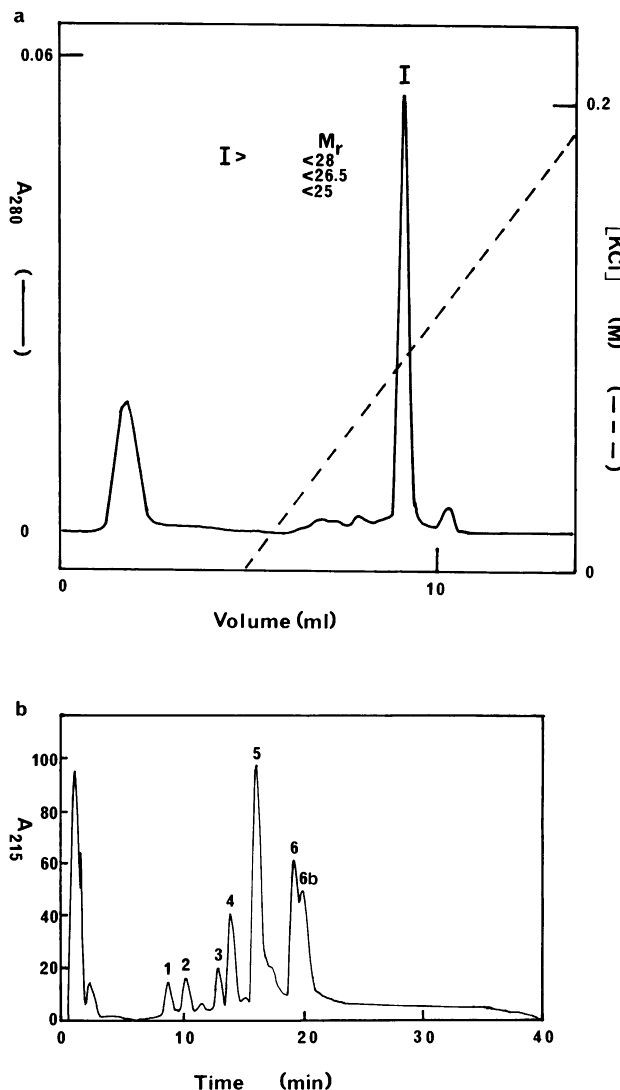

Fig. 3. (**a**) Purification of GSH transferase S.m.1-1 from adult worms.The GSH transferase pool from *S.mansoni* adults was analysed by anion exchange FPLC as described under Materials and methods. The predominant GSH transferase is labelled I. Inset: SDS–PAGE of peak I, arrows show rat GSH transferase subunits 1 (M_r25K), 3 (M_r26.5K) and 2 (M_r28K) (Beale *et al.*, 1983). (**b**) Analysis of GSH transferase S.m.1-1 after treatment with staphylococcal V8 protease. Micro-preparative separation by HPLC of 100 μl of the V8 digest after 2 h at 37°C, at A.U.F.S. of 0.3. The peaks, labelled 1-6, were subjected to sequence analysis (Table II).

digestion with staphylococcal V8 protease. The cleavage products were separated by RP-HPLC into six major peaks (Figure 3b) each of which was analysed by amino acid sequencing (Table II). Peaks 2 and 6 gave single sequences that could be assigned unequivocally to parts of the deduced sequence of the M_r28K antigen of *S.mansoni* (the shoulder labelled 6b had the same retention time as the starting material and probably represents uncleaved, and therefore N-terminal blocked, GSH transferase). The remaining four gave multiple analyses, but all the amino acid residues with the exception of some arising from the analysis of peak 5 could be assigned to peptide sequences contained within the M_r28K antigen. All these peptides would result from cleavage of the antigen at the C-terminus of glutamic acid

Table II. Sequences of peptides derived from staphylococcal V8 protease digest of *S.mansoni* GSH transferase

