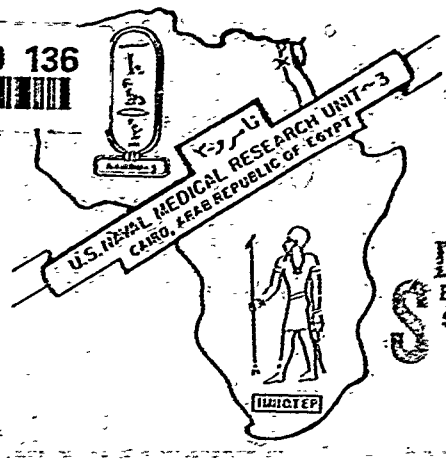

(2)

PUBLICATION REPORT

1584

66/89-90

ANTIBODY TO CARBOHYDRATE AND POLYPEPTIDE EPITOPES ON THE SURFACE OF SCHISTOSOMULA OF SCHISTOSOMA MANSONI IN EGYPTIAN PATIENTS WITH ACUTE AND CHRONIC SCHISTOSOMIASIS

BY

P. Omer Ali, M. Mansour, J.N. Woody,
S.R. Smithers and A.J.G. Simpson
U.S. NAVAL MEDICAL RESEARCH UNIT NO.3
(CAIRO, ARAB REPUBLIC OF EGYPT)
FPO NEW YORK 09527

Antibody to carbohydrate and polypeptide epitopes on the surface of schistosomula of *Schistosoma mansoni* in Egyptian patients with acute and chronic schistosomiasis

P. OMER ALI, M. MANSOUR*, J. N. WOODY*, S. R. SMITHERS and A. J. G. SIMPSON†

Division of Parasitology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA
**Biochemistry Department, U.S. Naval Medical Research Unit No. 3, Cairo, Arab Republic of Egypt*

(Accepted 17 November 1988)

SUMMARY

^{125}I-*Schistosoma mansoni* schistosomulum surface antigens were immunoprecipitated with human antibodies from individual Egyptian patients diagnosed as being either acutely or chronically infected with *S. mansoni*. Both sets of patients were found to have IgG antibodies in their sera capable of immunoprecipitating the major M_r > 200, 38 and 32K antigens. However, the immunoprecipitation of the M_r > 200K antigen was found to constitute a significantly greater proportion of the total precipitate achieved with acute sera than with chronic sera. The M_r 38 and 32K antigens were more variably precipitated by the acute sera than the chronic sera but the proportion of the total precipitation that these two antigens constituted was not found to be significantly different between the two sets of sera. Immunoprecipitation with pooled antibodies absorbed with egg and adult worm homogenates which had been treated to remove either carbohydrate or polypeptide epitopes demonstrated that the M_r > 200K antigen was the principal target of egg-cross-reactive anti-carbohydrate antibody amongst the antigens detected. The M_r 38 and 32K antigens were found to be precipitated by antibodies to protease-sensitive and periodate-insensitive polypeptide epitopes. These results are consistent with egg-cross-reactive anti-carbohydrate IgG antibody making a greater contribution to schistosomulum surface recognition in acute infection than in chronic infection. Indeed the presence of a higher level of egg-cross-reactive and anti-carbohydrate antibody directed against schistosomulum surface epitopes in an acute serum pool than in a chronic serum pool was confirmed by measurement of antibody binding to whole schistosomula.

Key words: *Schistosoma mansoni*, schistosomula, antigens, human antisera, carbohydrate epitopes, polypeptide epitopes

INTRODUCTION

Extensive analysis of *Schistosoma mansoni* schistosomulum surface recognition by murine IgG has demonstrated two distinct sets of epitopes. More than 90% of such epitopes are sodium metaperiodate sensitive carbohydrate structures which cross-react with the parasite egg. A smaller percentage are non-carbohydrate structures that do not cross-react with the egg (see Kelly (1987) for review). Epitopes of both types have been shown to be the target of protective murine monoclonal antibodies and are thus potential targets of protective immune responses (Omer Ali *et al.* 1988). Immunoprecipitation experiments have identified schistosomulum surface antigens of M_r > 200, 38 and 17K which express egg-cross-reactive carbohydrate epitopes and antigens of M_r 38, 32 and 20K which express non-carbohydrate epitopes (Omer Ali *et al.* 1988).

