

**SCHISTOSOMA MANSONI SHARES A PROTECTIVE  
CARBOHYDRATE EPITOPE WITH  
KEYHOLE LIMPET HEMOCYANIN**

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The production of mAbs of various isotypes to *Schistosoma mansoni* has greatly contributed to the appreciation of the function of antibodies in the immunity against schistosomiasis, and has allowed the characterization of several potential protective antigens. Using this approach, we clearly established in previous studies (1, 2) the close relationship existing between a 38,000  $M_r$  *S. mansoni* schistosomulum surface antigen and the expression of eosinophil-mediated killing of schistosomula. The 38,000  $M_r$  antigen was initially characterized by the IPLSm1 rat IgG2a mAb that exhibits a marked eosinophil-dependent cytotoxicity and passively transfers a high degree of protection towards a cercarial challenge (1, 2). This particular surface antigen was also shown to react with polyclonal antibodies present in various infected hosts including mice, rats, monkeys, and humans (3). Additional studies revealed that 97% of a group of 120 Brazilian patients with *S. mansoni* infection produced circulating antibodies against this antigen, suggesting that the 38,000  $M_r$  molecule corresponds to a potent immunogen in man. More interestingly, the antibody response was demonstrated to appear in young children, to be maximal in older patients, and showed a parallelism with the prevalence and the intensity of the infection. This indicated that the antibody response against the 38,000  $M_r$  antigen could be considered an important marker of *S. mansoni* infection (4).

Although the 38,000  $M_r$  antigen initially appeared as a good candidate for the molecular cloning and subsequent studies, the glycanic nature of the epitope recognized by the IPLSm1 mAb limited its in vitro production by recombinant DNA methodology. Secondly, the 38,000  $M_r$  antigen was shown (5) to bind the IPLSm3 mAb of IgG2c isotype capable of blocking both in vivo and in vitro effector function(s) of the IPLSm1 IgG2a mAb. We recently demonstrated (6) the existence of similar blocking antibodies specific to the 38,000  $M_r$  molecule

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in human infection and correlated the level of such blocking antibodies with the presence or absence of immunity to reinfection.

The protective role of the 38,000  $M_r$  molecule and particularly the effect of its IPLSm1-defined epitope was also supported by an antiidiotype antibody probe. An antiidiotype antibody Ab2 produced against the IPLSm1 antibody (Ab1) elicits by immunization polyclonal Ab3 antibodies exhibiting specificity for the 38,000  $M_r$  antigen. Ab3 antibodies induce a significant level of eosinophil-dependent cytotoxicity and passively transfer immunity. Most importantly, immunization with Ab2 led to a high degree of resistance to a cercarial challenge (7).

Recently, experiments performed on rats immunized with Ab2 mAb conjugated to KLH (currently used as a carrier in immunization with synthetic peptides) led us to make some unexpected observations. We could show that rats injected with KLH, used as control in the KLH/Ab2 immunization, produced anti-*S. mansoni* antibodies specific for the 38,000  $M_r$  schistosomula surface antigen. This observation was also supported by the demonstration that the carbohydrate epitope of the 38,000  $M_r$  antigen that is present at the early life cycle stages of *S. mansoni* (miracidia) is also expressed by the intermediate host *Biomphalaria glabrata* as well as by other freshwater snails (8).

In the present work, we provide new evidence for the existence of a shared epitope between KLH and the 38,000  $M_r$  antigen, and that KLH immunization elicits in rats the production of anti-*S. mansoni* antibodies with similar effector functions to those induced during the course of infection. We have exploited these latest results to investigate further the biological and immunological properties of this particular epitope.

## Materials and Methods

**Parasites.** The Puerto Rican strain of *S. mansoni* was used in all our experiments. The parasite cycle was maintained in our laboratory with the use of *B. glabrata* snails as invertebrate intermediate hosts and Golden hamsters, *Mesocricetus auratus*, as vertebrate definitive hosts. Schistosomula of *S. mansoni* prepared according to the method of Ramalho-Pinto et al. (9) and referred to as mechanically prepared schistosomula were used throughout the work. For all the cytotoxicity assays, however, schistosomula were prepared according to the skin penetration technique described by Clegg and Smithers (10) and referred to as skin-transformed schistosomula.

**Monoclonal Antibodies.** IPLSm1 mAb is a purified anti-*S. mansoni* antibody of IgG2a isotype (1). It was produced after fusion of spleen cells from rats infected with *S. mansoni* for 5 wk and the nonsecretory IR983 F myeloma cell line (11). It was purified by DEAE ion-exchange chromatography according to the method of Bazin et al. (12). For inhibition experiments, purified IPLSm1 antibodies were radiolabeled with  $^{125}\text{I}$ iodine by the chloramine T method (13).

JM8-36 antibody is a rat antiidiotype mAb of IgM isotype specific for the IPLSm1 antibody (7). Purified fractions of JM8-36 were obtained by gel filtration chromatography according to Bazin et al. (12).