	Sequence	Position
PEAK 1	H I K V I Y F D G R G R A	5−17
	S L − − S − − K L A V	142−152
PEAK 2	S L A I A R Y M A K K H	71−82
PEAK 3	N L L A S S P R L A K Y L S N	191−205
	Y − S V E K L I G Q A E D V D	92−106
PEAK 4	R I S F Q D W P K I K P I	35−46
	D E R I S F Q D W P − I K	33−45
	I L N G K V P V L L N M	127−138
	S − − M − − V A A G V D Y	19−31
PEAK 5	V I L P N N D R H Q I T D − − N G H	(protease)[a]
	S I R M T L V A A G V D Y E − − R I S F	19−38
PEAK 6	I L N G K V P V L L	127−136

The single-letter code is used for the amino acids. All peaks except 2 and 6 yielded multiple sequences. Assignments to the M_r28K antigen sequence of Balloul *et al.* (1987a) are listed (position). N-terminal yields were between 200 and 300 pmoles for each main sequence. Repetitive yields were less than 85% which is indicative of some washing out of peptide material during the run and which limited reliable assignments beyond 12 to 15 residues. Peptides are listed according to N-terminal yield although the relative quantities sometimes 'crossed over' during a run so that later residues were not necessarily in quantitative order. [a]V8 staphylococcal protease N-terminal sequence.

residues which is the principal site of cleavage of staphylococcal V8 protease under the conditions used here. The anomalous residues in the analysis of peak 5 were derived from the N-terminal of the V8 protease (Drapeau, 1978). In total, therefore, peptides which are identical to parts of the M_r28K antigen and which account for 48% of its primary structure, have been sequenced.

Nomenclature

Since the major *S.mansoni* GSH transferase is identical to the recombinant enzyme in substrate and inhibitor specificity, in its subunit retention time on RP-HPLC, in the apparent mol. wt of its subunit on SDS−PAGE, and was cleaved by V8 protease to peptides with sequences identical to regions within the primary structure of the recombinant enzyme, it is presumed that the native enzyme is a homodimer of subunit primary structure identical to the recombinant enzyme. On this basis it has been named GSH transferase S.m.1-1, according to the rules of nomenclature of Jakoby *et al.* (1984).

Western blot analysis of GSH transferase S.m.1-1, the M_r28K antigen of S.mansoni and GSH transferase from mammalian hosts

GSH transferase S.m.1-1 isolated from a crude homogenate of adult worms by affinity and adsorption chromatography, was separated by SDS−PAGE (see Figure 3a, inset), blotted onto nitrocellulose sheet and incubated with an antibody to M_r28K antigen expressed in *E.coli* (Figure 4). The enzyme is recognized by the antibody (Figure 4a) showing that GSH transferase S.m.1-1 and the M_r28K antigen share immunological cross-reactivity.

Purified GSH transferase subunits 1,2,3 and 4 from the rat, and α,μ and π from the human were analysed on SDS−PAGE (Figure 4b) and then blotted onto nitrocellulose sheet. The blot was incubated first with antibody raised to the M_r28K antigen expressed in *E.coli*. No cross-reaction was observed (Figure 4c) showing that the sequences in the above mammalian GSH transferases and homologous with sequences in the M_r28K antigen (see Figure 1) are not involved in induction of this antibody. The blot was incubated subsequently with a pool of rabbit antisera raised to rat GSH transferase subunits 1/2 and 3/4 (Figure 4d) which shows that the blotted enzymes were recognized by anti-GSH transferase antibodies and revealed cross-reactivity with purified human GSH transferases α and μ. In a similar experiment, there was no cross-reaction between the pool of rabbit antisera raised to rat GSH transferase subunits 1/2 and 3/4 and proteins in homogenates of *S.mansoni*.

These results were in agreement with immunogold electron microscopy carried out on sections of rat liver. Antibody reactivity was seen with rabbit polyclonal antibodies to either rat GSH transferase subunits 1/2 or subunits 3/4 while no reactivity was seen with antibody (Balloul *et al.*, 1987a) to the M_r28K recombinant fusion protein.

Distribution of the M_r28K antigen in the tissues of S.mansoni

Immunogold electron microscopy was carried out on sections from both adult schistosomes and schistosomula, and the results are shown in Figure 5. Antibodies to M_r28K isolated from the parasite and to a M_r28K recombinant fusion protein (Balloul *et al.*, 1987a) were used. Plates A to D, G and H illustrate results obtained with the former antibody while plates E and F were obtained by use of the latter.

