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SCHISTOSOMA MANSONI SURFACE PROTEINS

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This paper is concerned with the antigenicity of surface associated proteins of Schistosoma mansoni and their potential roles in immunity. The discussion is confined to those proteins which have been defined by surface iodination, antibody binding to intact schistosomula, monoclonal antibody reactivity and immunoprecipitation experiments in this laboratory.

Proteins exposed on 3 hour schistosomula.

Antibodies from infected hosts or experimental animals vaccinated with highly irradiated cercariae recognise a relatively complex set of proteins exposed on the surface of schistosomula, immediately after their transformation from cercariae (see table 1, a summary of our knowledge of the biochemical immunological properties of the surface associated antigens of S. mansoni). We have chosen to divide schistosomulum surface antigens into two categories for ease of discussion: those defined primarily by carbohydrate epitopes shared with the parasite egg and other schistosome species and those defined by species specific polypeptide epitopes shared with the adult worm.

Measurement of antibody binding to intact schistosomula using ^{125}I-protein A has demonstrated that >90% of surface epitopes are defined by sodium metaperiodate sensitive carbohydrate structures associated with antigens of M_r>200, 38 and 17K (Omer Ali et al, 1986). These epitopes are the targets of several protective monoclonal antibodies and are thus clearly potential targets of protective immunity in vivo (Omer Ali et al, 1988). In addition it has been found, by passively transferring an IgM antibody into mice subsequently inoculated with live schistosome eggs that monclonal anti-carbohydrate antibody can mediate anti-egg granuloma formation (Table 2).

Comparison of the levels of human anti-carbohydrate antibodies in patients with acute and chronic schistosomiasis indicates that such antibodies are an early response which is dramatically down regulated during the course of infection (Omer Ali et al, 1989b). Since acute schistosomiasis is associated with the presence of large, florid granulomas which subsequently diminish in size (see Von Lichtenberg, 1987), it may be that anti-carbohydrate antibodies, possibly acting as targets of anti-idiotypic T-cells, contribute to the mediation of immunopathology in man. It has been demonstrated that protective immunity to schistosome infection in man develops slowly and is only demonstrable some years after initial infection (Butterworth and

Hagan, 1987). Thus, individuals with acute schistosomiasis can be predicted not to be immune. It would thus seem that anti-carbohydrate antibody does not mediate protective immunity in man. A possible reason for this comes from the observation that some anti-carbohydrate antibodies, including those of the IgM isotype, have the ability to block the killing of schistosomula (Yi et al, 1986, Khalife et al, 1986). This phenomenon is probably due to the polyvalent IgM antibodies forming a complex with the abundant target epitopes which physically prevents access of effector antibodies to the schistosomulum surface. Butterworth and his colleaques have found that the levels of blocking antibodies in the sera of Kenyans infected with S. mansoni correlate with susceptibility to reinfection and have suggested that these antibodies may explain, at least in part, the slow development of immunity in man (Butterworth et al, 1987).

Three antigens of M_r38, 32 and 20K express species specific polypeptide epitopes that do not cross react with the egg. These antigens appear to be universally recognised by IgG from individuals with a chronic S. mansoni infection but are recognised less well by antibodies from acute schistosomiasis patients (Simpson et al, 1986, Omer Ali et al, 1989b). There is only limited data available from experiments using monoclonal antibodies to suggest that these antigens are targets of protective antibody in vivo, however resistance was observed in some experiments using antibodies to the M_r 32 and 20K antigens (Tavares et al, 1984, Bickle et al, 1986, Yi et al, 1986). In addition their species specificity is consistent with that of the protective immunity induced by vaccination with highly irradiated cercariae suggesting that they may be targets of protective antibody in this system (Omer Ali et al, 1989a). Likewise the pattern of their recognition by human antibodies is consistent with their playing a role in the mediation of protective immunity in man.

Modulation of the expression of surface proteins during early maturation.

Studies of protective immunity in vivo are leading to an appreciation that schistosomula 3 to 7 days after penetration may be more important targets of protective immunity than very young parasites. It is also becoming apparent that immunity is most likely due to the entrapment of migrating larvae within inflammatory foci which may be either antibody or T cell mediated (see McLaren and Smithers, 1987). These observations focus attention on antigens exposed on or released from the surface of developing schistosomula, rather than on only those on three hour schistosomula, as targets of protective immunity. In this regard it had been found that following in vitro culture of $\geq$ 24 hours of the antigens on 3 hour schistosomula only those of M_r38, 32, 20 and 15K remain available for surface labelling. In addition a novel antigen of M_r15K, precipitable by anti-adult tegumental membrane antibody, and a M_r8K antigen are expressed on 24 hour schistosomula. The dominant M_r>200K antigen and the M_r17K

antigen are no longer detectable (Simpson et al, 1984, Payares et al, 1985). These observations are consistent with the abundant carbohydrate epitopes, which are infact remnants of the cercarial glycocalyx, being rapidly lost from the developing parasite surface. It is perhaps of interest to note that it is those epitopes that persist on the developing schistosomula rather than the the dominant surface carbohydrates that stimulate antibody in mice protectively vaccinated with highly irradiated cercariae (Omer Ali, et al, 1986). Analysis of culture supernatants has resulted only in the detection of the M_r 38, 32, 20 and 15K antigens (Simpson et al, 1984). The antigens detected were found to be associated with released membrane vesicles as judged by their recoverability by high speed centrifugation. This has been recently supported by the observation that the shed antigens can be rendered soluble by digestion with phospholipase C (Pearce and Sher, 1989). The lack of detectable carbohydrate in culture supernatants is suprising but may indicate that the carbohydrate epitopes are lost from the parasite surface by internalization or degradation rather than by being shed.