Human antibodies have been shown to immunoprecipitate schistosomulum surface antigens of similar M_r to those recognized by mice (Simpson *et al.* 1986). Furthermore, antibody has been detected in the sera of Kenyan children infected with *S. mansoni* to both carbohydrate and non-carbohydrate epitopes extracted from schistosomula, although it has not been demonstrated whether these are expressed on the surface (Dunne *et al.* 1988). Nevertheless, Dunne *et al.* (1988) found that there was a correlation between the levels of antibody to carbohydrate epitopes extracted from the schistosomulum and the egg and a correlation between antibodies to non-carbohydrate epitopes extracted from the schistosomulum and the adult worm. These observations are consistent with the epitopes being shared between the respective life-cycle stages and suggest that similar surface epitopes may be recognized by human and murine antibodies.

In order to test this possibility the specificities of anti-schistosomulum surface IgG antibodies in the sera of Egyptian patients infected with *S. mansoni* have been examined. Serum was taken from both acutely and chronically infected individuals. Acute schistosomiasis typically occurs 1–2 months after initial exposure to infected water and is diagnosed on the basis of fever, eosinophilia, stool examination and serology (see von Lichtenberg, 1987). Comparison of the immune responses of such patients with those known to have been infected for several years enables early and persistent responses to be differentiated. It was considered that such analysis may be of value in determining responses likely to

† Reprint requests to Dr A. J. G. Simpson

contribute to resistance to reinfection which has been shown to develop only after several years of exposure in individuals in endemic communities (Butterworth & Hagan, 1987).

MATERIALS AND METHODS

Parasites

A Puerto Rican strain of *S. mansoni* is maintained in hamsters and *Biomphalaria glabrata* at the National Institute for Medical Research and was used throughout the study. Schistosomula were prepared by mechanical transformation of cercariae and incubation for 3 h at 37 °C in Earle's salts plus lactalbumin hydrolysate (Ramalho-Pinto *et al.* 1974).

Antisera

All human sera were collected from patients admitted to the FUO ward of the U.S. Naval Medical Research Unit No. 3 in the Abbassia Fever Hospital, Cairo, in 1985–86. All sera were stored at −20 °C prior to serological analysis. None of the patients had been previously treated for schistosomiasis.

Twelve sera were collected from patients who had been diagnosed as suffering from acute schistosomiasis. These patients were all town dwellers who were exposed briefly for the first time to infected Nile water. Eggs of *S. mansoni* were found in all patients, although repeated stool examinations or rectal snip were required to find the eggs in some cases. The patients ranged in age between 5 and 14 years and had fever of 3–8 weeks, duration with diarrhoea, high eosinophilia and palpable tender livers. The length of fever is consistent with all the patients being infected 8–13 weeks prior to serum collection.

Thirteen patients from rural communities had a well-documented active infection of at least 3 years. They were all excreting eggs in their faeces and ranged in age between 10 and 24 years. Precise age matching of the two groups of patients was not feasible since acute infection tends to occur in younger patients and patients with documented infection of at least 3 years of the same ages were not available.

Pools of the acute and chronic sera were constructed by mixing equal 50 μl samples of each serum. The serum pools were also stored at −20 °C

Normal sera were obtained from healthy laboratory workers at NAMRU 3, Cairo.

Antigen detection assays

Measurement of antibody binding to intact and sodium metaperiodate-treated schistosomula was undertaken exactly as described previously (Omer Ali *et al.* 1986) using ^{125}I-protein A (Sigma) or ^{125}I-protein G (Pentex Biologics) to detect bound antibody. Radio-isotope labelling was undertaken using Iodogen (Fraker & Speck, 1978), resulting in a specific activity of $1–4 \times 10^6$ cpm/μg.

Schistosomula were surface labelled using Iodogen and ^{125}I-labelled antigens ($1–2 \times 10^6$ cpm) precipitated with a 1/10 dilution of antibody and protein-A Sepharose (Pharmacia) (Knight *et al.* 1984). Electrophoretic analysis was undertaken using 12·5% SDS-polyacrylamide gels which were subsequently dried and exposed for autoradiography. For quantitative analysis the autoradiographs were scanned using a Joyce-Lobel densitometer and the intensity of individual bands calculated as a percentage of the total precipitation. The significance of the variation of the relative intensity of individual bands between the acute and chronic sera was calculated using Student's *t* test.

Depletion of sera by absorption with adult worm and egg homogenates

Adult worms and eggs were homogenized in phosphate-buffered saline, pH 7·4 (PBS) containing 10 mM ethylenediaminetetraacetic acid (EDTA) to give a suspension containing approximately 10 mg/ml of protein. The homogenates were divided into three parts. The first was digested with 100 μg/ml of proteinase K (the reaction being stopped by the addition of phenylmethylsulphonyl fluoride at a final concentration of 5 mM), and then boiled for 1 h. The second was incubated with 100 mM sodium metaperiodate for 1 h at room temperature and the reaction stopped by the addition of ethylene glycol at a final concentration of 100 mM. The third was diluted with PBS to give the same final protein concentration as the treated antigens. The treated antigens were dialysed overnight against PBS at 4 °C. For absorption each antigen was incubated overnight at 4 °C with an equal volume of antibody. The particulate antigen was then removed by centrifugation at 12000 *g* for 10 min and the supernatant fraction used for immunoprecipitation.