In plate A gold particles are seen to be densely distributed over excretory epithelial cells but absent from the flame cell. Plates B and C show the presence of gold particles in both the tegument and subtegumentary parenchymal cell extensions and plate D shows an interdigitation between a parenchymal cell and a caecal epithelial cell illustrating the presence of gold particles in the former, but their absence in the latter. Plates G and H confirm the presence of antigen in the tegument and associated parenchymal cell extensions. Plates E and F concern the schistosomulum and show gold particles not only in the tegument but also over granules of the head gland from which tegumental components may be derived. Careful analysis of other tissues of the parasite such as the digestive tract, reproductive organs or muscles has shown no evidence of labelling.

Discussion

The data presented here prove that the principle GSH transferase of *S.mansoni* (GSH transferase S.m.1-1) is a homodimer and its subunit is identical with a protein charac-

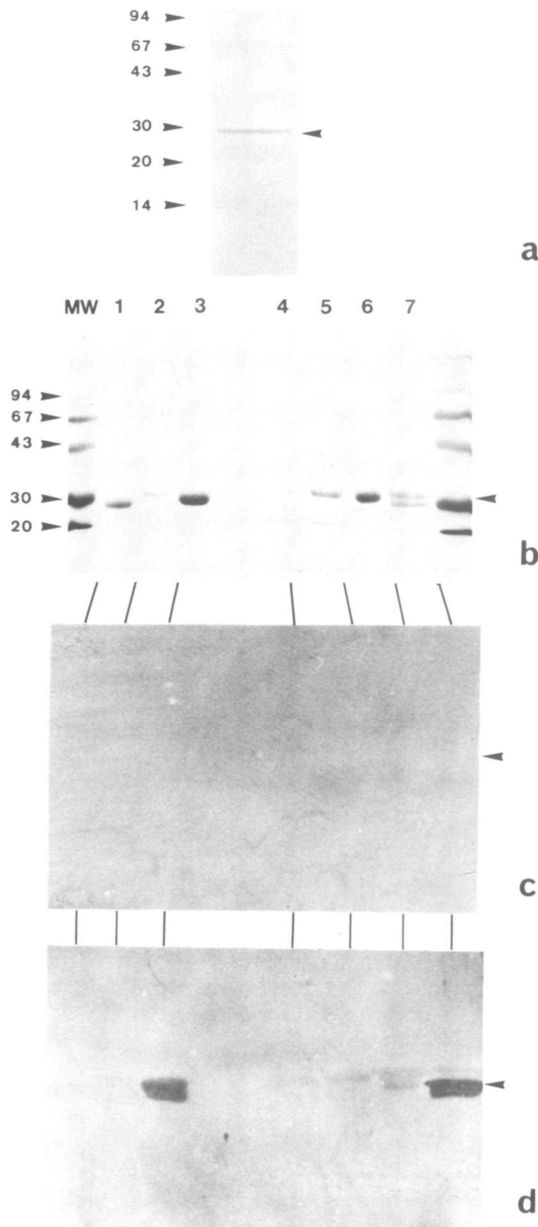

Fig. 4. Analysis of possible shared epitopes between GSH transferase from *S.mansoni* and GSH transferase from rat and man. (**a**) GSH transferase S.m.1-1 (see Fig.3a) was analysed by SDS–PAGE, transferred onto nitrocellulose sheet and incubated with antibody against recombinant M_r28K antigen. Immune complexes were revealed by subsequent incubation with an anti-rat IgG rabbit serum labelled with horseradish peroxidase. (**b**) GSH transferase subunits from: **1**. human GSH transferase π; **2**. human GSH transferase μ; **3**. human GSH transferase α; **4**. rat GSH transferase 4-4; **5**. rat GSH transferase 3-3; **6**. rat GSH transferase 2-2; **7**. rat GSH transferase 1-2. (**c**) Reaction between rat anti-recombinant M_r28K antigen serum with rat and human GSH transferase fixed onto nitrocellulose and ordered as in (b). (**d**) Reaction of pooled anti-rat GSH transferase (subunits 3/4 and subunits 1/2) rabbit sera with rat and human GSH transferases on blot (c). Human GSH transferases were purified as described in Ostlund Farrants *et al.* (1987).