**Antigens and immunization procedure.** Purified lyophilized KLH (mol wt,  $3 \times 10^6$  to  $7.5 \times 10^6$ ) was purchased from France Biochem. (Meudon, France). For inhibition experiments, KLH was radiolabeled with  $^{125}\text{I}$  by the chloramine T method (13). Degly-

cosylation of KLH (DKLH)<sup>1</sup> was performed according to the procedure of Edge et al. (14). Briefly, 5 mg of KLH was dissolved in 1 ml of anisole/trifluoromethanesulfonic acid (TFMS) (1 vol/2 vol) mixture and kept for 3 h at 0°C. The deglycosylated KLH was freed of reagents and low molecular weight sugars by extraction with diethylether in the presence of aqueous pyridine followed by dialysis against pyridine acetate buffer, pH 5.5. LOU/M rats (10 wk old, 180–200 g) were immunized twice at a 2 wk interval by intraperitoneal injection of 1 ml of physiological saline solution containing 100 µg KLH or 100 µg DKLH. Rats were bled every week from the start of the immunization. Sera were recovered and stored at –20°C.

*Indirect Immunofluorescence Assay.* Anti-*S. mansoni* antibodies were detected by indirect immunofluorescence on sections of *S. mansoni* schistosomula prepared as previously described (9). Sections of 8 µm were overlaid with 50 µl of immunized rat sera at a dilution of 1:10 in 10 mM PBS buffer. After 30 min contact and three washings in PBS, FITC-conjugated anti-rat Ig antisera prepared in rabbits (Miles Laboratories, Inc., Naperville, IL) were added for 30 min. The slides were then washed three times in PBS, the last wash containing Evans Blue (1:10,000 final dilution) as a counterstain. 4 wk infected rat sera and normal rat sera (NRS) were used respectively as positive and negative controls. The sections were examined under fluorescence microscopy.

*Immunoprecipitation and Western Blotting Analysis.* Schistosomula surface antigens were radioiodinated using the lactoperoxidase technique of Marchalonis (15) and immunoprecipitated after incubation of the labeled parasite detergent extract (NP-40, 0.05%) with rat antibodies. Immune complexes were adsorbed on protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden) and analyzed by SDS-PAGE exactly as previously described (16).

KLH was analyzed in 10% polyacrylamide-SDS gels in the presence of 5% 2-ME (17), then transferred onto nitrocellulose. Western blotting was performed according to the method of Burnette (18) using 5 µl of rat sera or 10 µg of IPLSm1 mAb. Fixed antibodies were revealed by autoradiography after incubation with  $5 \times 10^5$  cpm of <sup>125</sup>I-labeled rabbit anti-rat IgG ( $8 \times 10^6$  cpm/µg sp act).

*Inhibition of IPLSm1/KLH Binding.* Inhibition experiments were carried out on polyvinylchloride (PVC) microtiter plates (Falcon Labware, Oxnard, CA) coated with IPLSm1 antibody. Each well of the PVC plates was coated with 100 µl of a 10 µg/ml solution of purified IPLSm1 antibody diluted with 10 mM PBS. After 2 h of incubation at 20°C, the plates were washed twice in PBS and saturated for 30 min with 200 µl of a 2% BSA (Albumin, Fraction IV; Boehringer Mannheim Biochemicals, Mannheim, Federal Republic of Germany) solution in PBS. The plates were then washed twice in PBS/0.1% BSA (200 µl per well). For the test, 50 µl of <sup>125</sup>I-labeled KLH in PBS/0.1% BSA (100,000 cpm/well) were incubated with 50 µl of inhibiting factor diluted in the same buffer for 1 h at 37°C and 18 h at 4°C. The plates were washed three times in PBS/0.1% BSA and each well was counted in a gamma counter. The percentage of inhibition of binding was calculated using the following formula:  $100 \times (a - b)/a$ , where *a* is cpm obtained without inhibitor and *b* is cpm obtained in the presence of inhibitor. In additional studies, PVC plates were coated under the conditions described above with 100 µl of a 10 µg/ml solution of purified KLH in 10 mM PBS. 50 µl of <sup>125</sup>I IPLSm1 antibody in PBS/0.1% BSA (100,000 cpm/well) were incubated with 50 µl of inhibiting factor diluted in the same buffer. The experimental conditions of the assay and the evaluation of binding inhibition were identical to those described above.

*Inhibition of IPLSm1/38,000 Antigen Binding.* Inhibition of binding of radiolabeled IPLSm1 antibody to 38,000 M<sub>r</sub> antigen was performed according to the technique previously described (7). Briefly, PVC plates precoated with rat IgM C3-109 mAb recognizing the 38,000 M<sub>r</sub> antigen, were incubated with 100 µl of a NP-40 extract of schistosomula (100 µg protein/ml); this procedure allows a better fixation of the 38,000

<sup>1</sup> *Abbreviations used in this paper:* DKIRS, DKLH-immunized rat sera; DKLH, deglycosylated KLH; IRS, infected rat sera; KIR, KLH immunized rat; KIRS, KLH-immunized rat sera; NRS, normal rat sera; PVC, polyvinylchloride; TFMS, trifluoromethanesulfonic acid; TRI, synthetic tripeptide (glycyl-histidyl-lysine).