RESULTS

Immunoprecipitation of ^{125}I-schistosomulum surface antigens was undertaken with sera from individuals diagnosed as being acutely or chronically infected with *S. mansoni* (Fig. 1A and B). Both sets of sera produced variable precipitation of labelled proteins of M_r 15–20K and 60–100K. The major features of the precipitation profiles, however, were the M_r > 200, 38 and 32K antigens. The chronic sera consistently exhibited an intense precipitation of both the M_r 38 and 32K antigens, whereas the precipitation of these antigens was more variable amongst the acute sera. Four acute sera preferentially precipitated the M_r 38K antigen and one contained

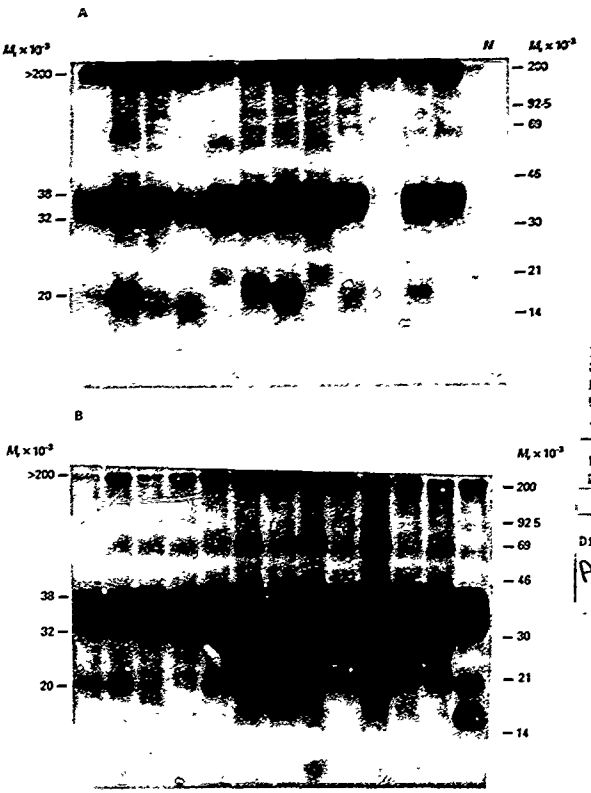

Fig. 1. (A) Immunoprecipitation of ^{125}I-schistosomulum surface antigens with antibodies from individual patients with acute schistosomiasis. *N*, precipitation with normal human serum. (B) Immunoprecipitation of ^{125}I-schistosomulum surface antigens with antibodies from individual patients with chronic schistosomiasis

no detectable IgG antibody against either of these two antigens. All the sera tested contained antibodies against the $M_r > 200$K antigen. However, the precipitation of this antigen by the acute sera was relatively more intense. Quantitative analysis of the immunoprecipitations was undertaken by scanning the autoradiographs of the gels with a Joyce-Lobel densitometer. The relative intensity of immunoprecipitation of individual bands was calculated as a percentage of the total precipitation in each lane. This method of quantitation was used to control for possible variations in the amount of antibody or radio-isotope labelled protein in individual precipitations. The percentage of total precipitation constituted by the $M_r > 200$K and the M_r 38 and 32K antigens combined is shown in Table I. Comparison of the results by Student's *t* test demonstrated that precipitation of the $M_r > 200$K antigen constituted a significantly greater percentage of the total precipitation in the acute sera than in the chronic sera