terized previously as an antigen of M_r28K which induces host immune protection to re-infection by the parasite (Balloul *et al.*, 1987a). The purity of the GSH transferase was confirmed by RP-HPLC and by SDS–PAGE, which showed a single component of M_r28K (Figure 3). This protein was shown to be blocked at the N-terminus, in common with the M_r28K antigen reported by Balloul *et al.* (1987a). After the enzyme was digested with staphylococcal V8 protease, amino acid sequences of peptides were obtained which were identical with sequences in the M_r28K antigen and accounted for over 48% of primary structure. Purified M_r28K antigen, expressed from its cDNA, possessed GSH transferase and GSH peroxidase activity similar to GSH transferase S.m.1-1.

The M_r28K antigen, over much of its length, has a low level of homology to subunits of mammalian class α and class μ GSH transferases although it is best described as a mosaic of short regions which have high homology to one or other of the multigene families (Figure 1). An overall homology to mammalian class μ GSH transferases has been reported for an M_r 26K antigen of *Schistosoma japonicum* (Smith *et al.*, 1986) although we note that this antigen also contains a mosaic of sequences homologous not only to the rat subunit 3/4/6 multigene family but also to the subunit 1/2 and 7 multigene families and again these homologous areas all occur at similar positions in the proteins (J.Taylor and S.Pemble, unpublished). At first sight these mosaics of sequences characteristic of two or more non-homologous multigene families, suggest that both antigens are the products of genes close to a primaeval precursor of rat GSH transferases. This is unlikely however, since there is no strong homology between the GSH transferases of *S.mansoni* and *S.japonicum*, and so the possibility arises that the parasites gain some advantage from imitating structures of the host's GSH transferases. Prediction of the antigenicity of the M_r28K molecule by computer analysis and confirmation by the synthesis of peptides indicate that the major epitopes of the protein are created, as expected, only in those regions which show no significant homology with rat and human GSH transferases (Balloul *et al.*, 1987a and unpublished). The existence of significant homologies between *S.mansoni* and vertebrate GSH transferases might provide an epitope capable of induction of humoral anaphylactic response in the course of vaccination against *S.mansoni* and result in auto-immune disease. In this study however, no evidence has been obtained, either by immunoblotting (Figure 4) or by immuno-electron microscopy, that significant cross-reactivity occurs, which incidentally provides supporting evidence that the immunogenic regions of the schistosome antigen are not localized in the conserved regions of the GSH transferases.

Functionally, GSH transferase S.m.1-1 has enzymic roles (Table I) which are similar to those of members of the mammalian supergene family (for review see Ketterer *et al.*, 1986, 1987) and which may aid parasite survival. For example, cell mediated immune attack on the schistosome is associated with the release of highly reactive oxygen species capable of initiating lipid peroxidation and the production of membrane decomposition products including cytotoxic hydroxyalkenals. GSH transferase S.m.1-1, having both fatty acid hydroperoxide-GSH peroxidase and GSH transferase activities, may neutralize immune attack by inhibiting lipid peroxidation and scavenging any hydroxyalkenals produced.

Immuno-electron microscope studies (Figure 5) show that in the adult, GSH transferase S.m.1-1 is present both in the tegument and its extensions into the parenchyma and the ex-