$M_r$  antigen. After 2 h of exposure at 37°C, plates were washed twice in PBS/0.1% BSA. For the inhibition test, 50  $\mu$ l of  $^{125}$ I-labeled IPLSm1 in PBS/0.1% BSA were incubated with 50  $\mu$ l of inhibiting factor. After 2 h at 37°C and 18 h at 4°C, plates were washed three times with PBS/0.1% BSA (100  $\mu$ l/ml) and the wells were counted in a gamma counter. The percentage of inhibition was calculated according to the formula previously defined.

*Inhibition of Radiolabeled IPLSm1 Binding to KLH by Sera from Infected Patients.* Inhibition experiments were performed on PVC microtiter plates precoated with KLH. Sera were obtained from untreated patients in an endemic area in Kenya and selected on the basis of egg counts. They were kindly donated by Dr. A. E. Butterworth, (Cambridge University, United Kingdom). Normal human sera were obtained from subjects without any parasitic infection. For the test, 50  $\mu$ l of  $^{125}$ I-labeled IPLSm1 were incubated 1 h at 37°C and 16 h at 4°C with 50  $\mu$ l of diluted infected or normal sera (dilution 1:50 in PBS/0.1% BSA). Plates were then washed three times in PBS/0.1% BSA and wells were counted in a gamma counter. The percentage of inhibition was measured according to the formula defined above.

*Eosinophil-dependent Cytotoxicity.* The cytotoxicity assays were performed on *S. mansoni* skin schistosomula, as previously described by Capron et al. (19). Briefly, effector cells were obtained from LOU rats stimulated 48 h previously by intraperitoneal injection of 10 ml 0.9% NaCl sterile physiological saline solution; peritoneal cavities were washed with 20 ml Eagle's MEM containing penicillin (100 U/ml), streptomycin (50 U/ml), 1% NRS, 20 ng/ml synthetic tripeptide (glycyl-histidyl-lysine [TRI], Calbiochem-Behring Corp., San Diego, CA) and 25 IU/ml calcium heparinate (MEM/NRS/TRI). Eosinophil-rich populations were prepared by allowing these peritoneal cells ( $5-7 \times 10^6$ /ml in MEM/NRS/TRI) to adhere for 2 h at 37°C in 25-cm<sup>2</sup> tissue culture flask (Corning Glass Works, Corning, NY). The nonadherent cells of each tissue culture flask were recovered, pooled, and washed twice in MEM/NRS/TRI. After staining with Discombe's diluent or toluidine blue, this cell population was shown to contain 40-90% eosinophils and 4-10% mast cells. The cytotoxic assay was carried out in sterile plastic microplates with flat-bottomed wells (Nunclon, Roskilde, Denmark). 50 schistosomula were added to each well and incubated overnight with 50  $\mu$ l of KLH-immunized rat sera (KIRS), DKLH-immunized rat sera (DKIRS), or 50  $\mu$ l NRS or infected rat serum (IRS) at a final dilution of 1:16 (sera were previously heat-inactivated for 2 h at 56°C). After 18 h incubation at 37°C, effector cells were added in a 100  $\mu$ l of MEM/NRS/TRI at a ratio of 6,000 effector cells for one schistosomulum. The plates containing effector cells and sensitized targets were incubated in a 5% CO<sub>2</sub> humidified atmosphere at 37°C. The percentage of cytotoxicity was measured after 24-48 h by microscopic examination.

*Passive Transfer Experiments.* 2 ml of sera from rats immunized with KLH (KIRS) were injected intravenously into male LOU rats (10 wk old, 180-200 g) that had been infected with 800 *S. mansoni* cercariae 4 h previously. Parasite burdens were evaluated 3 wk later by the liver-perfusion technique according to Smithers and Terry (20). The number of worms obtained from rats injected with KIRS were compared with those obtained from control groups (rats injected with 2 ml of physiological saline solution or 2 ml of NRS). The percentage of protection was calculated by the formula (21):  $100 \times (a - b)/a$ , where  $a$  is the number of worms recovered from rats injected with 2 ml of physiological saline solution and  $b$  is the number of worms recovered from rats injected with KIRS.

*Active Immunization.* For active immunization experiments, male LOU M rats (10 wk old, 180-200 g) were immunized twice at 2 wk intervals by intraperitoneal injection of 1 ml of physiological saline solution containing 100  $\mu$ g of KLH. 3 wk after the second injection, rats were infected with 800 *S. mansoni* cercariae. The worm recovery and the evaluation of the percentage of protection were performed as described above, 3 wk after the cercarial challenge. In these experiments, rats injected with 1 ml of physiological saline solution were used as negative controls.

