The EMBO Journal vol.3 no.1 pp.213 – 219, 1984

Cell-free synthesis of *Schistosoma mansoni* surface antigens: stage specificity of their expression

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Communicated by A.E.Smith

Messenger RNA has been extracted from all stages of the life cycle of the parasitic multicellular helminth *Schistosoma mansoni*. *In vitro* translation of these mRNA preparations in rabbit reticulocyte lysates yielded in each case a large number of polypeptides. Immunoprecipitation of translation products either by serum from immune mice or from human patients demonstrated that relatively few, ~10, polypeptides are recognised as antigens. Two of the *in vitro* synthesised antigens, of mol. wts. 22 000 and 14 000, were demonstrated to correspond to schistosomula surface antigens. The expression of these antigens may show stage specificity. Both are readily detected from adult and sporocyst translation products, neither from schistosomula and only the 22 000 antigen from miracidia. This is an unexpected finding since similar polypeptide antigens occur on the surface of schistosomula. These results indicate that not only are schistosomula surface antigens preformed at the preceding sporocyst stage, i.e., within the snail host, but they also remain invariant throughout the life cycle in the vertebrate host. Two other prominent schistosomula surface antigens of mol. wts. 38 000 and 32 000, were not recognised amongst cell-free translation products directed by RNA from any life cycle stage. The demonstration that at least two schistosomula surface antigens are detectable amongst adult mRNA cell-free translation products demonstrates the feasibility of identifying the genes encoding them in cDNA libraries from adult worm mRNA.

Key words: Schistosoma mansoni/surface antigens/cell-free translation/immunoprecipitations

Introduction

Schistosomiasis, or bilharzia, is a chronic debilitating disease widespread in Africa, South America and the Far East and is caused by a parasitic helminth of the genus *Schistosoma*. It is estimated that at least 200 million people are afflicted by the disease and since transmission is effected by a variety of freshwater snails (see Figure 1), it has become a major concern in areas where irrigation projects have been developed. Efforts are currently being directed towards the development of an effective vaccine. Irradiation-attenuated cercariae [the invasive form of the parasite which penetrate skin directly (Figure 1)], can induce a high degree of immunity against subsequent schistosome infection, both in laboratory animals and in cattle (Hsu *et al.*, 1969; Minard *et al.*, 1978; Majid *et al.*, 1980). As yet, however, a vaccine consisting of dead parasite material has not been developed.

It is believed that the immature larval forms of the parasite, the schistosomula (Figure 1) are the target of the protective immune response. This stage of the parasite can bind anti-parasite antibody and is readily killed *in vitro* by a variety of immune effector mechanisms. Adult worms on the other hand, fail to bind antibody from immune animals and are also resistant to the effector mechanisms which kill the immature schistosomula (see Smithers and Doenhoff, 1982). Polypeptide antigens, exposed on the surface of the vulnerable schistosomula, are recognised by sera obtained from chronically infected animals and from those immunised with irradiation-attenuated cercariae (Taylor *et al.*, 1981; Dissous *et al.*, 1981; Simpson *et al.*, 1983). Predominant amongst these are antigens of approximate mol. wts. 98 000, 38 000, 32 000, 20 000 and 15 000 daltons. Monoclonal antibodies have been raised against two of these defined schistosomula polypeptide surface antigens (mol. wts. 38 000 and 20 000) which can mediate *in vitro* killing of schistosomula (Grzych *et al.*, 1982; Dissous *et al.*, 1982; Tavares *et al.*, 1983). Furthermore, the 38 000-dalton antigen also confers immunity *in vivo* by passive transfer (Grzych *et al.*, 1982).

Although mechanisms of immunity in schistosomiasis are far from clear such polypeptide surface antigens might form the basis of a dead anti-schistosome vaccine but to test this it will be necessary to isolate and purify these antigenic surface polypeptides. Schistosomes, however, have a complex life cycle (Figure 1) and cannot multiply *in vitro*. Thus, it will be very difficult to obtain the quantities of parasite material required to purify these molecules by conventional protein

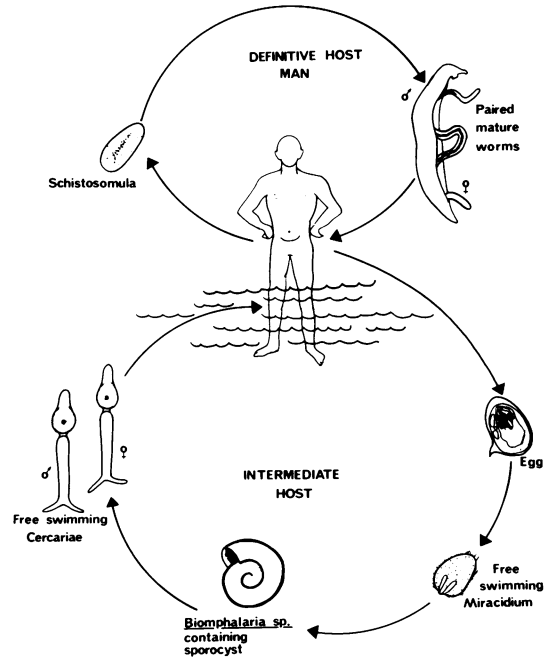


Fig. 1. Life cycle of *Schistosoma mansoni*.

purification methods.