Surface iodination of schistosomula recovered from the lungs of mice 5 days after infection results in the labelling of the M_r32, 20, 15 and 8K antigens as well as an antigen of M_r25K which is not detectable on the surface of three hour or 24 hour schistosomula (Payares 1985).

Antigenic proteins associated with adult tegumental membranes.

Surface labelling of intact adult S. mansoni does not result in the detectable iodination of schistosome proteins possibly due to their being masked by acquired host macromolecules (Payares et al, 1985). Nevertheless, iodination of isolated adult tegumental membranes followed by immunoprecipitation demonstrated that these structures have multiple antigenic proteins associated with them. By this methodology the most dominant antigen is the M_r25K protein also detectable on lung stage parasites. The M_r32 and 20K schistosomulum surface antigens are also present as well as the antigens of M_r15 and 8K (Payares et al, 1985). The dominant schistosomulum surface carbohydrate epitopes, however, are not detectable in adult membrane preperations. Furthermore, anti-adult tegumental membrane antibodies only bind to periodate insensitive epitopes on the surface of schistosomula (Smithers et al, 1989). These observations reflect a general relationship between adult tegumental membrane, egg and schistosomulum surface antigens. Measurement by ELISA of the cross reactivities of antibodies raised against these structures has revealed that although both egg and adult worm antigens cross react extensively with those of the schistosomulum surface, there is a minimal level of cross reaction between adult tegumental membrane and egg antigens (Simpson et al, 1989). These observations are supported by those of Dunne et al, (1988), who showed, using antibodies from Kenyan schoolchildren, that epitopes shared by schistosomula and adult worms are different from those shared by schistosomula and eggs. Immunoprecipitation and absorption

experiments have confirmed that none of the adult tegumental membrane antigens are detectable in the schistosome egg (Simpson et al , 1989). This lack of cross reactivity between adult tegumental membrane and egg antigens may be highly significant. It provides a potential molecular basis for the dissociation of protective responses from those which mediate anti-egg immunopathology thus enabling the two manifestations of the immune response in infected hosts to be independently regulated. To substantiate this hypothesis, two questions require answering: firstly, whether adult tegumental membrane antigens can stimulate protective immunity in vivo and secondly, whether the nature and extent of anti-adult tegumental membrane immune responses during natural infection in man are compatible with their playing a significant protective role? To date our attention has been focused on the first of these two questions.

An extensive series of experiments has led to the development of a vaccination protocol that results in the stimulation of reproducible levels of protective immunity in the permissive mouse model (Smithers et al , 1989). Highest levels of protection were achieved following subcutaneous vaccination of antigens in the presence of saponin as adjuvant. Using this protocol significant levels of protection were achieved using homogenates of schistosomula, cercariae or adult worms. Egg homogenates, however, failed to stimulate immunity. Fractionation of the adult worm homogenate by high speed centrifugation demonstrated that protection could be stimulated with membrane bound but not soluble antigen. Furthermore, equivelent levels of immunity (up to 39%) could be achieved with isolated tegumental surface membranes as with whole adult worm homogenate. The levels of protection achieved compare well with those achieved using irradiated cercariae in the same strain of mice (up to 45%).

Analysis of the antibody response in mice protectively vaccinated with tegumental surface membranes by both immunoprecipitation and immunoblotting demonstrated that the presence of IgG antibodies to antigens of M_r 32, 25, 22, 20, 15, 13 and 8K. The M_r13K antigen was only detectable by immunoblotting, the M_r22K antigen appears to be a soluble tegumental protein, a percentage of which is extrinsically associated with isolated membranes. It is not included in Table 1. Highest levels of antibodies appeared to be directed against the M_r 25, 15 and 13K antigens. Our present work thus focuses on these antigens as being the most likely candidates for stimulating the protective immunity that results from vaccination with tegumental membranes. Indeed, comparison of levels of antibody to the M_r25K antigen and the level of protective immunity stimulated in individual experiments showed these to be closely correlated ($p<0.05$, using Student's T test).

The M_r25, 15 and 13K antigens are all acidic molecules with pI<4.0 and are integral membrane proteins as judged by their partitioning in TX-114. The M_r25K antigen is composed of approximately 20% N-linked carbohydrate side chains as demonstrated by alteration of electrophoretic mobility following digestion with N-glycanase. The M_r15 and 13K molecules are apparently unaffected by this enzyme and do not detectably bind any of a range of lectins tested suggesting that they may not be

glycosylated.

