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IDENTIFICATION OF SNAILS INFECTED WITH SCHISTOSOMA MANSONI
BY THE USE OF MONOCLONAL ANTIBODIES AND DNA PROBES

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Executive Summary: Schistosomiasis, a water-borne disease transmitted by freshwater snails afflicts more than 200 million people globally. It is a lingering public health problem in many developing countries because chemotherapy does not prevent reinfection, and because the vicious circle of human-water-snail interactions that leads to transmission still prevails in these countries.

Determination of schistosome infections in humans and snails is the hallmark of epidemiological surveys, but so far snail infection rates cannot be accurately determined. Firstly, because routine tests only examine whether the snails shed infective larvae—cercaria, thus leaving out infected snails in which infection is not yet patent. Secondly, because cercarial shedding tests do not provide differential identification of human schistosomes larvae from animal schistosomes' larvae which may develop in the same snail species.

The present project was aimed at identifying infected snails by detecting schistosome-derived molecules (antigens, DNA) rather than the parasites themselves. For this purpose, we initially developed monoclonal antibodies (Mab) to Schistosoma mansoni and used them in a simple test system for detecting hitherto undefined schistosomal antigens in the hemolymph of infected snails. The test made it possible to differentially identify S. mansoni-infected snails from uninfected ones and from snails infected with other trematodes. Both laboratory studies in Jerusalem and field studies in Kenya proved
the high degree of sensitivity and specificity of the test and its ability to detect snails even before they shed cercariae.

DNA-DNA hybridization was also employed for detecting infected snails. For this purpose a simple procedure for preparing snails for DNA hybridization tests was developed, and highly repeated, tandemly arranged DNA sequences representing at least 12% of the *S. mansoni* genome were cloned in bacteria and used as probes. These probes proved to be species specific and capable of detecting *S. mansoni* infection in snails with high sensitivity.

The results of this project clearly demonstrate the feasibility of our approach and open the way for development of similar probes for other schistosome species. They justify further research and development and extended field trial in search of cost effective detection procedures.

This project helped the transfer of hybridoma technology as well as modern concepts and approaches of diagnostic biotechnology to the collaborating Kenyan laboratory.
Research Objectives. The overall aim of the present project was to prepare and apply Mab and DNA probes for the differential identification of snails infected with *S. mansoni*. None of these means of molecular identification of schistosome infection in snails existed when the project was started. Snail infection was and still is routinely monitored by examining the capacity of the snails to shed carcariae. Prepatent infections in the snails are not tested routinely at all although they may constitute a significant proportion of an infected snail populations in active transmission sites. Microscopical examination of crushed snails for presence of early larval stages or serial shedding tests over several weeks after transfer of the snails to the laboratory were carried out experimentally for detecting prepatent infections in snails. These approaches, however, are not considered suitable for routine testing because they are cumbersome and/or time consuming, and/or inaccurate.

More accurate determination of snail infection rates (including prepatent infections) was considered important because it should help more accurate application of mathematical models of transmission. Also, upscaling of transmission monitoring was obviated since mass control measures, particularly mass chemotherapy, were on the increase when this project was planned. Furthermore, rapid developments in schistosomiasis vaccine research also required a balancing development of suitable technologies for mass monitoring. It was argued that identification of active transmission sites by community surveys and studies on human
behaviour is expensive and often results in a decline in community participation. Snail populations, on the other hand, can be sampled more frequently and should allow more rapid detection of resurgent transmission after a mass control campaign.

The use of antibodies for detecting schistosomal antigens in infected snails was one of the approaches taken by us. The feasibility of this approach depended on the availability of antibodies that can clearly differentiate between schistosomal and snail antigens. We initially prepared polyclonal antibodies by immunizing rabbits with extracts of adult worms and cercariae. These antibodies exhibited stronger binding to infected snails extracts than to extracts of normal uninfected snails, but there was still a very high degree of cross reactivity. The widespread antigenic cross reactivity, between schistosomes and snails was well known (1-9) and since our polyclonal antibodies could not provide sufficient discrimination between infected and normal snails this work was discontinued, and a search for suitable monoclonal antibodies took place. Monoclonal antibodies that can differentially bind to schistosomal antigens in snails extracts have not been previously described but presence of schistosome-specific antigens in infected Biomphalaria glabrata was demonstrated in a single publication (10) by employing suitably absorbed infected mouse serum. Although the use of monoclonal antibodies for detecting parasites (e.g. malaria) in vectors (mosquitoes) is being applied for large scale field studies to our knowledge Mab are not yet used for detecting snails infected with schistosomes.
The use of DNA probes for detecting parasites was gaining momentum at the time our study was started. As far as schistosomes are concerned, cloned ribosomal gene fragments were employed for determining species, strain and sex of schistosomes (11,12). Specific identification was possible by different banding patterns exhibited when schistosomal DNA was digested by restriction enzymes then run electrophoretically and underwent southern blot analysis with rDNA probes. We did not consider this approach suitable for testing multiple samples. Furthermore, since ribosomal genes are represented by only 100 copies in the S. mansoni genome this test was not expected to provide a sufficiently high sensitivity. We were therefore seeking a suitable probe that is highly represented in the schistosomal genome, exhibits species specificity and can be used in a dot hybridization test. Such highly repeated sequences were identified in filariae (13). It was also important to develop a simple procedure for preparing snails for dot hybridization because extraction of DNA from each snail by the conventional method (14) involves multiple steps (including mechanical disruption, Proteinase K digestion, Phenol extraction and alcohol precipitation) and is not practical for examining multiple samples. The use of rDNA probes for detecting infected snails by dot hybridization was attempted but detection sensitivity was low (14) and species specificity cannot be accomplished by rDNA probe in this test configuration due to the presence of highly conserved sequences. Species specific schistosomal sequences that are unique to females (15) cannot be considered for practical purposes not only because
this sequence is represented by only 75 copies/genome but mainly because most snail infections in nature arise from a single micracidium and therefore constitutes a single sex. The large proportion of snails infected with male schistosomes is bound to be missed if these probes were to be used. Since our probe represents a highly repeated DNA sequence that is present in both sexes and is species specific, it appears to be suitable for detecting infected snails.

