

CHARACTERIZATION OF PROTECTIVE AND NON-PROTECTIVE SURFACE MEMBRANE CARBOHYDRATE EPITOPES OF *SCHISTOSOMA MANSONI*

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We have produced a number of monoclonal antibodies, protective and non-protective, which recognize a complex of schistosomula antigens, including the 38 kDa antigen. Eight different protective and non-protective monoclonal antibodies, varying in isotypes, were used in the binding assays. Lectin inhibition studies suggested that the monoclonal antibodies probably recognized carbohydrate epitopes on the antigen(s). Immunoprecipitation studies showed that at least two of the monoclonal antibodies recognized different epitopes on the same molecule. Additionally, we tested for monoclonal antibody binding after the antigens were treated with; 1) proteases, 2) periodate, 3) various exo- and endoglycosidases, 4) mild acid hydrolysis. We also tested for binding of the antibodies to keyhole limpet hemocyanin (KLH). Using the 8 monoclonal antibodies as probes, we were able to define at least 4 different carbohydrate epitopes related to the protective monoclonal antibodies, and at least one epitope which is seen by the non-protective antibodies. The epitope seen by the non-protective antibodies was shown to be cross-reactive with epitopes on KLH. These results demonstrate the importance of epitope mapping studies for any defined vaccine.

Antigens present on the surface membranes of developing schistosomes have been considered targets of the host immune response based on the results of a large number of studies *in vitro*, which have shown that these stages are susceptible to a variety of host effector mechanisms (Butterworth et al., 1982; Capron et al., 1982). A more direct correlation between surface antigens and protection has been shown in studies on passive transfer of immunity *in vivo*, using monoclonal antibodies directed against surface membrane antigens, reviewed in Harn et al. (1987a). Thus, surface membrane antigens are likely candidates for a defined vaccine against schistosomiasis.

One schistosomula surface membrane antigen complex has been detected by protective rat and mouse monoclonal antibodies (Gryzch et al., 1982; Harn et al., 1984; Harn et al., 1987b; Kelley et al., 1986). The apparent molecular weights of this antigen complex on schistosoma are: > 200, 200, 180-160 and 38 kDa. In addition to the surface membranes of schistosomula, the protective monoclonal antibodies also bind to the cercarial glycocalyx, internal structures of adult worms and the ciliary plates of miracidia (Harn et al., 1987b).

We have produced a number of monoclonal antibodies, of varying isotypes, that recognize

a schistosomula antigen complex which included a 38 kDa molecule. Passive transfer of immunity experiments showed that not all of the monoclonal antibodies were protective, and that the ability to protect did not appear to correlate with isotype (Harn et al., 1987a). Thus, to examine the possible differences in the antibodies, we initiated these studies on mapping of the individual epitopes.

MATERIALS AND METHODS

Parasites — Cercariae were mechanically transformed into schistosomula (MS), as previously described (Harn et al., 1984). A deoxycholate extract of mechanically transformed schistosomula (DOC-MS), was prepared as previously described (Harn et al., 1984). A soluble schistosomular preparation, (SCHLAP), was prepared by homogenizing MS in phosphate buffered saline (PBS) on ice, followed by centrifugation at 15,000 g for 90 minutes. The supernatant was collected as SCHLAP.

Monoclonal antibodies — Anti-egg monoclonal antibodies, E.1-E.5, were produced as previously described (Harn et al., 1984). Antibodies E.1, E.2 and E.5 are partially protective *in vivo*, antibodies E.3 and E.4 are non-protective *in vivo* (Harn et al., 1987a). Protective

anti-membrane monoclonal antibodies M.3 and M.4 were produced as described (Harn et al., 1985). All hybridomas were grown in culture, and hybridoma culture supernatants were used as the source of antibodies for all experiments.

Immunoprecipitation – Living MS, or DOC-MS, were radiolabeled with ^{125}I using iodogen as previously described (Harn et al., 1987b). After labeling of the intact parasites or the detergent extracts, immunoprecipitations were carried out with hybridoma culture supernatants as described (Harn et al., 1987b). Immunoprecipitated samples were analyzed via SDS-PAGE (Laemmli, 1972), followed by autoradiography.

Protease digestion – The 38 kDa complex of antigens were purified from ^{125}I labeled DOC-MS by immunoaffinity chromatography using E.1 covalently linked to protein A-sepharose. Immunoaffinity purified antigen was then subjected to proteolytic degradation separately with each of the following: trypsin, chymotrypsin, proteinase K or staphylococcal V8 protease.