By screening adult S. mansoni cDNA expression libraries constructed in Xgt11 with total anti-adult tegumental membrane antibody as well as antibodies purified from immunoblots by antibody select, cDNA clones corresponding to the M_r25K (Knight et al, 1989), and more recently the M_r15 and 13K antigens have been isolated. The three cDNAs correspond to different mRNA species suggesting that the proteins are distinct gene products. The cDNAs for the M_r 25 and 13K antigens hybridize to mRNAs of 0.8 and 0.6 kb respectively, sizes compatible with those of the mature proteins. In contrast the cDNA for the M_r15K antigen hybridizes to a mRNA of >3kb suggesting that the antigen is a highly processed product of a higher M_r precursor. The nucleotide sequences of the cDNAs for the M_r25 and 15K proteins have been determined. In both cases the predicted amino amino acid sequence is rich in glutamic acid residues which may account for the acidic pI of the mature gene products. Antibodies raised against the ß-galactosidase fusion protein resulting from the expression of the cDNA for the M_r25K protein in Xgt11 have been used to examine the distribution of the antigen within the adult worm. Detection of antibody binding to sections of adult worms by immunofluorescence as well as ELISA assays using different antigenic fractions both suggest that the protein is tegument specific (Knight et al, 1989).

The cDNA clones represent an important starting point for a detailed examination of the expression, structure and distribution of the proteins as well as their individual contributions to the stimulation of protective immune responses in experimental models. In addition, they will provide reagents for the detailed analysis of immune responses to these antigens in man.

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Table 1

Schistosoma mansoni surface antigens

M_r	Immunodominant Epitopes[a]	Expression H S L At E[b]	Antibody Reactivity CMS VMS AHS CHS[c]
>200K	Carbohydrate	- + - - +	+ - + -
38K[d]	Carbohydrate	- + - - +	+ - + -
	Polypeptide	+ + - - -	- + - +
32K	Polypeptide	+ + + + -	- + - +
25K	Polypeptide	+ - + + -	+ - ?[e] ?
20K	Polypeptide	+ + + + -	- + - +
17K	Carbohydrate	- + - - +	+ - - -
15K(i)[f]	?	- + - - -	- + - -
15K(ii)	Polypeptide	? + + + -	+ + ? ?
13K	Polypeptide	? - ? + -	? ? ? ?
8K	Polypeptide	? + + + -	+ + ? ?

a, determined by absorption, immunoprecipitation and monoclonal antibody binding experiments (Omer Ali *et al*, 1986; Omer Ali *et al*, 1989a).

b, determined by monoclonal antibody reactivity and immunoprecipitation with antibodies from schistosomiasis patients, infected mice and mice vaccinated with highly irradiated cercariae (Simpson *et al*, 1984; Yi *et al*, 1986; Omer Ali *et al*, 1988; Omer Ali *et al*,1989a). H= *S. haematobium* schistosomula, S=3 hour *S. mansoni* schistosomula, L=5 day lung stage schistosomula, At= adult tegumental membranes, E=Eggs.

c, determined by immunoprecipitation (Omer Ali *et al*, 1989a; Omer Ali *et al*, 1989b). CMS=infected mouse serum, VMS=serum from mice vaccinated with highly irradiated cercariae, AHS=serum from patients with acute schistosomiasis, CHS=serum from patients with chronic schistosomiasis.

d, The M_r38K antigen has both polypeptide and carbohydrate epitopes. The characteristics ofthe two sets of epitopes are tabulated seperately.

e, ?=not determined.

f, 15K (i) and (ii) are thought to be distinct antigens of similar M_r.

Table 2

Mediation of granuloma formation by a monoclonal anti-carbohydrate IgM antibody.

Experiment 1.

Group	Treatment	Mean diameter of 100 granulomas (um) Day 8	Day 16	p[c]
A	Irr.McAb[a]	N.D.[b]	5.58 + 9.07	-
B	Anti-CHO	N.D.	17.99 + 15.50	<0.001

Experiment 2.

Group	Treatment	Day 8	Day 16	p
A	-	5.06 + 6.97	5.93 + 4.94	-
B	Irr.McAb	5.63 + 6.30	6.16 + 3.78	N.S. (AvB)
C	Anti-CHO	11.02 + 11.57	10.28 + 7.05	<0.001 (BvC)
D	Eggs	9.72 + 6.65	9.97 + 4.67	<0.001 (AvD) N.S. (CvD)

The experiments exploited the lung model of egg granuloma formation (von Lichtenberg, 1962). CBA/Ca mice, 6 per group, recieved 0.4ml of monoclonal ascites every two days between day 0 and day 12. Two thousand live eggs were injected intravenously on day 0. In experiment 2, mice were sensitized with 1,000 live eggs injected intravenously on day -21.

a, an irrelevant anti-plasmodium monoclonal IgM antibody.

b, not determined.

c, significance of diference in mean granuloma size between groups as determined by Student's T-test.