As an alternative, cloning of genes which encode these antigens, with their subsequent expression in microorganisms, may provide the amounts required. With this aim, our initial investigations have been directed at attempting to identify polypeptides corresponding to surface antigens amongst cell-free translation products. Schistosomes are large and complex multicellular organisms and the parasite surface contributes only a minute proportion to the total parasite tissue (Simpson *et al.*, 1981). Moreover, the surface antigens are integral membrane proteins and are in some cases heavily glycosylated (G.Payares, unpublished observations). It seems likely, therefore, that the surface antigens are minor gene products which will be difficult to detect amongst cell-free translation systems. Nevertheless, we have sought to identify surface antigens amongst polypeptides synthesised in rabbit reticulocyte lysates programmed with parasite RNA, and have found that at least two surface antigens are major products. Furthermore, analysis of the stage-specificity of synthesis of these molecules has shown that both are synthesised by the adult worms, the only life cycle stage from which large amounts of RNA can be readily prepared. These results demonstrate the feasibility of cloning the genes encoding these surface antigens utilising assays based on *in vitro* translation.

Results

Identification of schistosomula surface antigens

To demonstrate and characterise antigens exposed on the surface of schistosomula, these organisms were surface labelled with ^{125}I by the Iodogen method and labelled polypeptides immuno-precipitated with antisera. The schistosomula surface antigens recognised by serum from chronically infected mice (CMS), vaccinated mice (VMS) and rabbit anti-membrane serum are shown in Figure 2. All three sera recognise major antigens of ~92 000, 38 000, 32 000 and 20 000 daltons. In addition, the CMS (lane 1) recognised an antigen of 17 000 and the VMS (lane 2) an antigen of 15 000. The pooled human infection serum precipitated surface antigens indistinguishable from those recognised by CMS (data not shown).

During a chronic infection it is the mature adult worms that stimulate the production of antibody against the invading schistosomula (Smithers and Terry, 1969; Simpson *et al.*, 1983). This is further demonstrated here by the observation that antibodies raised in rabbits against purified adult worm surface membrane, precipitate schistosomula surface antigens (Figure 2, lane 3). Due to the antigenic cross-reactivity between schistosomula and adult worm (Figure 2, lane 3) and the relative abundance of the latter stage, from which functional RNA can readily be obtained (Tenniswood and Simpson, 1982), we initially attempted to identify polypeptides corresponding to surface antigens amongst cell-free translation products directed by adult worm RNA.

Identification of schistosome antigens amongst total adult worm cell-free translation products

Total RNA prepared from adult schistosomes was used to direct protein synthesis in rabbit reticulocyte lysates. Examination of the total translated products by SDS-polyacrylamide gel electrophoresis showed the synthesis of many parasite proteins with mol. wts. ranging from between ~100 000 to 14 000 (Figure 3, lane 1). Of the large number

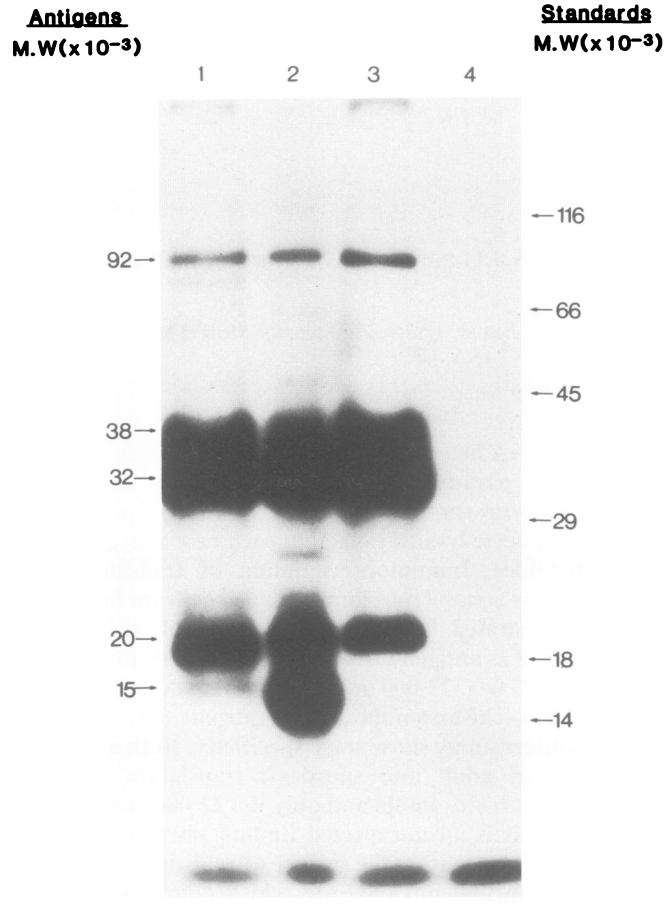


Fig. 2. Identification of schistosomula surface antigens; the surfaces of young schistosomula were labelled with ^{125}I and solubilised as described in Materials and methods. ^{125}I-labelled surface antigens were immunoprecipitated and analysed by SDS-PAGE followed by autoradiography. Immunoprecipitations were carried out with the following antisera **(1)** CMS, **(2)** VMS, **(3)** anti-adult worm membrane serum and **(4)** serum from control non-infected mice.