Phase separation – ^{125}I -labeled, immunoaffinity purified E.1 antigens were equilibrated with 1.0% Triton X-114 at 4C, and then separated into aqueous and detergent phases by equilibrating the temperature of the solution to 30C. The aqueous and detergent phases were collected and then analyzed via SDS-PAGE followed by autoradiography.

Periodate treatment of E.1 antigen – ^{125}I -labeled, immunoaffinity purified E.1 antigens were treated with increasing concentrations of NaIO_4 , or control buffer and then re-immunoprecipitated with E.1, or rabbit anti-SWAP sera, and analyzed by SDS-PAGE.

Mild Acid hydrolysis of E.1 antigen – ^{125}I -labeled, immunoaffinity purified E.1 antigen was treated with H_2O , 0.05N or 0.10N HCl for 1 hr at 80C. The treated antigens were then re-immunoprecipitated with either E.1 or E.3 monoclonal antibodies then analyzed via SDS-PAGE followed by autoradiography.

RIA on total schistosomula antigens before and after mild acid hydrolysis – Polyvinyl chloride (PVC), 96 well, flex plates were coated with SCHLAP, 75 ng/well, then treated with either H_2O or various concentrations of HCl,

ranging from 0.025N-0.10N, at 80C for 1 hr. The plates were next washed with PBS-0.05% Tween 20, then incubated with hybridoma supernatants from all of the E and M monoclonal antibodies, washed with PBS-Tween, and probed with 10^6 cpm of rabbit anti-mouse IgG, IgA and IgM. The plates were washed again, and individual wells were cut out and counted.

Glycosidase treatment of E.1 antigen – ^{125}I -labeled, immunoaffinity purified E.1 antigen was treated with N-glycanase, endo-F, endo-H, or control buffers, then re-immunoprecipitated with E.1 or E.3. The immunoprecipitates were analyzed via SDS-PAGE followed by autoradiography.

Binding of E and M monoclonal antibodies to KLH – PVC plates were coated with KLH, 10 $\mu\text{g}/\text{ml}$ in PBS, then washed with PBS-Tween. Plates were next incubated with the E and M monoclonal antibodies, washed, then probed with 10^6 cpm of rabbit anti-mouse IgG, IgA, IgM. Plates were washed a final time, then cut into wells and counted.

RESULTS

Immunoprecipitations – Initial experiments were to examine the epitopes which were immunoprecipitated from cercariae or schistosomula by E.1 and E.3, the protective and non-protective antibodies respectively. The first experiment was designed to show which epitopes were accessible to antibody on intact, living cercariae or schistosomula. On cercariae, both E.1 and E.3 immunoprecipitated a very high molecular weight material which did not migrate past the stacking gel, as well as antigens at > 200, 200, and 180-160 kDa. On intact MS, E.1 immunoprecipitated the > 200, 200 and 160 kDa antigens, but the high molecular weight material in the stacking gel disappeared. E.3 immunoprecipitated the same high molecular weight antigens from schistosomula as E.1, but also strongly immunoprecipitated the 38 kDa antigen. Thus, on intact organisms, there was a distinct difference in the target antigens immunoprecipitated by the protective and non-protective monoclonal antibodies.

We next looked at antigens immunoprecipitated by E.1 and E.3 from DOC-MS. In contrast to the results with intact parasites, we found that both of the monoclonal antibodies primarily

immunoprecipitated the 38 kDa antigen from the radiolabeled detergent extract. This result shows that the epitope recognized by E.1 exists on the 38 kDa molecule, but was not accessible to antibody on the intact parasite. Partial immunoprecipitation characteristics for all the E and M antibodies are summarized in the Table.

Phase separation experiments — Because of the results from the immunoprecipitation studies, we decided to look for physical differences between the high molecular weight molecules and the 38 kDa molecule. We separated immunoaffinity purified E.1 antigens from DOC-MS via Triton X-114 phase separation. We found that there was indeed a physical difference in the molecules, with the 38 kDa antigen partitioning exclusively into the detergent phase, and conversely, the 200 kDa antigen partitioned exclusively in the aqueous phase.

Proteolytic digestion of immunoaffinity purified E.1 antigens — Earlier studies suggested that our monoclonal antibodies recognized carbohydrate epitopes. To determine if the schistosomula antigens also contained peptide, we treated purified E.1 antigens from radiolabeled DOC-MS with a number of different proteases. All of the E.1 schistosomula antigens were shown to be sensitive to protease digestion by their altered migration in SDS-PAGE, and thus, are comprised in part, of peptide.