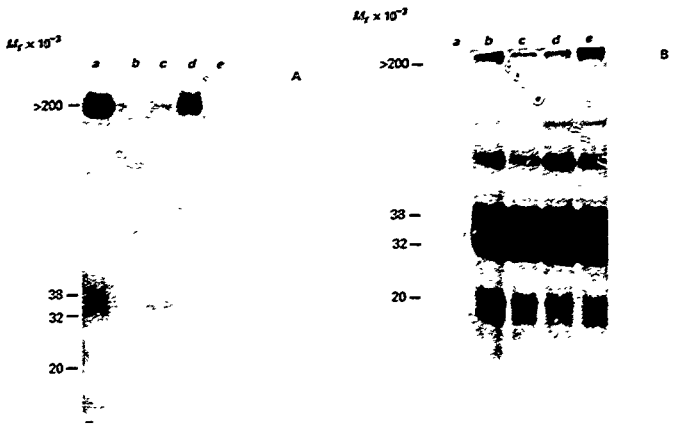

Fig. 2 (A) Immunoprecipitation of ^{125}I-schistosomulum surface antigens with antibodies from sera pooled from patients with acute schistosomiasis and the effect of absorption with whole egg homogenate. *a*, Intact acute serum (diluted with PBS to control for the dilution that occurs during absorption, see Materials and Methods section); *b*, acute serum absorbed with intact whole egg homogenate; *c*, acute serum absorbed with homogenate digested with proteinase K and boiled; *d*, acute serum absorbed with homogenate treated with sodium metaperiodate; *e*, normal human serum. (B) Immunoprecipitation of ^{125}I-schistosomulum surface antigens with antibodies from sera pooled from patients with chronic schistosomiasis and the effect of absorption with whole egg homogenate. *a*, Normal human serum, *b*, intact chronic serum (diluted with PBS to control for the dilution that occurs during absorption, see Materials and Methods section), *c*, chronic serum absorbed with intact homogenate, *d*, chronic serum absorbed with homogenate digested with proteinase K and boiled, *e*, chronic serum absorbed with homogenate treated with sodium metaperiodate

Table 1 Statistical analysis of the relative levels of immunoprecipitation of the $M_r > 200$K and M_r 38/32K schistosomulum surface antigens by antibodies in the sera of patients with acute and chronic schistosomiasis

(The values for the immunoprecipitation of the antigens were calculated by dividing the absorbance corresponding to each antigen band on the autoradiograph shown in Fig 1, by the total absorbance for all bands in the immunoprecipitate)

Patient type	Number	$M_r > 200$K	M_r 38/32K
Acute	12	40·7±21·3	42·4±15·8
Chronic	13	13·4±4·1	49·0±5·4
Significance		$t = 4·52$ $P < 0·001$	$t = 1·2$ P n.s.

The M_r 38 and 32K antigens, on the other hand, did not constitute a significantly different proportion of the total precipitation in the two groups of patients

The cross-reactivity between life-cycle stages and the contribution of carbohydrate and polypeptide moieties to the epitopes recognized on the principal schistosomulum surface antigens of $M_r > 200$, 38 and 32K were assessed by absorption experiments using serum pools. Absorption of the acute serum pool with a schistosome egg homogenate greatly reduced its ability to precipitate the ^{125}I surface antigens (Fig. 2A) Pre-treatment of the egg homogenate by boiling and digestion with proteinase K, to destroy polypeptide epitopes, did not affect its ability to absorb anti-$M_r > 200$K antibody but did reduce its ability to absorb anti-M_r 38 and 32K antibody Pre-treatment of the homogenate with periodate, on the other hand, prevented the absorption of the anti-$M_r > 200$K antibody but not the anti-M_r 38 and 32K antibodies Thus the anti-$M_r > 200$K antibody in the acute serum pool is directed against periodate sensitive, protease insensitive epitopes which are shared with the schistosome egg The anti-M_r 38 and 32K antibodies, on the other hand, are directed against periodate insensitive, protease sensitive polypeptide epitopes

Absorption of the chronic serum pool with egg homogenate only resulted in a reduction in the

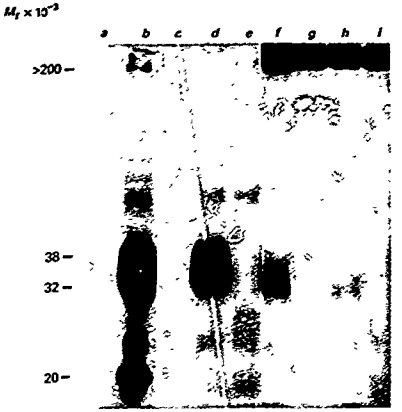

Fig. 3. Immunoprecipitation of ^{125}I-schistosomulum surface antigens with antibodies from sera pooled from patients with acute and chronic schistosomiasis and the effect of absorption with whole adult worm homogenate. *a*, Normal human serum; *b*, intact chronic serum (diluted with PBS to control for the dilution that occurs during absorption, see Materials and Methods section); *c*, chronic serum absorbed with intact homogenate, *d*, chronic serum absorbed with homogenate digested with proteinase K and boiled, *e*, chronic serum absorbed with homogenate treated with sodium metaperiodate, *f*, intact acute serum (diluted with PBS to control for the dilution that occurs during absorption); *g*, acute serum absorbed with intact homogenate, *h*, acute serum absorbed with homogenate digested with proteinase K and boiled, *i*, acute serum absorbed with homogenate treated with sodium metaperiodate.

relatively weak precipitation of the $M_r > 200$K antibody (Fig. 2B). As with the acute serum pool this absorption was abolished by pre-treatment of the egg homogenate with periodate. There was no other reduction of precipitation. This indicated that whereas anti-egg antibody contributed the majority of the anti-schistosomulum surface IgG in the acute serum pool it made a negligible contribution to surface recognition in the chronic serum pool.