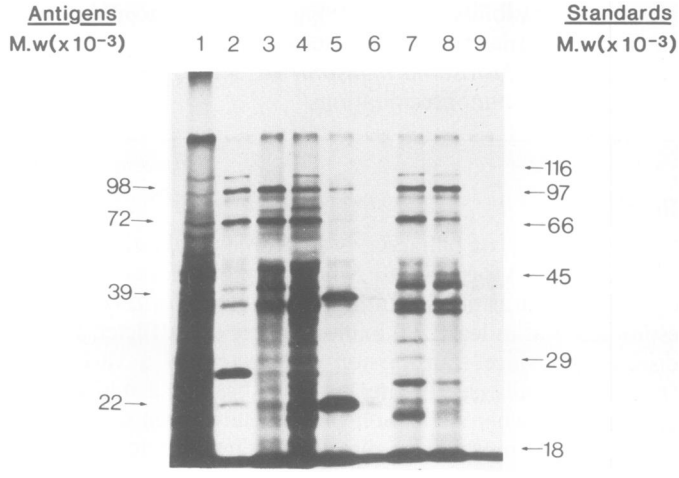


Fig. 3. Immunoprecipitation of [^{35}S]methionine adult worm *in vitro* translation products; adult worm RNA translation products were analysed by SDS-PAGE before **(1)** and after immunoprecipitation with VMS **(2)**, CMS **(3)**, infected human anti-serum **(4)**, rabbit anti-membrane serum **(5)**, normal rabbit serum **(6)**, male-specific antiserum **(7)**, female-specific antiserum **(8)** and normal mouse serum **(9)**. Translations and immunoprecipitation were done as described in Materials and methods.

of parasite polypeptides synthesised, however, only relatively few were found to be antigenic, as shown, by immunoprecipitation with VMS (Figure 3, lane 2), or with CMS (Figure 3, lane 3). Several dominant antigens, 98 000, 72 000, 45 000, 39 000 and 22 000 were recognised by both types of mouse antisera although the VMS recognised unique antigens, 100 000, 35 000 and 30 000 daltons (lane 2), which were not immunoprecipitated by CMS. Conversely, CMS immunoprecipitated a unique 50 000 dalton antigen (lane 3). An intense 14 000-dalton antigen which was not resolved on this gel was also immunoprecipitated by both types of mouse antisera (see below). Interestingly, immunoprecipitation of cell-free translation products using serum from chronically infected patients (Figure 3, lane 4) demonstrated that most antigens recognised by either mouse antisera were also recognised by the human serum. This is an indication that antigens recognised by antisera from both types of immunised mice may be relevant to the development of schistosome immunity in the normal human host.

To determine the possible occurrence of sex-specific antigens among cell-free translation products, antisera from single-sex infections were utilised. Figure 3, lanes 7 and 8 show that there is no striking difference between the antigens recognised by sera from all male or all female infections.

Demonstration of surface antigens amongst in vitro synthesised polypeptides

To establish whether surface antigens are detectable amongst cell-free translation products, undiluted samples of VMS and anti-membrane sera were absorbed with live schistosomula to remove antibodies directed against exposed components. When absorbed VMS was utilised for immunoprecipitation (Figure 4, lanes 6 and 7) most antigen bands appeared as intense as when precipitated by unabsorbed sera. This established that antibody had not been non-specifically removed by the absorption process. The 22 000-dalton antigen however was not detectably precipitated by the absorbed VMS (lanes 6 and 7) thus indicating that this molecule is exposed on the schistosomula surface. Antibodies to the 22 000-dalton antigen were also present in the anti-membrane serum, but these were not completely absorbed out by incubation with live schistosomula, indicating that this serum had a higher titre of this antibody than did VMS (Figure 4, lanes 3 and 4). In addition to the specific absorption of antibody recognising the 22 000-dalton antigen from VMS, a strong antigenic band of mol. wt. ~14 000 was precipitated considerably less intensely by the absorbed VMS, suggesting that this antigen is also a surface molecule (Figure 4, lanes 6 and 7). The same antigen appears to be precipitated by CMS and human sera but not by anti-membrane sera. Another antigen of mol. wt. ~72 000 daltons which is routinely precipitated by VMS, CMS and human serum (Figure 3, lanes 2, 3, 4) was perhaps slightly less intense when precipitated by absorbed VMS (Figure 4, lanes 6 and 7) and may also represent a surface antigen amongst cell-free translation products, although further studies are needed to confirm this.

In addition to precipitating the 22 000-dalton surface antigen, the anti-membrane serum also strongly precipitated a 39 000-dalton polypeptide which was less intensely precipitated by absorbed anti-membrane serum (Figure 4, lanes 3 and 4). This implies that the 39 000-dalton antigen is exposed on the surface of schistosomula. Curiously, however, this antigen was not recognised by VMS, CMS or the human serum, suggesting that the antigen is not exposed during infection.