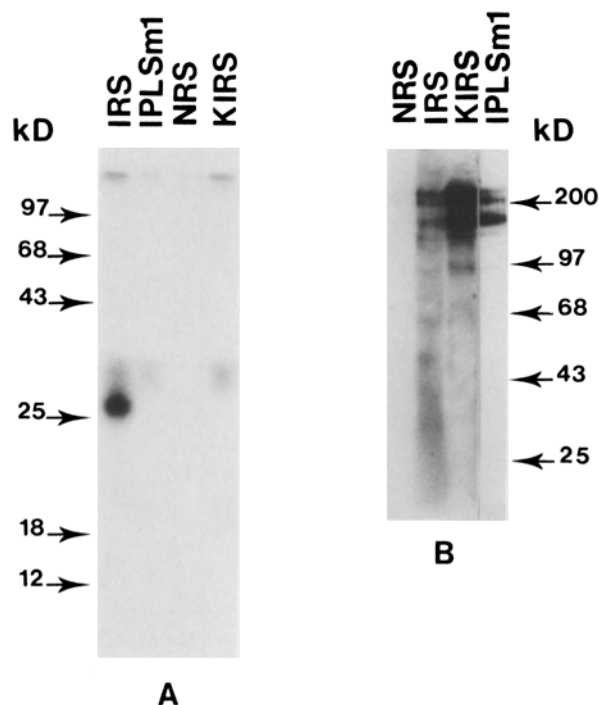


FIGURE 1. Epitopic identity between *S. mansoni* 38,000  $M_r$  antigen and KLH. (A)  $^{125}\text{I}$ -labeled schistosomula surface antigens were immunoprecipitated by 10  $\mu\text{l}$  of NRS, IRS, KIRS, or 10  $\mu\text{g}$  of purified IPLSm1 mAb. (B) KLH-purified preparation was analyzed in a 10% SDS-polyacrylamide gel under reducing conditions and then transferred to nitrocellulose. Immunodetection was performed with 5  $\mu\text{l}$  of NRS, 5  $\mu\text{l}$  IRS, 5  $\mu\text{l}$  of KIRS, or 10  $\mu\text{g}$  of purified IPLSm1 antibody.  $M_r \times 10^{-3}$  are shown.

## Results

**Antigenic Community between KLH and the 38,000  $M_r$  Antigen.** The existence of a common antigenic structure shared by KLH and *S. mansoni* was investigated by immunoprecipitation and Western blotting methodologies. Immunoprecipitation of radiolabeled schistosomulum antigen (Fig. 1A) showed that sera from rats immunized with KLH recognize a 38,000  $M_r$  antigen present on the schistosomulum surface and previously demonstrated as the target antigen of the IPLSm1-protective mAb (2). Additionally, the analysis of a purified fraction of KLH by SDS-PAGE under reducing conditions (Fig. 1B) revealed by Western blotting that IPLSm1 mAb and infected rat sera recognize in KLH two molecules of  $\sim 150\text{--}200 \times 10^3$  mol wt.

**Inhibition of IPLSm1 Binding to KLH.** Binding of  $^{125}\text{I}$ -labeled IPLSm1 to KLH coated on PVC plates was shown to be strongly inhibited in a dose-dependent manner by various concentrations of unlabeled IPLSm1. Similar levels of inhibition could be obtained in the presence of *S. mansoni*-infected rat sera (Fig. 2).

**Epitopic Community.** The structural identity between KLH and the 38,000  $M_r$  antigen epitope defined by the IPLSm1 mAb was further studied in inhibition experiments performed in the presence of the JM8-36 antiidiotype antibody specific for IPLSm1 antibody. Results in Fig. 3A show that JM8-36 antibody

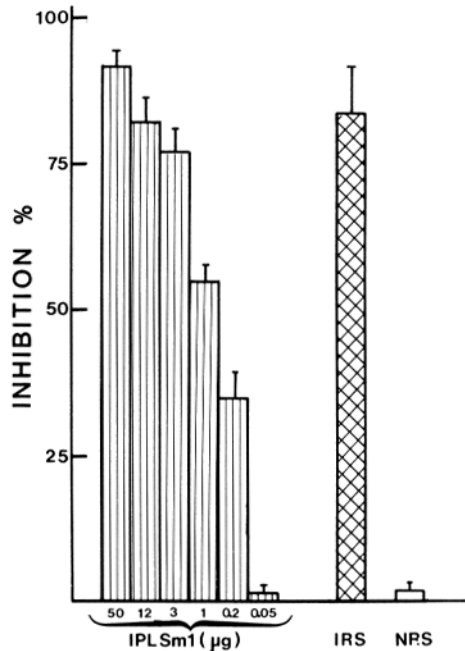


FIGURE 2. Dose-dependent inhibition of IPLSm1/KLH binding. 50  $\mu$ l of  $^{125}$ I-labeled IPLSm1 antibody were incubated with 50  $\mu$ l of various concentrations of unlabeled IPLSm1, 50  $\mu$ l of 4 wk IRS, or 50  $\mu$ l of NRS.