Absorption experiments were also undertaken using an adult worm homogenate (Fig. 3). Absorption of the chronic serum pool completely abrogated its ability to immunoprecipitate schistosomulum surface antigens. Boiling and protease treatment, but not periodate treatment, prevented the absorption of anti-M_r 38 and 32K antibody, demonstrating that these were entirely directed at polypeptide epitopes. Absorption of the acute serum pool with the adult homogenate did not absorb antibody to the $M_r > 200$K antigen although the precipitation of the M_r 38 and 32K was abolished. As in previous experiments absorption of these antibodies was prevented by protease but not periodate treatment.

The analysis of surface antigen recognition by immunoprecipitation of ^{125}I-surface antigens thus demonstrated that the $M_r > 200$K antigen is the principal target of egg-cross-reactive anti-carbohydrate antibody in the patients examined and that there is a significantly greater proportion of anti-surface antibody directed against this antigen in the sera of acute patients than chronic patients. If the epitopes recognized on this antigen are exposed on the schistosomum surface and constitute a significant proportion of the total surface epitopes as they have been shown to do using mouse sera (Omer Ali *et al.* 1986, 1988), a significant difference in the binding of the serum pools to whole schistosomula would be predicted. To test this prediction binding assays to whole schistosomula were undertaken. The binding of the acute serum pool to intact schistosomula was found to be approximately 8-fold higher than that of the chronic pool. This high level of binding of the acute serum pool was reduced by 83% by absorption with egg homogenate (Fig. 4). In contrast, no change in the level of binding of the chronic serum pool was detectable. A similar reduction in binding of the acute serum pool resulted from the pre-treatment of the schistosomula with sodium metaperiodate which did not alter the level of binding

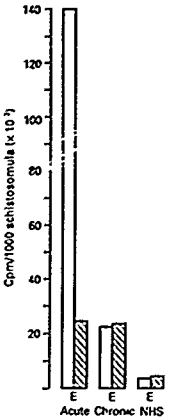

Fig 4 The binding of antibodies from sera pooled from patients with acute and chronic schistosomiasis to the surface of schistosomula. Hatched bars represent binding of antibody absorbed with whole egg homogenate. NHS = normal human serum. Each serum pool was used at a 1/10 dilution Each bar is the average of duplicates

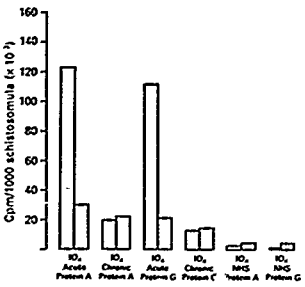

Fig. 5. The binding of antibodies from sera pooled from patients with acute and chronic schistosomiasis to the surface of schistosomula as measured with ^{125}I-protein A and ^{125}I-protein G. Bars marked IO_4 represent binding following treatment of schistosomula with sodium metaperiodate. NHS = normal human serum Each serum pool was used at a 1/10 dilution. Each bar is the average of duplicates

exhibited by the chronic pool (Fig 5) In this experiment protein-G, which binds to all human IgG subclasses was used to exclude the possibility of significant levels of anti-carbohydrate IgG_3 antibody in the sera of the chronic patients which would not be recognized by protein-A

DISCUSSION

It has been demonstrated here that antibodies from Egyptian patients both acutely and chronically infected with *S. mansoni* bind to the schistosomulum surface and immunoprecipitate surface antigens. Immunoprecipitation of ^{125}I-schistosomulum surface antigens with individual sera demonstrated that all those tested contained antibodies against the M_r > 200K antigen However, quantitative analysis of the immunoprecipitations revealed that there was a statistically significant difference in the relative intensity of precipitation of the M_r > 200K antigen by antibodies in the acute sera than antibodies in the chronic sera This indicated that there is more anti-M_r > 200K antibody in acute sera than in chronic sera.