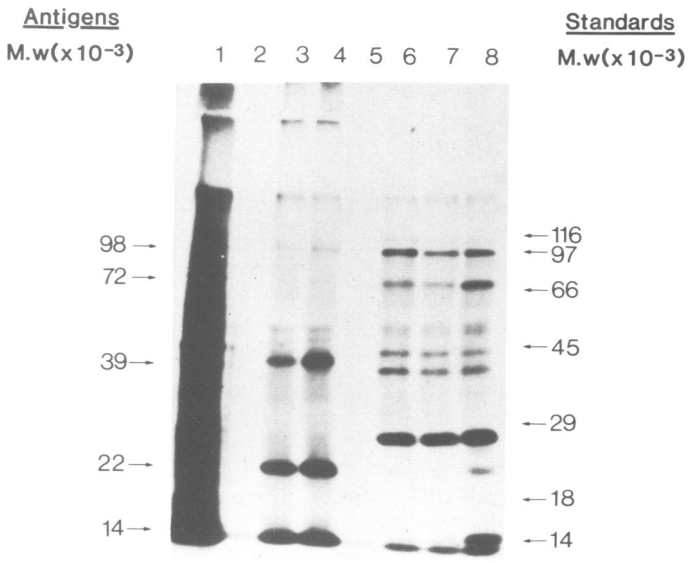


Fig. 4. Identification of surface antigens amongst [^{35}S]methionine-labelled adult worm *in vitro* translation products; total [^{35}S]methionine-labelled *in vitro* translation products were analysed by SDS-PAGE directly **(1)** or following immunoprecipitations with either absorbed **(3)** or unabsorbed **(4)** rabbit anti-membrane serum. Unabsorbed VMS **(8)** or VMS absorbed by two different methods **(6** and **7)** were also employed in immunoprecipitations. **Lanes (2)** and **(5)** represent immunoprecipitation of translation products with normal rabbit and mouse serum, respectively. The absorption of antibodies corresponding to surface antigens on live schistosomula has been described in detail in Materials and methods.

Further studies are needed before conclusions about the properties of this antigen can be reached. Finally, an antigen of 98 000 daltons was weakly (and not in all experiments) precipitated by the anti-membrane serum. This antigen was recognised by all the infection sera, but antibodies to it could not be absorbed by living schistosomula (Figure 4, lanes 3 – 8). It is unlikely therefore that this polypeptide is an exposed antigen.

From these absorption experiments, it is clear that at least two schistosomula surface antigens, 22 000 and 14 000 daltons, are relatively major gene products and can be detected amongst cell-free translation products directed by adult worm RNA.

Comparison of cell-free translation and internal schistosome biosynthesis

As shown above, CMS, VMS and anti-membrane serum precipitated from surface-labelled schistosomula two dominant antigens of 32 000 and 38 000 daltons. There is as yet no evidence for the precipitation of these surface antigens from cell-free translation products programmed by adult worm mRNA or from any other life cycle stage (see below). These two surface antigens are glycosylated (G.Payares and A.J.G. Simpson, unpublished observation), and may thus require extensive post-translational processing before being recognised as antigenic. It is possible that these molecules are in fact major cell-free translation products but are not precipitated by the antisera employed. To investigate this, we have compared antigens synthesised by cell-free translations with those synthesised by the intact organism (Figure 5). When adult parasites were incubated *in vitro* in the presence of [^{35}S]-methionine, a large number of radiolabelled polypeptides were detected by SDS-polyacrylamide gel electrophoresis and fluorography. Immunoprecipitation of these *de novo* syn-

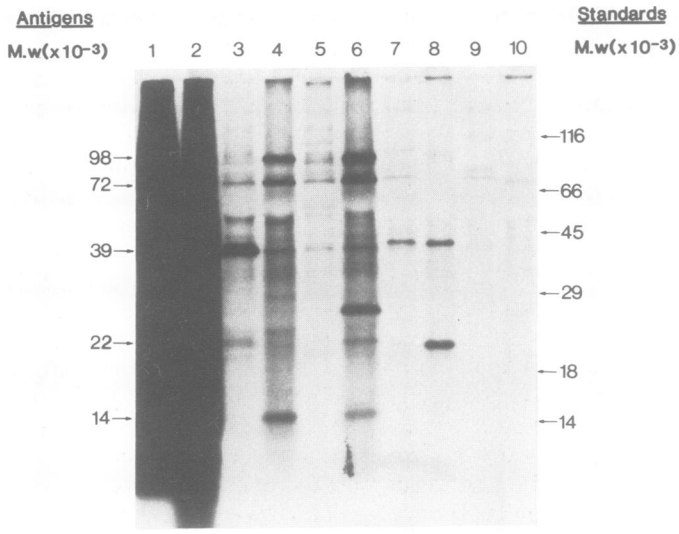


Fig. 5. Immunoprecipitation of cell-free synthesised and metabolically-labelled antigens from adult worms. [^{35}S]Methionine-labelled polypeptides from adult worms (**lanes 1, 3, 5, 7** and **9**) and cell-free translation (**lanes 2, 4, 6, 8** and **10**) were electrophoresed before (**lanes 1** and **2**) and after immunoprecipitation with CMS (**lanes 3** and **4**), VMS (**lanes 5** and **6**), anti-membrane serum (**lanes 7** and **8**) and normal mouse serum (**lanes 9** and **10**). Analysis of immunoprecipitations were by SDS-PAGE followed by fluorography and autoradiography (Materials and methods).

thesised polypeptides with VMS and CMS (Figure 5, lanes 3 and 5) resulted in similar antigens being precipitated when the same sera were used to precipitate cell-free translation products (lanes 4 and 6). There is considerable correspondence between the mol. wts. of the major antigenic polypeptides produced by the two systems although most metabolically synthesised antigens are less intense than those synthesised *in vitro*. Precipitation of [^{35}S]methionine metabolically-labelled polypeptides with VMS, CMS and the anti-membrane serum (lanes 3, 5, 7) again did not reveal the major 38 000 and 32 000 glycosylated surface antigens. Furthermore, there was close resemblance between the metabolically-labelled and *in vitro* synthesised antigens precipitated by the anti-membrane serum (lanes 7 and 8). Thus, cell-free translation was apparently adequate to synthesise the major surface antigens. The absence of the 38 000 and 32 000 glycosylated surface antigens amongst the metabolically-labelled polypeptides was either due to their low rate of synthesis or to their representing very minor gene products.