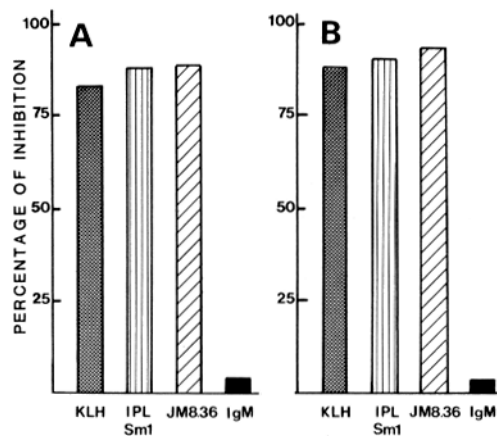


FIGURE 3. Inhibition of IPLSm1/KLH binding by JM8-36 antiidiotype antibody. (A) 50  $\mu$ l of  $^{125}$ I-labeled KLH were incubated with 50  $\mu$ l of a solution containing either 50  $\mu$ g of KLH, IPLSm1 antibody, JM8-36 antiidiotype antibody or unrelated IgM, on plates coated with purified IPLSm1 antibody. (B) 50  $\mu$ l of  $^{125}$ I-labeled IPLSm1 were incubated with 50  $\mu$ l of the same solutions on plates coated with native KLH.

inhibits the binding of  $^{125}$ I-labeled KLH to IPLSm1 antibody coated on PVC plates at a level comparable to that obtained either with unlabeled KLH or with purified IPLSm1 antibody. Moreover, JM8-36 antiidiotype antibody can also inhibit  $^{125}$ I-labeled IPLSm1 binding to KLH coated on PVC plates at a level

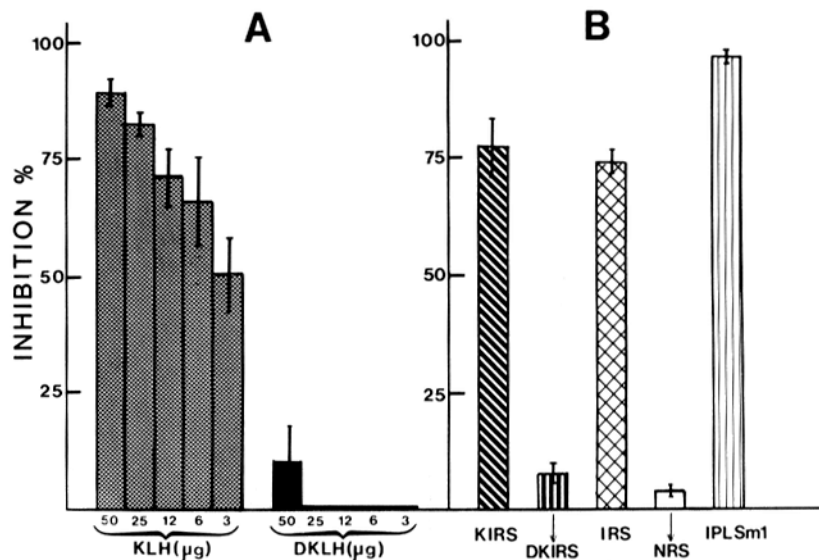


FIGURE 4. Inhibition of <sup>125</sup>I-IPLSm1 binding to the 38,000  $M_r$  antigen. (A) 50  $\mu$ l of various concentrations of native KLH or DKLH were incubated with 50  $\mu$ l of <sup>125</sup>I-labeled IPLSm1 mAb (100,000 cpm/well). (B) 50  $\mu$ l of 1:50 diluted sera collected from KIRS, DKLH immunized rats, 4 wk IRS, NRS, or IPLSm1 ascitic fluid were incubated with 50  $\mu$ l of <sup>125</sup>I-labeled IPLSm1 antibody ( $10^5$  cpm/well).

comparable to that seen with unlabeled IPLSm1 or KLH. These results therefore suggest a close structural identity between the antigenic determinant of KLH, the internal image of the antigen expressed on JM8-36 antiidiotype antibody, and the 38,000  $M_r$  antigen epitope defined by the IPLSm1 mAb.

*Involvement of Carbohydrate Moieties.* The previous demonstration that IPLSm1 mAb binds to the 38,000  $M_r$  antigen via a glycanic epitope (2) led us to compare the immunological properties of native and deglycosylated KLH. Native KLH preparations used at various concentrations inhibit the binding of <sup>125</sup>I-labeled IPLSm1 to its 38,000  $M_r$  target antigen. This activity is completely abolished when KLH is deglycosylated by TFMS treatment (Fig. 4A). Additionally, a marked inhibition of binding of <sup>125</sup>I-labeled IPLSm1 could be induced with KIRS or IRS, but not with the sera from rats immunized with DKLH or NRS (Fig. 4B).