All the chronic sera and the majority of the acute sera were also able to precipitate the M_r 38 and 32K antigens. Although the M_r 38 and 32K antigens were not precipitated by all the acute sera the statistical analysis revealed that there was no significant difference between the two groups Indeed the consistency of precipitation of the M_r 38 and 32K antigens is striking both by antibodies from the chronically infected patients included in the present study and from infected individuals in endemic areas of St Lucia (Simpson *et al.* 1986) and Brazil (Dissous *et al.* 1984) It is clear that the recognition of these two antigens contrasts that of the majority of antigenic polypeptides generated by the cell-free translation of mRNA where the recognition of individual proteins varies greatly between sera (Butterworth *et al* 1985; Simpson *et al* 1986, Newport *et al* 1988). Indeed, in this study, the immunoprecipitation of the M_r 20K antigen as well as minor antigens of M_r 40-100K were inconsistently precipitated by the individual sera

The chemical nature and life-cycle stage cross-reactivity of the epitopes on the M_r > 200, 38 and 32K antigens were examined using separate serum pools constructed from the acute and chronic sera It was thus demonstrated that the M_r > 200K antigen was recognized via carbohydrate epitopes which could be absorbed with an egg homogenate In contrast, antibody to the M_r 38 and 32K antigens was found to be against polypeptide epitopes that could be absorbed with an adult worm homogenate In the case of the acute serum pool, but not the chronic serum pool, there was also an apparent cross-reaction of epitopes recognized on the M_r 38 and 32K antigens with the egg Thus there may be low levels of these antigens present in the egg which were sufficient to absorb the anti-M_r 38 and 32K

antibody from the acute serum pool but not from the chronic serum pool. It is possible, however, that the apparent absorption is due to proteolytic activity associated with the egg homogenate which degrades the lower level of antibody to the M_r 38 and 32K antigens in the acute serum pool but is not sufficient to detectably reduce the higher levels of antibodies against other antigens. The reduction in absorption as a result of digestion with proteinase K and boiling is consistent with this hypothesis.

The principal findings from the immunoprecipitation experiments are the following. (1) There are different levels of IgG to the $M_r > 200$K antigen in the sera of the acute and chronic patients examined. (2) The epitopes recognized on the $M_r > 200$K antigen are dependent on carbohydrate moieties that are cross-reactive with the schistosome egg. (3) There are no significantly different levels of anti-M_r 38 and 32K antibody in the sera of the acute and chronic patients although the level of antibodies to these antigens is more variable in the acute sera and, as a result, they are found at a lower level in the acute pool. (4) The epitopes on the 38 and 32K antigens recognized by human sera are polypeptide and are strongly cross-reactive with the adult worm but weakly, if at all, with the egg. These findings are consistent with egg-cross-reactive anti-carbohydrate antibody making a significantly greater contribution to anti-schistosomulum surface antibody in the sera of acute patients than in the sera of chronic patients. This was indeed demonstrated by surface-binding experiments using sera pooled from the two patient types. These demonstrated that pre-absorption of the sera with egg homogenate or pre-treatment of the schistosomulum surface with periodate both greatly reduced the binding of the acute serum pool but did not alter the binding of the chronic serum pool.

These experiments demonstrate, that the correlations between human antibody levels to carbohydrate epitopes extracted from the schistosomulum and the egg as well as between antibody levels to non-carbohydrate epitopes extracted from the schistosomulum and the adult worm (Dunne *et al* 1988) can be accounted for by the recognition of cross-reacting epitopes, and that at least some of the epitopes in question are exposed on the schistosomulum surface and establish the polypeptide nature of epitopes previously defined as 'non-carbohydrate' (Omer Ali *et al* 1986, 1988; Dunne *et al* 1988). The findings are also consistent with the observation of Dunne and his colleagues that anti-egg and anti-carbohydrate IgG both decline with age and thus, by inference, with longevity of infection. They are also in agreement with the work of Gazzinelli and colleagues (Gazzinelli *et al* 1985) who demonstrated higher levels of anti-egg antibody in acute than in chronic schistosomiasis patients in Brazil.

A strong IgG response to schistosomulum surface carbohydrate epitopes, including those expressed on the $M_r > 200$K antigen have been detected in mice 12–15 weeks after infection (Omer Ali *et al.* 1986). Additionally, anti-polypeptide antibodies specific for the M_r 38 and 32K antigens have been found both in infected mice and mice vaccinated with highly irradiated cercariae (Omer Ali *et al.* 1989). Thus there are strong similarities between the anti-schistosomulum surface antibody responses in man and the mouse model.