The stage specificity of schistosome antigen synthesis

Although the schistosome polypeptide target antigens are exposed on the young schistosomulum stage (Figure 2) we have demonstrated that some of these can also be synthesised by adult parasites. To further investigate the stage-specificity of protein and antigen expression, RNA has been extracted from all the distinct stages of the complex *S. mansoni* life cycle and utilised for *in vitro* translation. We found that translatable RNA was readily available from all stages except free-swimming cercariae (Table I). As an alternative, however, the intact infected *Biomphalaria* hepatopancreas containing schistosome sporocysts was found to be an extremely rich source of RNA. As judged by agarose gel electrophoresis, ~50% of the total RNA extracted this way was parasite-specific (data not shown).

The major polypeptides synthesised by mRNA extracted from the different life cycle stages were compared by gel

Table I. Starting material and final yield of total RNA from life cycle of *S. mansoni*

Life cycle stage	Yield total RNA (mg)
100 infected snail hepatopancreas (sporocysts)	19.3
28 non-infected snail hepatopancreas	4.00
1 x 10^6 cercariae	0
1 x 10^6 schistosomula	0.185
4 g wet weight, adult worms	5.3
3.62 g wet weight, egg	1.04
0.61 g wet weight, miracidia	1.5

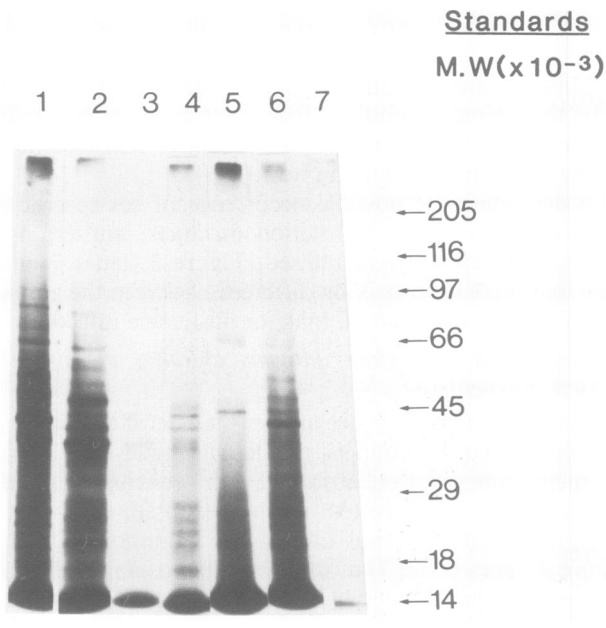
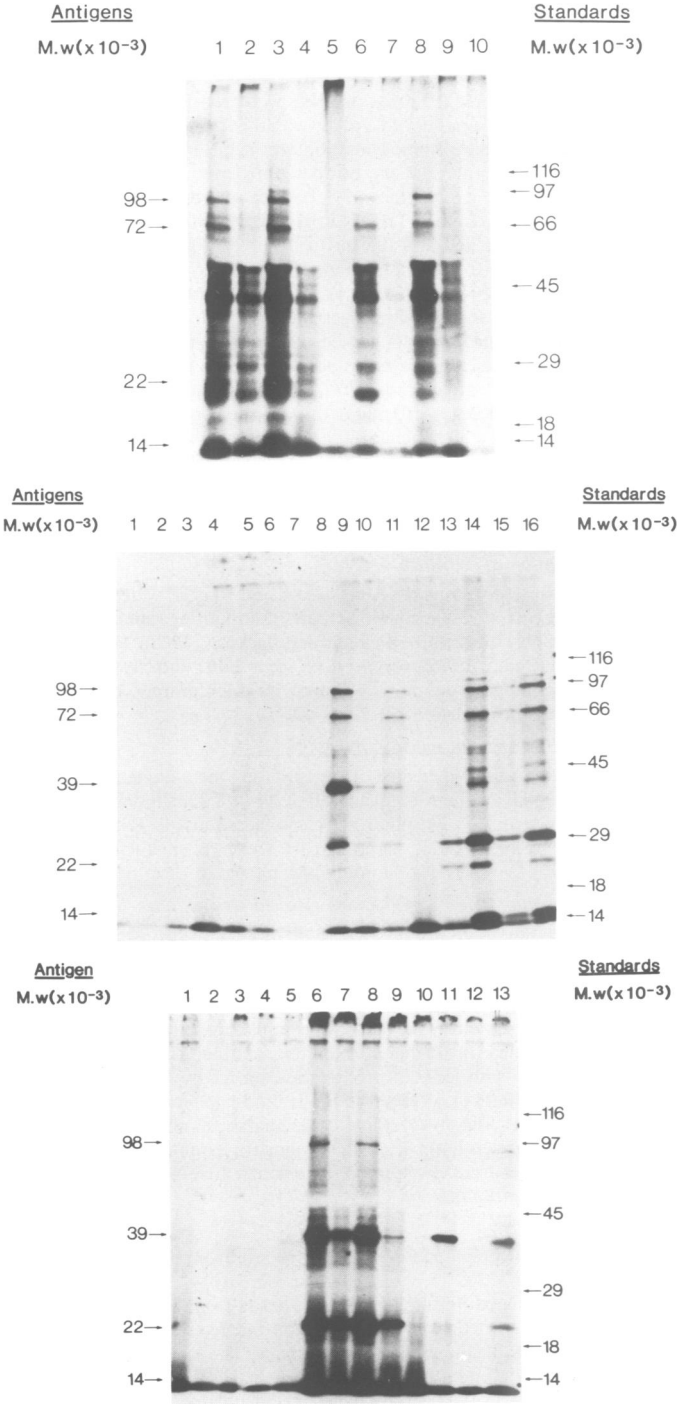