*Eosinophil-dependent Cytotoxicity.* The relationship between the existence of antibody-mediated eosinophil-dependent cytotoxicity for schistosomula and the 38,000  $M_r$  antigen prompted us to investigate the presence of cytotoxic antibodies in the sera collected from rats immunized with KLH. The cytotoxic activity of anti-KLH rat sera was investigated in the presence of normal rat eosinophils. In these particular conditions, anti-KLH rat sera were shown to exhibit a marked killing activity towards *S. mansoni* skin schistosomula ranging between 46 and 94% and comparable to that usually obtained using 4 wk-IRS (19) or the protective mAb IPLSm1. No cytotoxicity was observed with sera collected from rats immunized with DKLH (Fig. 5).

*Passively Transferred Immunity.* The potential protective role of anti-KLH rat sera was investigated by passive transfer experiments in naive LOU rats. The

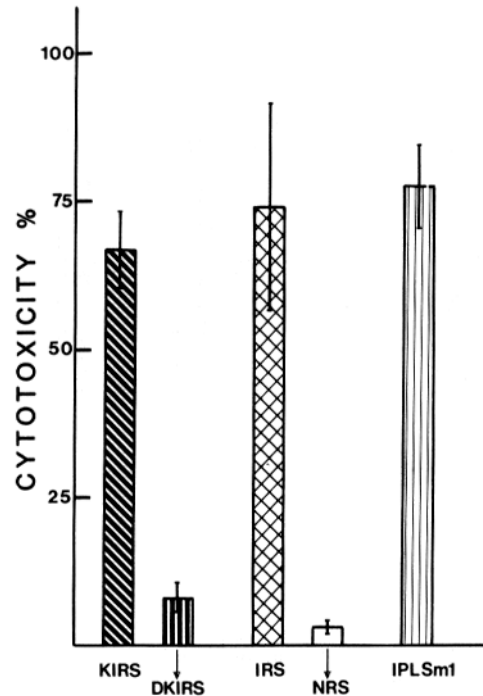


FIGURE 5. Eosinophil-dependent cytotoxicity for *S. mansoni* schistosomula. The percentage of cytotoxicity was measured after 48 h of incubation with effector cells (LOU rat peritoneal cells enriched in eosinophils); schistosomula were preincubated either with KIRS, DKIRS, 4 wk IRS, NRS, or IPLSm1 mAb (mean of 10 duplicate experiments  $\pm$  SD).

parasite burden was evaluated by liver perfusion 3 wk after a challenge infection with 800 *S. mansoni* cercariae. Results presented in Fig. 6A indicated that antibodies produced in KLH-immunized rats passively transferred immunity to a degree (48%) very close to that previously observed for the IPLSm1 mAb. In these experiments, rats receiving KIRS presented a lower number of worms when compared with rats treated with NRS or physiological saline solution (Fig. 6A).

**Active Immunization.** The protective effect of immunization with KLH was studied in naive LOU rats immunized twice at 2 wk intervals with 100  $\mu$ g of KLH and infected 3 wk after the second injection by 800 *S. mansoni* cercariae. The effectiveness of the procedure was evaluated 3 wk after the challenge infection by liver perfusion. Two series of experiments involving a total of 24 rats demonstrated that rats previously immunized with KLH presented a significant decrease in the parasite burden when compared with appropriate controls (rats treated with physiological saline solution). The level of protection obtained in these experiments ranged between 50 and 70% (Fig. 6B).

**Studies on Sera from Humans Infected with *S. mansoni*.** Previous studies (4) have demonstrated that the 38,000  $M_r$  antigen glycoprotein is a major immunogen in human infection and induced the production of specific antibodies in 97% of *S. mansoni*-infected patients. The potential use of KLH for the detection of anti-*S. mansoni* antibodies in human sera was explored using a competitive RIA with



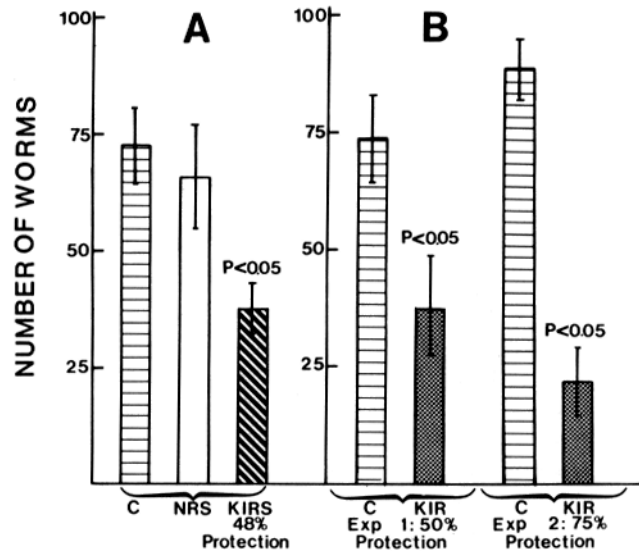


FIGURE 6. In vivo protective role of anti-KLH antibodies. (A) 1.5 ml of KIRS, 1.5 ml of NRS, or 1.5 ml of physiological saline solution were injected intravenously into each rat (groups of six animals in each test) that had been infected 4 h previously with 800 *S. mansoni* cercariae. (B) KIR or rats injected with physiological saline solution (C) were infected with 800 *S. mansoni* cercariae. In A and B, the number of worms was estimated by liver perfusion 21 d after the challenge infection.