Periodate treatment of E.1 antigens — To partially confirm earlier studies which suggested that our monoclonal antibodies recognized carbohydrate epitopes, we performed experiments to partially deglycosylate the E.1

antigens. We treated purified E.1 antigens from iodinated DOC-MS with sodium periodate and then tried to re-immunoprecipitate the antigens. We found that the 38 kDa molecule was degraded by periodate treatment to a molecule of approximately 31 kDa. We also found that treatment of the antigens with concentrations of greater than 5mM NaIO₄, destroyed the E.1 epitopes. However, the rabbit anti-SWAP sera was still capable of immunoprecipitating the antigens after 20mM periodate treatment, indicating that the periodate treatment did not indiscriminately destroy epitopes. Results are summarized in the Table.

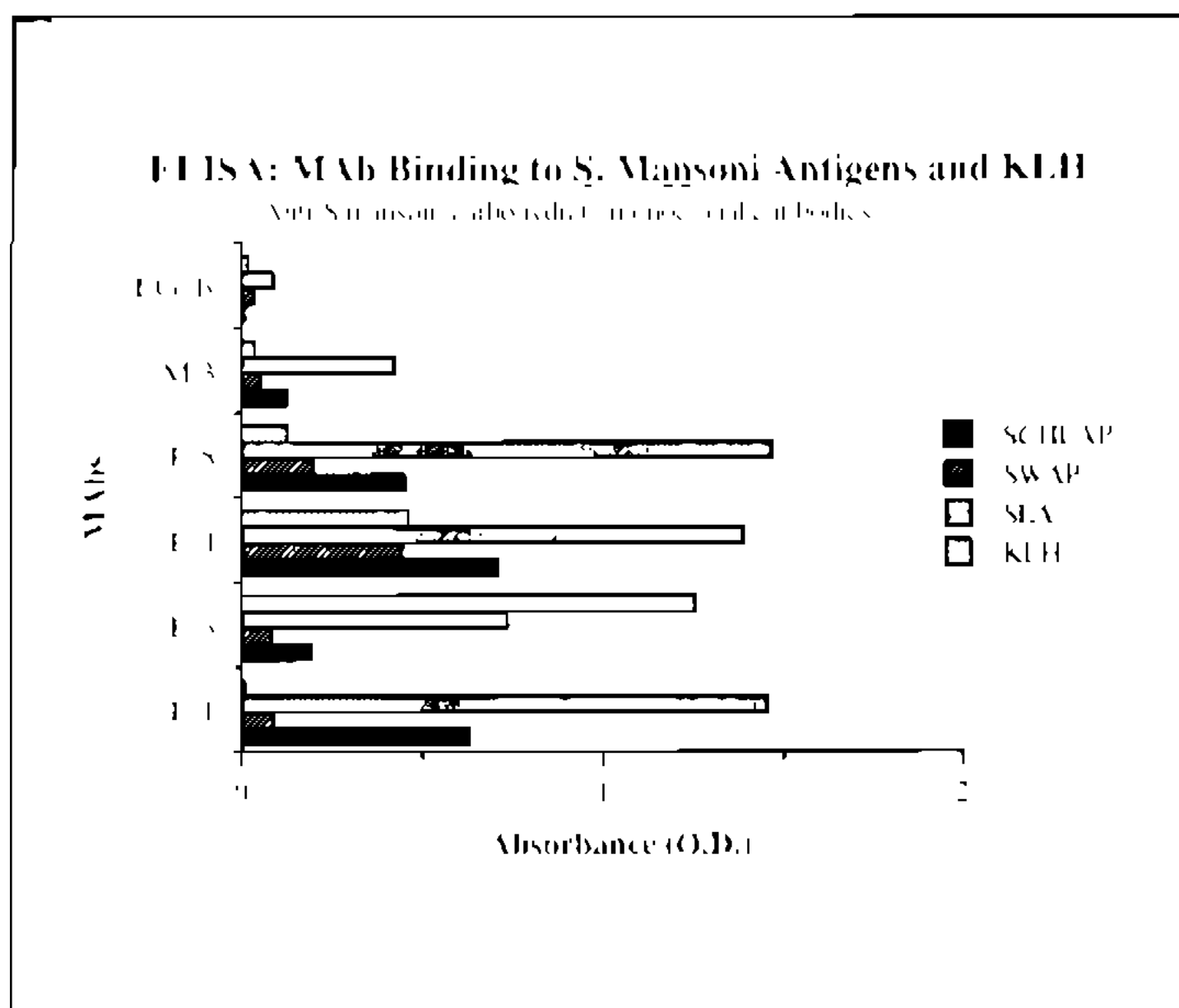
Mild acid hydrolysis of E.1 antigens — To further characterize the nature of the E.1 epitope, we subjected radiolabeled E.1 antigen to treatment with mild hydrochloric acid. This treatment preferentially destroys sialic acid residues or fucose residues, while other hexoses are quite stable under the hydrolytic conditions employed here. Mild acid hydrolysis effected the mobility of the purified E.1 antigens in SDS gels. After treatment with 0.1N HCl the antigens could not be re-immunoprecipitated with E.1.