Murine monoclonal antibodies specific for carbohydrate epitopes on the $M_r > 200$, 38 and 17K antigens as well as a monoclonal antibody against a non-carbohydrate epitope on the M_r 32K antigen have all been found to passively transfer resistance (Omer Ali *et al.* 1988). Thus it is possible that the human antibodies described in this paper may mediate immune killing in man. However, resistance to reinfection develops slowly after initial infection (Butterworth & Hagan, 1987) and it is reasonable to postulate that persistent rather than transient early responses are more likely to contribute to protective immunity in man. Based on the findings in the present study, using a small number of sera, the anti-polypeptide antibodies directed against the M_r 38 and 32K antigens rather than the anti-carbohydrate antibodies directed against the $M_r > 200$K antigen are more likely to contribute to protective immunity in man. Nevertheless, the finding that these antigens are also recognized by acute patients is consistent with the hypothesis that potentially protective responses are blocked early in infection (possibly by anti-carbohydrate antibodies) as suggested by Butterworth and colleagues (see Butterworth & Hagan, 1987). It is also possible that there are differences in antibody isotype or affinity in the anti-M_r 38 and 32K IgG response, during the course of infection, both of which have not been measured in the present work.

We thank Fiona Hackett for providing the parasite material used in this study. This work was supported in part by a grant from the U.S. Naval Medical Research and Development Command, Bethesda, MD, work unit No. 3M161102BS13-AK311. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the Naval Service at large.

REFERENCES

BUTTERWORTH, A. E. & HAGAN, P. (1987) Immunity in human schistosomiasis. *Parasitology Today* 3, 11–16.

BUTTERWORTH, A. E., CAPRON, M., CORDINGLEY, J. S., DALTON, P. R., DUNNE, D. W., KARIUKI, H. C., KIMANI, G., KOECH, D., MUGAMBI, M., OUMA, J. H., PRENTICE, M. A., RICHARDSON, B. A., ARAP SIONGOK, T. K., STURROCK, R. F. & TAYLOR, D. W. (1985) Immunity after treatment of human schistosomiasis mansoni. II. Identification of resistant individuals, and analysis of their immune responses. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 79, 393–408.

DISSOUS, C., PRATA, A. & CAPRON, A. (1984). Human antibody response to *Schistosoma mansoni* surface antigens defined by protective monoclonal antibodies. *Journal of Infectious Diseases* 149, 227–33.

DUNNE, D. W., GRABOWSKA, A. M., FULFORD, A. J. C., BUTTERWORTH, A. E., STURROCK, R. F., KOECH, D. & OUMA, J. H. (1988). Human antibody responses to *Schistosoma mansoni*, the influence of epitopes shared between different life-cycle stages on the response to the schistosomulum. *European Journal of Immunology* 18, 123–31.

FRAKER, P. J. & SPECK, J. C. JR. (1978). Protein and cell membrane iodination with a sparingly soluble chloramide, 1,3,4,6-tetrachloro 3a, 6a-diphenylglycoluril. *Biochemical and Biophysical Research Communications* 80, 849–57.

GAZZINELLI, G., LAMBERTUCCI, J. R., KATZ, N., ROCHA, R. S., LIMA, M. S. & COLLEY, D. G. (1985). Immune responses during human schistosomiasis mansoni. XI. Immunologic status of patients with acute infections and after treatment. *Journal of Immunology* 135, 2121–7.

KELLY, C. (1987). Molecular studies of schistosome immunity. In *The Biology of Schistosomes* (ed Rollinson, D. and Simpson, A. J. G.), pp. 265–293. London and New York: Academic Press.

KNIGHT, M., SIMPSON, A. J. G., PAYARES, G., CHAUDHRI, M. & SMITHERS, S. R. (1984). Cell-free synthesis of *Schistosoma mansoni* surface antigens: stage specificity of their expression. *EMBO Journal* 3, 213–19.

NEWPORT, G. R., HEDSTROM, R. C., KALLESTAD, J., TARR, P., KLEBANOFF, S. & AGAB'AN, N. (1988). Identification, molecular cloning and expression of a schistosome antigen displaying diagnostic potential. *American Journal of Tropical Medicine and Hygiene* 38, 540–6.

OMER ALI, P., HAGAN, P., HACKETT, F., SMITHERS, S. R. & SIMPSON, A. J. G. (1989). Variable species and stage specificities of schistosomulum surface epitopes recognized by mice vaccinated with highly irradiated cercariae. *Parasite Immunology* (in the Press)

OMER ALI, P., MAGEE, A. I., KELLY, C. & SIMPSON, A. J. G. (1986). A major role for carbohydrates preferentially recognised by chronically infected mice in the determination of *Schistosoma mansoni* schistosomulum surface antigenicity. *Journal of Immunology* 137, 3601–7.