TABLE I  
Inhibition of  $^{125}\text{I}$  IPLSml Binding to KLH by Human Serum

Human sera source	Percent inhibition $\pm$ SD
<i>S. mansoni</i> -infected human sera ( $n = 20$ )	$68 \pm 13.3$
Normal human sera ( $n = 20$ )	$0.5 \pm 1.1$

50  $\mu\text{l}$  of  $^{125}\text{I}$ -labeled IPLSml ( $10^5$  cpm/well) were incubated on plates coated with KLH for 1 h at  $37^\circ\text{C}$ , and for 16 h at  $4^\circ\text{C}$  with 50  $\mu\text{l}$  of 1:50 diluted infected or normal sera.

PVC plates coated with KLH. Results summarized in Table I and concerning a limited series of human infection sera show that the latter significantly inhibit the binding of radiolabeled IPLSml antibody to KLH ( $68 \pm 13.3\%$ ). No significant levels of inhibition were observed with the various control sera ( $0.5 \pm 1.1\%$ ).

### Discussion

The experimental data reported here clearly demonstrate that the immunization of naive LOU rat with purified hemocyanin of *Megathura crenulata* (KLH) elicits the production of specific anti-*S. mansoni* antibodies exhibiting both in vitro and in vivo effector functions towards *S. mansoni* schistosomula, indicating therefore the presence of a functional crossreactive epitope between KLH and the 38,000  $M_r$  antigen.

These observations found a molecular basis in the demonstration that sera

collected from KLH-immunized rats contain antibodies able to immunoprecipitate from a schistosomula extract the 38,000  $M_r$  surface antigen previously reported as the target antigen of the protective IPLSm1 mAb and of antibodies from human or experimental infection sera. The antigenic similarity was confirmed by Western blotting analysis of KLH in which two molecules of about 150,000 and 200,000  $M_r$  were recognized both by IPLSm1 mAb antibody or *S. mansoni*-infected rat sera.

The existence of a shared epitope between KLH and the 38,000  $M_r$  antigen was fully supported by the results obtained in inhibition experiments performed using the JM8-36 antiidiotype antibody. The latter induced both a marked inhibition of binding of  $^{125}\text{I}$ -labeled KLH to IPLSm1 antibody or of  $^{125}\text{I}$ -labeled IPLSm1 to KLH, suggesting therefore a close structural identity between the epitope of KLH, of the 38,000  $M_r$  antigen and the internal image of the 38,000  $M_r$  epitope presented by the JM8-36 antiidiotype antibody.

The glycanic nature of the portion of the KLH molecule recognized by the IPLSm1 antibody was established by further inhibition experiments. Deglycosylation of the native KLH by TFMS treatment abolished the inhibitory effect of KLH on the binding of labeled IPLSm1 to its target antigen. On the other hand, sera collected from rats immunized with DKLH were completely devoid of inhibitory activity on IPLSm1/38,000  $M_r$  antigen binding when compared with the sera obtained from rats immunized with native KLH, or rats infected with *S. mansoni*. These results confirmed the close structural and chemical identity between KLH and the 38,000  $M_r$  antigen previously demonstrated to bind the IPLSm1 mAb through a glycanic epitope.

The functional properties of the carbohydrate epitope present on native KLH molecules were studied in the light of the relationship previously reported between killing of schistosomula mediated by normal rat eosinophils and the 38,000  $M_r$  schistosomula surface antigen (1). A marked level of eosinophil-dependent cytotoxicity was observed in the presence of sera collected from KLH-immunized rats, comparable to that obtained with IRS or IPLSm1 mAb; no cytotoxicity was observed with the sera from rats immunized with DKLH. These results indicated that, together with a structural identity between KLH and 38,000  $M_r$  carbohydrate moieties, the KLH epitope recognized by IPLSm1 antibodies expressed similar immunological functions and elicited by immunization the production of cytotoxic antibodies. The effector functions of anti-KLH antibodies were also confirmed in vivo by passive transfer experiments. Sera collected from rats immunized with KLH were found to be protective, when passively transferred to rats, to a degree similar to that induced by the IPLSm1 antibody. More importantly, the prior immunization of naive LOU rats with purified KLH gave a high level of protection towards a subsequent *S. mansoni* challenge. The parasite burden estimated by liver perfusion methodology was significantly reduced when compared with appropriate controls.

These latest data increase the interest for the well-defined characterization and isolation of this glycanic epitope shared both by KLH molecules and the 38,000  $M_r$  schistosomula surface antigen, and potentially available for an immunoprophylactic approach towards schistosomiasis. From these encouraging results, observations in various experimental infection models and particularly

in primates to evaluate the immune response against this glycanic epitope should follow.