Fig. 6. Total [^{35}S]methionine-labelled *in vitro* translation products from all life cycle stages of *S. mansoni*. RNA extracted from adult worm **(1)**, miracidia **(2)**, egg **(3)**, schistosomula **(4)**, non-infected snail hepatopancreas **(5)** and infected snail hepatopancreas (sporocysts) **(6)** were used to direct protein synthesis in a mRNA-dependent rabbit reticulocyte lysate. Cell-free translation in the absence of RNA is shown in **lane (7)**.

electrophoresis (Figure 6). Despite the different morphology of the life cycle stages, most major detectable polypeptides were common to all the parasite forms although differences in intensity made the overall pattern of synthesis of each stage distinguishable. In addition, a major polypeptide of ~40 000 daltons was specific to the eggs and miracidia (Figure 6, lanes 2 and 3). The comparison of translation products directed by RNA from infected and non-infected hepatopancreas RNA emphasised the intensity of parasite-specific biosyntheis within the infected snail (Figure 6, lanes 5 and 6).

To compare the overall antigenicity of the various stages, human serum, which precipitated the widest range of antigens from adult, mRNA-directed protein synthesis (Figure 3, lane 4), was utilised to immunoprecipitate *in vitro* synthesised antigens from all parasite stages (Figure 7a). As judged by their mol. wts., the antigens precipitated from the cell-free translation products directed by the RNA from each parasite stage were, remarkably, almost indistinguishable. There were, however, some apparent variations in the translation of low mol. wt. antigens. Of particular interest was the low amount of the 22 000 and 14 000 daltons surface antigens in the schistosomula and miracidia stages of the parasite. This

observation was investigated more thoroughly by immunoprecipitation with VMS and anti-membrane serum (Figure 7b, c). The VMS precipitation (Figure 7b) clearly demonstrated that although some antigens are synthesised throughout the parasite life cycle such as the 29 000 dalton antigen, others are sex and stage specific. The antigen of highest mol. wt. (100 000), for example, is apparently synthesised only by adult male worms. In addition, the two surface antigens also show stage specificity. The 22 000-dalton antigen is clearly synthesised by adult worms, sporocysts and miracidia but apparently not by schistosomula. (This antigen was not detected amongst the egg translation products but since eggs and miracidia represent essentially the same life cycle stage, the inability to detect this antigen was thought to be due to the poorer yield of egg RNA obtained.) From these results, it is clear that the 22 000-dalton surface antigen is less intensely immunoprecipitated from schistosomula RNA directed cell-free translation, i.e., the very stage at which it is expressed at the parasite surface (Figure 2). To confirm this result the anti-membrane serum was used to immunoprecipitate translation products from all life cycle stages (Figure 7c). Although the 39 000-dalton antigen was clearly synthesised by RNA isolated from all stages, the 22 000-dalton antigen was only intensively synthesised by the adult, sporocyst and miracidia stages, and was barely precipitable from schistosomula RNA-directed translation products. The 14 000-dalton antigen which was immunoprecipitated by VMS (Figure 7b) showed a similar stage specificity. It was synthesised by adult worms and sporocysts but neither by schistosomula nor by miracidia. Thus, although most detectable antigens were synthesised to similar extents by all life cycle stages, the two surface antigens (mol. wts. 22 000 and 14 000) showed stage specificity in their *in vitro* synthesis, being abundantly synthesised by adult worms and sporocysts and less abundantly by miracidia and schistosomula stages.



Discussion

Polypeptides are present on the surface of schistosomula which are recognised as antigens by immune animals and man and these molecules might provide the basis of a protective vaccine against schistosomiasis. To date, however, very little information about these putative protective antigens is available. The work presented here shows that at least two of the surface antigens are major gene products of the total organism. They require no post-translational processing before being recognised by antibodies from immunised or chronically-infected animals, and can be readily detected among cell-free translation products of rabbit reticulocyte lysates under the direction of schistosome mRNA.

Although we have shown that these two molecules are surface antigens their relationship to the defined fully-processed schistosomula surface antigens remains to be established. One of the two surface antigens detected *in vitro* had a mol. wt. of 22 000. It was precipitated by sera from chronically infected and vaccinated mice, as well as by anti-membrane serum, and

Fig. 7. (a) Immunoprecipitation of [^{35}S]methionine-labelled *in vitro* translation products from all life cycle stages with infected human serum. Translation of RNA extracted from adult worms **(1)**, female adult worms **(2)**, male adult worms **(3)**, infected snail hepatopancreas (sporocysts) **(4)**, non-infected snail hepatopancreas **(5)**, schistosomula **(6)**, egg **(7)** and miracidia **(8)** were employed to direct cell-free translations in a mRNA-dependent rabbit reticulocyte lysate. **Lanes (9)** and **(10)** represent immunoprecipitation of adult RNA with two different batches of normal non-infected human sera. **(b)** Normal mouse serum **(lanes 1–8)** or VMS **(Lanes 9–10)** were used to immunoprecipitate [^{35}S]methionine-labelled polypeptides synthesised *in vitro* by RNA isolated from miracidia **(1** and **9)**, egg **(2** and **10)**, schistosomula **(3** and **11)**, non-infected snail hepatopancreas **(4** and **12)**, infected snail hepatopancreas (sporocysts) **(5** and **13)**, adult male worms **(6** and **14)**, adult female worms **(7** and **15)** and a mixed population of male and female adult worms **(8** and **16)**. **(c)** Immunoprecipitation of [^{35}S]methionine-labelled *in vitro* translation products from all life cycle stages with rabbit anti-membrane serum. Normal rabbit serum **(Lanes 1–5)** or serum raised in rabbits against partially purified adult worm membranes was used to immunoprecipitate *in vitro* synthesised polypeptides under the direction of adult worm RNA **(6)**, female only adult worms **(7)**, male only adult worms **(8)**, infected snail hepatopancreas **(9)**, non-infected snail hepatopancreas **(10)**, schistosomula **(11)**, egg **(12)** and miracidia **(13)**. The extraction of RNA, translations, immunoprecipitations and analysis by SDS-PAGE are described in detail in Materials and methods.