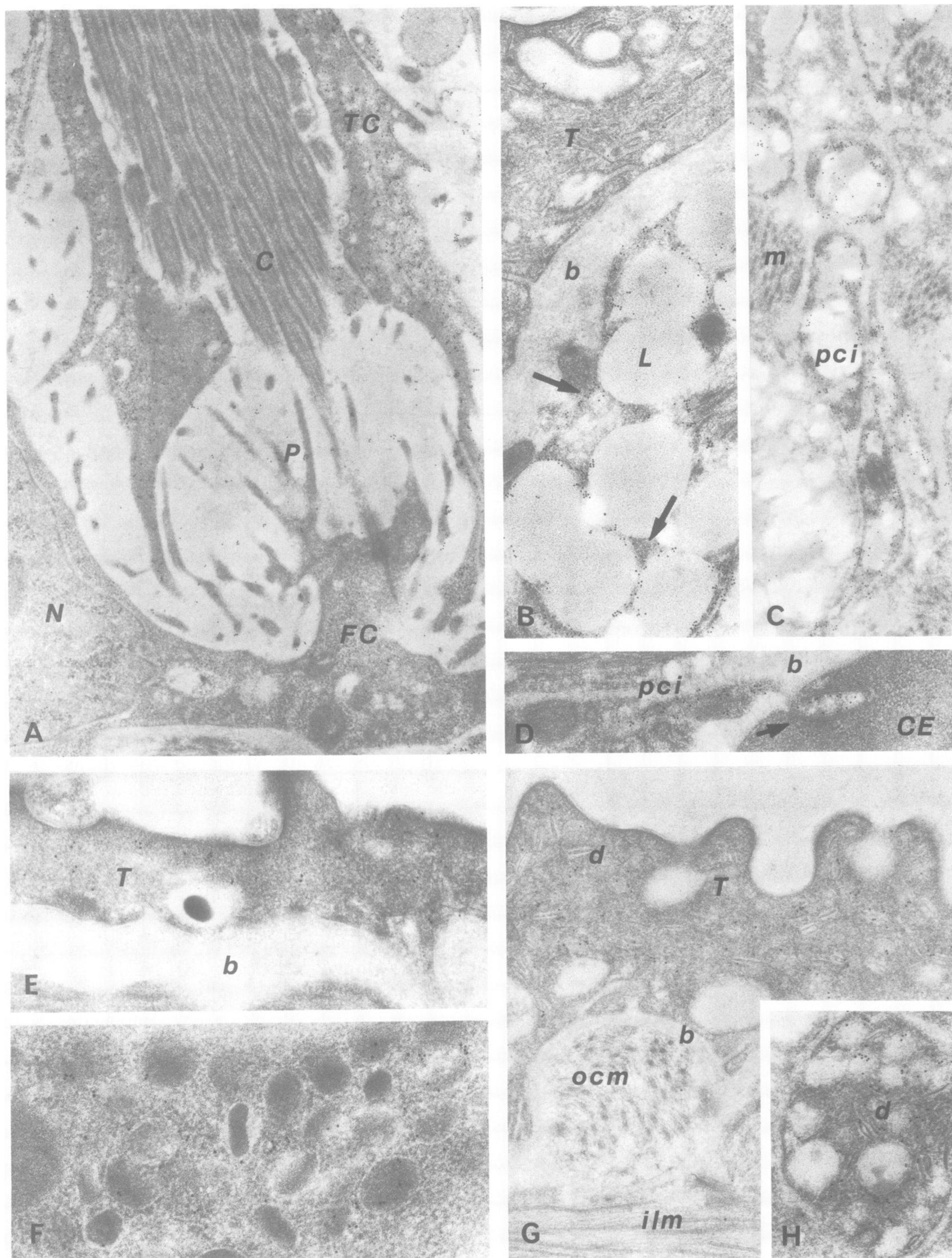

Fig. 5. Distribution of the 28K antigen in *S.mansoni* adult worms (A–D, G and H, and schistosomula (E and F) as observed by indirect immuno-electron microscopical labelling. Lowicryl sections of fixed parasites were labelled with anti-M_r28K antigen rabbit serum (A–F) and anti-M_r28K recombinant fusion protein (Balloul *et al.*, 1987a) rat serum (G–H) followed by appropriate anti-rabbit IgG and anti-rat IgG-colloidal gold. (**A**) In the protonephridial region, gold particles are densely distributed over the cytoplasmic phase of the excretory tubule cell (TC). Gold particles are absent from the apical flame cell body (FC), processes (P) from the chamber wall and tuft of cilia forming the flame (C). N, Nucleus. × 21 000. (**B**) In the dorsal tubercle region of male *S.mansoni*, gold particles are mostly associated with cytoplasmic areas (arrows) in close vicinity to the lipid droplets (L). b, basement lamina. × 31 000. (**C**) Anti-M_r28K antigen is densely distributed throughout the cytoplasm of parenchymal cell interdigitations (pci). m, body musculature. × 23 000. (**D**) Closely associated with the parenchymal side of the caecal epithelium (CE), a discrete paddy-form area (arrow) of the end of a parenchyma cell interdigitation (pci) labelled with gold particles. b, basement lamina. × 34 000. (**E**) The label for anti-M_r28K antigen is also localized on the tegument (T) of schistosomulum *S.mansoni*. b, basement lamina. × 39 000. (**F**) Using the anti-M_r28K antigen fusion protein rat serum, cross-reactivity can equally be detected over the granules of the head gland of schistosomulum. × 50 000. (**G**) Anti-M_r28K antigen fusion protein is fairly uniformly distributed throughout the tegument of adult worm (T). Gold particles are generally associated with discoid granules (d). b, basement lamina; ocm, outer, circular cortical muscle; ilm, inner, longitudinal cortical muscle. × 33 000. (**H**) In the tegumentary cells observed below the peripheral musculature, gold particles associated with discoid granules (d) are present over the cytoplasm extensions joining the tegument. × 37 000.