In man, preliminary investigation performed using a competitive RIA allows the characterization of specific antibodies that can inhibit the binding of IPLSm1 antibody to KLH in the sera of *S. mansoni*-infected patients. Although the existence of natural antibodies to KLH in some individuals has been reported (22), no significant level of inhibition was observed with the various control sera used. The fact that almost all subjects infected by *S. mansoni* produce antibodies against the glycanic epitope of the 38,000  $M_r$  antigen also present in KLH opens an entirely new pathway towards the development of a simple, easily standardized and cheap reagent for the seroepidemiology of human schistosomiasis. We are at present applying this methodology to a large series of well-documented sera.

This paper brings new evidence that the carbohydrate moieties of *Megathura crenulata* hemocyanin (KLH) express the epitope previously characterized on a major immunogen 38,000  $M_r$  antigen of the parasite *S. mansoni* and clearly demonstrate its capacity to elicit by immunization the production of functional antibodies both in vitro and in vivo. Such observations appear to have several major consequences.

The close structural and functional identity between KLH carbohydrate moieties and the glycanic epitope of the 38,000  $M_r$  antigen provides new opportunities to analyze further in man and animal models the isotypes induced towards a potentially protective epitope. KLH could represent in this context a valuable tool for the identification of the human antibody isotype(s) directly involved in the eosinophil-mediated killing of schistosomula.

The characterization on the KLH molecule of a functional epitope recognized by the protective IPLSm1 mAb offers new opportunities to purify a large amount of the oligosaccharide for the study of its chemical structure. Our current work using mass spectroscopy analysis of the purified KLH oligosaccharides renders access to a well-defined structure feasible in the near future, a first step in the production of synthetic oligosaccharides. Such material could certainly provide essential information concerning the potential relations existing between the chemical nature of a functional epitope and the preferential induction of specific antibody isotype(s). This latest point appears to be essential since we previously demonstrated in schistosomiasis the production of blocking mAbs towards the 38,000  $M_r$  glycoprotein antigen. Two ways of investigation could be proposed at this stage, either a slight modification of the initial chemical structure of KLH carbohydrate moieties involved in the production of functional antibodies or the production of neoglycoproteins obtained by a chemical condensation of KLH oligosaccharides with various carrier proteins. These experiments could certainly lead to the appreciation of the real role played by the carrier proteic structure in the orientation of the immune response towards a specific epitope. In this context, we plan to apply such an approach to the P28 antigen of *S. mansoni* recently described (23) as promoting a protective immune response in rats and mice against *S. mansoni* infections.

Moreover, our findings have to be considered in the general framework of the antigenic community existing between parasites and their hosts. This concept established in our laboratory some 20 yr ago indicates that some antigenic

determinants were present both on *S. mansoni* and its snail intermediate host *B. glabrata* (24) and elicited the production of antibodies appearing early during experimental or human infection (25). These results, confirmed in several laboratories (26–30), were recently updated by the demonstration that the glycanic epitope of the 38,000  $M_r$  glycoprotein that is a major immunogen of *S. mansoni* schistosomula is in fact expressed by the intermediate host *B. glabrata* and several freshwater and marine molluscs (8). Such observations raised the problem of the conservation of this oligosaccharide structure throughout evolution and the expression in a human parasite of a major antigenic epitope whose origin was found in a marine mollusc *Megathura crenulata* that has been in existence for several million years.

Finally, it will certainly be of interest to immunologists, who have used KLH for the last 20 yr as a carrier for the immunization of humans or animals, to know that it can lead to the production of anti-*S. mansoni* antibodies.

### Summary

The glycanic epitope of the 38,000  $M_r$  *Schistosoma mansoni* schistosomula major immunogen defined by the IPLSm1 protective mAb was identified in the hemocyanin of the marine mollusc *Megathura crenulata*, better known as KLH. This antigenic community was exploited to investigate further the biological properties of this epitope. KLH was shown to strongly inhibit the binding of IPLSm1 mAb to its 38,000  $M_r$  target antigen. Immunization of naive LOU rats with KLH elicited the production of anti-*S. mansoni* antibodies capable of immunoprecipitating the 38,000  $M_r$  schistosomulum antigen. Antibodies to KLH mediated a marked eosinophil-dependent cytotoxicity and passively transferred immunity towards *S. mansoni* infection. Finally, rats immunized with KLH were significantly protected against a challenge with *S. mansoni* cercariae.

The deglycosylation of KLH completely abolishes its immunological and functional KLH properties, indicating the participation of an oligosaccharidic epitope of the native KLH that is also recognized by the sera of *S. mansoni*-infected patients.

These observations provide new opportunities of access to the well-defined structure of a glycanic epitope potentially available for the immunoprophylaxis and seroepidemiology of schistosomiasis, and a new approach to the isotypic response towards a well-chemically defined epitope.

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