OMER ALI, P., SMITHERS, S. R., BICKLE, Q., PHILLIPS, S. M., HARN, D. & SIMPSON, A. J. G. (1988). Analysis of the anti-*Schistosoma mansoni* surface antibody response during murine infection and its potential contribution to protective immunity. *Journal of Immunology* 140, 3273–9.

RAMALHO PINTO, F. J., GAZZINELLI, G., HOWELLS, R. E., MOTA-SANTOS, T. A., FIQUEREDO, E. A. & PELLEGRINO, J. (1974). *Schistosoma mansoni*: defined system for stepwise transformation of cercariae to schistosomula *in vitro*. *Experimental Parasitology* 36, 360–72.

SIMPSON, A. J. G., HACKETT, F., KELLY, C., KNIGHT, M., PAYARES, G., OMER-ALI, P., LILLYWHITE, J., FLECK, S. L. & SMITHERS, S. R. (1986). The recognition of *Schistosoma mansoni* surface antigens by antibodies from patients infected with *S. mansoni* and *S. haematobium*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 80, 261–70.

VON LICHTENBERG, F. (1987). Consequences of infection with schistosomes. In *The Biology of Schistosomes* (ed Rollinson, D. and Simpson, A. J. G.), pp. 185–232. London and New York: Academic Press.

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; Distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) 86/89-90	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION U.S. Naval Medical Research Unit No. 3	6b OFFICE SYMBOL (If applicable) NAVMEDRSCHU THREE
6c. ADDRESS (City, State, and ZIP Code) PSC 452, Box 5000 FPO, AE 09835-0007	7a NAME OF MONITORING ORGANIZATION 7b. ADDRESS (City, State, and ZIP Code)
8a NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command	8b OFFICE SYMBOL (If applicable) NAVMEDRSCH 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code) National Naval Medical Center Building 1, Tower 12 Bethesda, MD 20889-5044	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 61102A; PROJECT NO 3M1611-02BS13; TASK NO AK; WORK UNIT ACCESSION NO

11. TITLE (Include Security Classification) Antibody to Carbohydrate and Polypeptide Epitopes on the Surface of Schistosomula of Schistosoma mansoni in Egyptian Patients with Acute and Chronic Schistosomiasis. (UNCLASSIFIED)

12. PERSONAL AUTHOR(S) Omer-Ali, P.*, Mansour, M., Woody, J.N., Smithers, S.R.* and Simpson, A.J.G.*
* Division of Parasitology, National Institute for Medical Research Mill Hill, London

13a. TYPE OF REPORT	13b TIME COVERED FROM ____ TO ____	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
		1988, November, 6	8

16. SUPPLEMENTARY NOTATION

Published in: Parasitology, 98:417-424, 1989; Acc. No. 1584.

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Schistosoma mansoni, Schistosomula; Antigens; Human antisera; Carbohydrate epitopes; Polypeptide epitopes.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

^{125}I-Schistosoma mansoni schistosomulum surface antigens were immunoprecipitated with human antibodies from individual Egyptian patients diagnosed as being either acutely or chronically infected with S. mansoni. Both sets of patients were found to have IgG antibodies in their sera capable of immunoprecipitating the major M_r >200, 38 and 32K antigens. However, the immunoprecipitation of the M_r >200K antigen was found to constitute a significantly greater proportion of the total precipitate achieved with acute sera than with chronic sera. The M_r 38 and 32K antigens were more variably precipitated by the acute sera than the chronic sera but the proportion of the total precipitation that these two antigens constituted was not found to be significantly different between the two sets of sera. Immunoprecipitation with pooled antibodies absorbed with egg and adult worm homogenates which had been treated to remove either carbohydrate or polypeptide epitopes demonstrated that the M_r >200K antigen was the principal target of egg-cross-reactive anti-carbohydrate antibody amongst the antigens detected. The M_r 38 and 32K antigens were found to be

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	UNCLASSIFIED
22a NAME OF RESPONSIBLE INDIVIDUAL Research Publications Branch	22b TELEPHONE (Include Area Code) 202-284-1381
	22c. OFFICE SYMBOL R.P B.

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

66/89-90 (Contd.)

19. precipitated by antibodies to protease-sensitive and periodate-insensitive polypeptide epitopes. These results are consistent with egg-cross-reactive anti-carbohydrate IgG antibody making a greater contribution to schistosomulum surface recognition in acute infection than in chronic infection. Indeed the presence of a higher level of egg-cross-reactive and anti-carbohydrate antibody directed against schistosomulum surface epitopes in an acute serum pool than in a chronic serum pool was confirmed by measurement of antibody binding to whole schistosomula.