cretory epithelial cells. It is, however, absent from the caecal epithelium and the flame cells. In the schistosomulum, which is a stage that is sensitive to the immune response, the antigen is also found in the tegument and associated structures. Although in mammals GSH transferases are regarded as intracellular proteins, since GSH transferase S.m.1-1 raises an immune response in the host some of the enzyme must be accessible to the immune system. In previous work the M_r28K antigen in the intact parasite larva has proved accessible to radio-iodine labelling and is presumably at the tegument surface (Balloul *et al.*, 1985). It might be carried to the tegumental surface in the course of membrane formation since in schistosomes the structure of the multilaminate tegumental membrane is such that its outer surface has its origins in the cytoplasmic leaflet of the membrane bilayer (Torpier *et al.*, 1977, 1979; McLaren, 1980). Alternatively it might become exposed to the host immune system by incidental leakage from a tegumental surface which is by nature fragile and is constantly being shed into the environment of the parasite (Kusel and Mackenzie, 1975; Senft *et al.*, 1986).

Materials and methods

Structural analyses by computer

Sequence comparisons were undertaken using a MicroGenie Sequence Analysis Program (Queen and Korn, 1984) obtained from Beckman Instruments (Palo Alto, California). The algorithm locates regions >40 residues in length and containing at least 40% identity.

Purification of recombinant M_r28K antigen produced from cDNA

An extract from *E.coli* prepared by sonication in PBS and centrifuged at 20 000 g_{av} for 10 min was applied directly to a 7 ml S-linked GSH-agarose affinity column (Sigma Chemical Co. Ltd, Poole, UK, Catalogue No. G4510) and the GSH transferase fraction (48 mg, M_r28K) was eluted with 7 mM GSH, 50 mM Tris−NaOH, pH 9.6 (Vander Jagt *et al.*, 1985; Ostlund Farrants *et al.*, 1987).

The activity of this recombinant GSH transferase from *E.coli* was tested against the following substrates: 1-chloro-2,4-dinitrobenzene, linoleate hydroperoxide, ethacrynic acid, cumene hydroperoxide, *trans*-4-phenyl-3-buten-2-one, 1,2-epoxy-3(*p*-nitrophenoxy) propane, 1,2-dichloronitrobenzene and androst-5-en-3,17-dione (Meyer *et al.*, 1985). In addition inhibition by the ligands haematin and lithocholate-3-sulphate was determined. Protein produced in *S.cerevisiae* (27 mg, M_r28K) was submitted to a similar procedure. The mol. wt of the recombinant enzyme was determined by gel filtration on Sephacryl S-200 using Soy bean trypsin inhibitor, ovalbumin and BSA as standards.