RIA on mild acid hydrolyzed SCHLAP — We next wanted to examine whether the other monoclonal antibodies also detected mild acid sensitive epitopes. For these experiments, SCHLAP was treated with varying concentrations of HCl on PVC plates, then probed with the various monoclonal antibodies. The results of this experiment, are summarized in the Table. Interestingly, the epitope for E.3, the non-protective monoclonal antibody, was not sensitive to mild acid hydrolysis.

TABLE

Evidence for several protective carbohydrate epitopes

MAb	Isotype	%protection passive transfer	Recognizes gp38	KLH binding	Epitope sensitive to 0.1N HCl
E.1	G2B	41%	yes	—	yes
E.3	G3	NS	yes	+	no
E.4	M	NS	yes	+/-	no
M.3	G3	46%	yes	—	no
M.4	G1	40%	no	—	no
E.5	M	30-40%	no	—	no



ELISA for binding of E and M monoclonal antibodies to KLH – To further characterize the carbohydrate epitopes which the E and M monoclonal antibodies recognize, we tested for binding to KLH. These results are summarized in the Table and in the Figure. Both E.3 and E.5 bound to KLH in an ELISA. Both antibodies are non-protective. None of the protective monoclonal antibodies bound to KLH. Thus, these results would suggest that the KLH epitope is not protective *in vivo* in the mouse model.

DISCUSSION

In this report we partially characterized carbohydrate epitopes for 8 different monoclonal antibodies which recognize the 38 kDa antigen complex. By employing both protective and non-protective monoclonal antibodies, we were also able to differentiate between protective and non-protective epitopes.

Previously, Grzych et al. (1984), demonstrated using rat monoclonal antibodies, that IgG_{2a} but not IgG_{2c} antibodies to the 38 kDa, were effective *in vitro* and *in vivo*. They suggested that binding of the IgG_{2c} antibodies to the parasites was sufficient to block the protective antibody from binding to the parasites. Additionally, *in vitro* studies have also shown that certain patient sera also contain antibodies which are able to block the rat monoclonal antibodies from killing schistosomula (Khalife et al., 1987; Butterworth et al., 1987). However, the relevance of this test to immunity must be questioned, since all patient sera, when tested individually, functioned well in all of the

in vitro killing assays (Butterworth et al., 1985; Butterworth et al., 1987).

In earlier studies on passive transfer of immunity (Harn et al., 1987a), we found that there was no correlation with protection and the isotypes of mouse monoclonal antibodies to the 38 kDa antigen complex. However, we did find that we had several monoclonal antibodies to the 38 kDa antigen which did not passively transfer protection; these were either IgG₃ or IgM's.

In this study, we have shown that the inability of these monoclonal antibodies to passively transfer immunity in the mouse model, is more likely due to the specific carbohydrate epitope which they recognize. We demonstrated that the non-protective monoclonal antibodies bound to KLH in an ELISA. None of the protective monoclonals recognized KLH. In contrast, the protective rat monoclonal antibody does recognize a carbohydrate epitope on KLH (Dissous et al., 1986). Perhaps the differences between seen between the two systems can be ascribed to the use of the rat model of immunity versus our use of the mouse model.

In conclusion, we have characterized the carbohydrate epitopes found on the 38 kDa antigen complex which are relevant to both protective and non-protective mouse monoclonal antibodies. These studies have shown that there are at least 4 different carbohydrate epitopes related to protective monoclonal antibodies and at least one which is seen by non-protective monoclonal antibodies.

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