thus had similar precipitation characteristics to the 20 000-dalton antigen detectable on the schistosomulum surface. A monoclonal antibody has been prepared against this 20 000 mol. wt. antigen (Tavares *et al.*, 1983). Both this monoclonal antibody and the anti-membrane serum utilised here, precipitated the corresponding polypeptide produced by [^{35}S]methionine labelling of adult worms. Although the anti-membrane antibody precipitated this antigen in cell-free translations, the monoclonal antibody did not (data not shown). This may be due to the absence of membranes in the cell-free system employed, which may prevent the normal tertiary structure of the molecule from being adopted. Thus, the unequivocal identification of the 22 000-dalton antigen is not possible until polyvalent antisera against the purified 20 000-dalton surface antigen or sequence data, are available. This also applies to the 14 000-dalton antigen which was precipitated by the infection sera but not by the anti-membrane serum. It should be noted, however, that a molecule with similar mol. wt. and precipitation characteristics was detected on the schistosomulum surface.

The relative abundance of these two low mol. wt. surface antigens and our inability to detect other known major surface antigens (mol. wt. 32 000 and 38 000) suggest that there are two different types of surface antigen. It is possible that the unexpectedly high abundance of the low mol. wt. surface antigens (22 000 and 14 000) is because these molecules are not only exposed on the parasite surface, but can also occur within the internal tissues of the parasite and represent physiologically important molecules. This is further suggested by the observation that the molecules are detectable not only on the surface of the schistosomula but also within adult parasite translation products. This lack of antigenic variation within the life cycle is encouraging in terms of vaccination and useful from a practical point of view, since adult worm RNA, which is readily available, can be utilised for all selection and cloning procedures aimed at antigen gene identification.

We find that although the surface antigens are only detectable on the surface of the schistosomula stage of the parasite, their synthesis in cell-free translation directed by RNA extracted from this stage is barely detectable. This implies that the schistosomula surface antigens are pre-synthesised at the sporocyst stage and are not the product of *de novo* synthesis following transformation of cercariae to schistosomula.

In conclusion, although the surface forms a relatively small portion of the large multicellular schistosome, at least two surface constituent proteins are sufficiently major gene products of the total parasite to be readily detectable among cell-free translation products. In addition, both antigens are translated by adult worm mRNA, which is readily available in large quantities. It should be possible to identify the genes corresponding to these antigens within cDNA gene libraries constructed from adult worm mRNA, either by hybridisation selection or by direct expression in *Escherichia coli*. Once this is achieved, evaluation of the role of these molecules in schistosome immunity and their value as candidates for a vaccine can be undertaken.

Materials and methods

The parasite

A Puerto Rican strain of *S. mansoni* maintained at NIMR was used throughout these experiments.

Sporocysts were used within the hepatopancreas of *Biomphalaria glabrata* (water snails) which had been exposed to 4–6 miracidia 5 weeks previously. Each hepatopancreas was dissected from the snail and plunged directly into liquid nitrogen.

Cercariae were induced to shed from infected snails by exposure to a bright light source. They were left at 4°C for 2 h, washed once in saline, pelleted by centrifugation and stored in liquid nitrogen.

Schistosomula were obtained from cercariae by mechanically-induced transformation (Ramalho-Pinto *et al.*, 1974) and were maintained overnight at 37°C in Earles salt plus lactalbumin hydrolysate and stored in liquid nitrogen until required.

Adult worms were obtained by perfusion from hamsters infected 6 weeks previously with 800 cercariae (Smithers and Terry, 1965). The worms were washed twice in saline and stored in liquid nitrogen until required.

Eggs were obtained by digestion of the livers and intestines of infected hamsters with trypsin and purified by filtration and centrifugation (Smithers, 1960). Miracidia were collected by inducing eggs to hatch in dechlorinated tap water and pelleted by centrifugation at 4°C. Both eggs and miracidia were stored in liquid nitrogen until required.

Preparation of schistosome RNA

RNA was prepared from all schistosome life cycle stages as described by Taylor *et al.* (1983). The amount of starting material and the yield of total RNA are shown in Table I. Frozen parasites were transferred to a mortar on dry ice and crushed to a paste with a pestle with the frequent addition of liquid nitrogen. When complete homogenisation was achieved the liquid nitrogen was allowed to evaporate and the powder transferred to a 50 ml capacity polypropylene tube. Five volumes of 1:1 water saturated phenol: 200 mM NaCl, 2 mM EDTA, 20 mM Tris-HCl pH 7.8. 1% SDS (NETS) preheated to 85°C were then added and the mixture shaken vigorously until homogenous. After cooling to room temperature, the extract was centrifuged for 5 min at 10 000 *g* in a Sorvall SS-34 rotor at room temperature and the aqueous layer retained. The phenol layer was back extracted with a further half volume of NETS and the aqueous layers pooled. Following a second phenol extraction of the pooled aqeuous layers, nucleic acid was precipitated with 2.5 volumes of ethanol overnight at −20°C. The nucleic acids were collected by centrifugation at 10 000 *g* for 10 min at 0°C, washed once with 70% ethanol, centrifuged and dried under vacuum. Final pellets were dissolved in 0.1–1 ml of 10 mM HEPES, pH 7.5 and RNA was isolated by precipitation in 3 volumes of 4 M LiCl for 1 h on ice and centrifugation at 10 000 *g* for 10 min. The pellet was washed with 70% ethanol, dried again and dissolved in 10 mM HEPES pH 7.5.