Purification of the major GSH transferase from S.mansoni

A soluble fraction from 1 g of adult worms, collected from infected hamsters and homogenized in PBS (Balloul *et al.*, 1985), was stored at −70°C. It was thawed, diluted 2-fold into 1.15% KCl, 0.5 mM phenyl methyl sulphonyl fluoride, 1 mM EDTA, 10 mM potassium phosphate, pH 7.0 and centrifuged at 30 000 *g* for 15 min. The supernatant was applied to a 2.5 ml S-linked GSH-agarose affinity column and the bound GSH transferase pool was eluted as described above. During fractionation the temperature was maintained at 0−4°C. The major GSH transferase was then separated from minor GSH transferases by anion exchange FPLC using a Mono Q 5/5 column (Pharmacia, Upssala, Sweden) in 0.1 mM dithiothreitol, 20 mM diaminopropane-HCl, pH 8.55 eluting with a gradient of KCl at room temperature (Figure 3a).

Reverse-phase high pressure liquid chromatography (RP-HPLC)

Protein and polypeptide fragments from the major *S.mansoni* GSH transferase were separated by RP-HPLC (Gilson, Madison, WI, USA) using a 2 mm × 30 mm Aquapore RP-300 column (Applied Biosystems, California, USA). The solvents were 0.5% trifluoracetic acid (TFA) in water (solvent A) and 0.05% TFA in acetonitrile (solvent B). Gradients of 10−70% solvent B were run in 30 min at a flow rate of 0.2 ml/min. Protein and polypeptide fragments were detected at 215 nm.

Staphylococcal protease digestion

The principal *S.mansoni* GSH transferase was digested with staphyloccocal V8 protease. The major GSH transferase in 200 mM sodium phosphate, 10 mM $CaCl_2$ was adjusted to pH 8.0 by the addition of 10 μl of 1 M Tris-base. 5 μl of 1 mg/ml solution of staphyloccocal strain V8 protease (Sigma, Poole, UK) was added to a 200 μl aliquot (~60 μg) of the enzyme and the final solution incubated at 27°C. Proteolysis was monitored by analytical RP-HPLC at 30 min intervals. Although this analysis indicated that starting material was not completely cleaved by 2 h, a preparative run was made at this time in order to minimize the number of fragments generated for sequence analysis.

Amino acid sequencing

An Applied Biosystems 470A gas-phase sequencer was used with the 120A on-line PTH analyser (California, USA). Sample disks were loaded with 3 mg polybrene (Applied Biosystems) prior to peptide application.

Western blotting

GSH transferase preparations were mixed with an equal volume of 2 × sample buffer (3% SDS, 5% 2-mercaptoethanol, 62.5 mM Tris−HCl, pH 6.8) and boiled for three minutes. Samples were separated on 13% SDS−PAGE slab gels and then transferred onto nitrocellulose paper (Towbin *et al.*, 1979). Protein blots were probed with either anti-recombinant M_r28K rat serum or anti-rat GSH transferase. Bound antibodies were detected with antibodies conjugated with horseradish peroxidase (Pasteur Production, France).

Expression systems

The cDNA insert encoding the M_r28K antigen, which has previously been used to express a fusion protein in *E.coli* (Balloul *et al.*, 1987a) was engineered so as to be expressed as a non-fused protein in a similar system (Tessier *et al.*, 1984). Expression systems used in *S.cerevisiae* have been described by Lemoine *et al.* (1987).

Immuno-electron microscopy

Tissues and parasites were fixed in 0.1% glutaldehyde in 0.1 M sodium cacodylate, pH 7.4, for 45 min and subjected to low temperature dehydration followed by Lowicryl KHM resin embedding as in Roth *et al.* (1981). Grids holding sections were floated for 15 min on a solution of 0.5% ovalbumin in Tris−HCl-buffered saline (20 mM Tris−HCl, 0.5 M NaCl, pH 7.4, TBS) containing 1% decomplemented goat serum. This was followed by incubation with the tested antibodies diluted in TBS−0.5% OVA for 2 h at room temperature and then washed in TBS−OVA (4 × 15 min). Antibody reaction was revealed by incubation with colloidal gold-labelled goat anti-rabbit IgG and rat IgG (GARG10 and GARaG10, Janssen Pharmaceutica, Beerse, Belgium) (1:40 to 1:50) in TBS−0.5% OVA for 1 h at room temperature. All grids labelled with immunogold were thoroughly washed with buffered ovalbumin and finally with distilled water.

Lowicryl sections were then stained with 1% uranyl acetate and with lead citrate. Observations were made at 80 KV with a Philips EM 420 electron microscope.

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