In vitro translation of schistosome RNA

Samples of total schistosome RNA between 1 and 5 μg/μl, were translated *in vitro* in the presence of [^{35}S]methionine (Amersham), using rabbit reticulocyte lysates as previously described (Pelham and Jackson, 1976). Following the translation, the radiolabelled polypeptides were either directly analysed by polyacrylamide gel electrophoresis followed by fluorography, or were subjected to immunoprecipitation before analysis.

Intrinsic labelling of adult worm polypeptides

Adult parasites were incubated overnight in methionine-free Eagle's Minimum Essential Medium contaiing 50 μCi/ml [^{35}S]methionine and 10% rhesus monkey serum. Polypeptides were solubilised in 1% Triton X-100 in Krebs Ringer saline pH 7.4, containing 0.5 mM phenylmethylsulphonyl fluoride (PMSF). Samples were immunoprecipitated as described below.

Immunoprecipitation of [^{35}S]methionine-labelled polypeptides

Samples of the translation products (1–3 μl) or intrinsically labelled proteins (10–20 μl) were added to 5 μl of antiserum in a final volume of 50 μl made up with 0.05% Triton X-100, 50 mM Tris-hydrochloride, 150 mM NaCl, 10 mM EDTA, pH 8.0 (precipitation buffer). After incubation overnight at 4°C, 50 μl of 50% (v/v) protein A-Sepharose in precipitation buffer was added and the samples incubated for 60 min at room temperature. The beads were then washed four times with ice-cold precipitation buffer by centrifugation and finally suspended in 25 μl of electrophoresis buffer (1% w/v) SDS, 50 mM Tris-hydrochloride, 10% (v/v) β-mercaptoethanol, 2 mM PMSF, 1 mM EDTA, 1% (v/v) glycerol, pH 7.5. After boiling for 2 min to solubilise bound antigens the samples were electrophoresed through 10% polyacrylamide gels and fluorographed (Bonner and Laskey, 1974).

Preparation of antisera

All mouse sera were prepared in CBA mice. Serum from chronically infected mice (CMS) was collected 15 weeks after exposure to ~20 normal cercariae. Serum from vaccinated mice (VMS) was collected from animals exposed to 1000 cercariae attenuated by exposure to 20 Krad from a ^{60}Co source and boosted with an identical exposure 4 weeks later. The serum was collected 6 weeks after the initial immunisation.

Absorbed VMS was obtained by either: (a) incubating 0.5 ml of serum 3

times for 8 h at 4°C with ~50 000 mechanically transformed schistosomula, or (b) twice for 2 h at room temperature with ~80 000 mechanically-transformed schistosomula.

Serum from mice infected with either all male or all female worms was prepared by exposure of the mice to 130–300 cercariae of one sex obtained from snails exposed to a single miracidium. The sex of the infection was determined by perfusing one or two mice from each group 6 weeks after infection and the sera was collected 17 weeks after infection.

Rabbit anti-membrane serum was prepared by immunising Sandylop rabbits with purified adult worm surface membranes prepared as described by Simpson *et al.* (1981). Absorbed rabbit anti-membrane sera were obtained by incubating 0.5 ml of serum 3 times for 8 h at 4°C with ~50 000 mechanically transformed schistosomula.

Pooled human infection serum was obtained from a group of Kenyan patients known to be chronically infected with *S. mansoni* and was kindly provided by Dr A.Butterworth of the University of Cambridge.

Iodogen labelling of the schistosomula surface

Schistosomula were surface labelled using 1,3,4,6 tetrachloro-3,6 diphenylglycoluril (Iodogen) (Fraker and Speck, 1978). 100 μg of Iodogen, dissolved in chloroform, was plated on the base of a Bijou bottle by drying under a stream of nitrogen at room temperature. 50 000 3-h mechanically transformed schistosomula were concentrated in 100 μl phosphate-buffered saline (PBS) and mixed in the Bijou bottle with an equal volume of PBS containing 500 μCi of [^{125}I]Na carrier free (Amersham) and incubated on ice for 5–7 min. The schistosomula were transferred to 10 ml cold PBS, washed 3 times and resuspended in 0.5 ml of 1% Triton X-100 in Krebs Ringer Tris pH 7.4 containing 1 mM PMSF and 0.5 mM EDTA. Radiolabelled schistosomula surface membranes were solubilised on ice with occasional swirling.

Solubilised ^{125}I-labelled membranes were immunoprecipitated and processed as described above.

Acknowledgements

We should like to thank Mrs. Fiona Hackett for the inexhaustible supply of parasite material. M.K. is supported by a grant from the Edna McConnell Clark Foundation and G.P. is supported by The Council of Scientific and Humanistic Development of the Central University of Venezuela.

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Received on 19 October 1983