The project was initially supported for 3 years by AID/PSTC and was extended for about 2 1/2 more years. This was made possible partly by a no-cost extension of the initial AID/PSTC grant, and partly by using funds from the NIAID Egypt/Israel/USA project on vector borne diseases in the Middle East. Part of these funds, intended for developing a DNA probe for detecting *W. bancrofti* infection, were used for doing a similar study on *S. mansoni* as a model system pending supply of *W. bancrofti* material by the Egyptian team. Completion of the study on *S. mansoni* DNA probes enabled us later on to proceed and identify highly repeated sequences in *W. bancrofti* (work now in progress).
Methods and Results. This project, produced 4 major publications entitled as follows (and arranged according to a subject sequence).


This publication describes the production of monoclonal antibodies and the identification of the ones that are specific for *S. mansoni* antigens within infected snails. These Mab were employed in a simple ELISA configuration for identification of abundant schistosomal antigens in extracts and hemolymph of infected *B. glabrata* during prepatency and patency. Characterization of the antigens recognized by the selected Mab was carried out by Western blotting, and their glycoprotein nature shown by their periodate sensitivity.

b) Identification of snails infected with schistosomes by ELISA employing monoclonal antibodies: *Schistosoma mansoni* in laboratory snails (*Biomphalaria glabrata*) and in field snails (*Biomphalaria pfeifferi*) from Kenya.

This publication described the employment of Mab (see above) in a simple one-step ELISA for identification of infected snails by detection of schistosomal antigens in the haemolymph of infected snails. Infected laboratory snails were differentially identified from uninfected laboratory snails (*B. glabrata*) during prepatency and through patency with 100% sensitivity and specificity. Infected field snails (*B. pfeifferi*) were differentially
identified with 100% sensitivity and specificity from uninfected field snails and from snails naturally infected with other trematodes (eschinostomes and strigeids). Prepatent infections were readily identified in field snails.


A simple procedure was developed for preparing snails for dot hybridization. The procedure involves tituration of each snail in presence of NaOH+detergent following by neuralization, heating and centrifugation and finally spotting. The procedure enables easy preparation of multiple samples and can be considered suitable for field work. Using a total *S. mansoni* DNA probe it was possible to detect infected snails as soon as one week after their exposure to miracidia. No cross reaction was observed with normal snail extracts.

d) Highly repeated short DNA sequences in the genome of *Schistosoma mansoni* recognized by a species specific probes. Mol. Biochem. Parasitol., in press.

A cloned 0.6 kb DNA sequence of *S. mansoni* contains 121 bp tandem repeats and comprises at least 12% of the schistosomal genome of both sexes. It exhibits a high degree of species specificity and could detect with high sensitivity schistosomal DNA sequences in infected snails.
Impact, Relevance and Technology Transfer: The findings of our research clearly demonstrate the feasibility of the use of monoclonal antibodies and DNA probes for the identification of infected snails. Cercarial shedding, which is currently used for this purpose, is expected to prevail as the major test for routine identification of snails infected with _S. mansoni_. However, our approach will be considered for routine use if the detection procedure is further simplified and the test made cost effective. In practice even if "molecular identification" of infected snails is made cost effective and usable by community health workers in endemic areas it is not expected to replace shedding tests for identifying snails patently infected with _S. mansoni_. It may, however, be useful in these areas for periodic quality control of shedding tests in particular where the routine differentiation of _S. mansoni_ cercariae from a large variety of non-human cercariae is difficult. Adaptation of "molecular" approach for monitoring _S. haematobium_ infection in snails will make it the approach of choice because shedding tests in this case do not provide specific identification of the human schistosome vis a vis a number of animal schistosomes which can develop in the same snail species. The immediate use of our Mab for the identification of schistosomal antigens (_S. mansoni_) in snail hemolymph can be considered for research purposes wherever accurate data are required on the effective influx of the parasite from the vertebrate to the invertebrate hosts of _S. mansoni_. This should be possible as soon as
our results gain credence by large scale trials in which the prepatent/patent infection ratio should be examined as a measure for such an influx.

The use of Mab for detecting infected snails may gain a quicker adaptation for field studies in LDC since ELISA is a well-rehearsed routine procedure in many countries where schistosomiasis is endemic. DNA hybridization technology on the other hand, will be adopted only if non-radioactive probes prove to be effective. Such non-radioactive detection should constitute part of the further research and development required.

The introduction of modern diagnostic biotechnology for routine diagnostic/epidemiological purposes of parasitic diseases (schistosomiasis being only one example) will require some organizational changes. Public Health Laboratories will have to acquire these technologies by close collaboration with research institutions who may already be involved in the use of the relevant technologies. In this context training as well as redefinition of job responsibilities of technical and scientific staff will become an important matter. Technical staff should be given avenues for further training through acquisition of an academic degree since much of the modern diagnostic biotechnology requires a broader academic training. Thus, more academicians will be required at the laboratory bench instead of the managerial desk. Training on location should receive more emphasis. Accordingly, research institutions and central Public Health Laboratories should consider organizing central facilities as well as suitable personnel
(including invited scientists) for providing a much needed bridge for technology acquisition. Such facilities will also serve as convenient central training sites.

Specifically relating to technology transfer within the context of this project it should be taken into account that the establishment of a hybridoma unit in a developing country is dependent on the availability of suitable space and supplies. A separate room with UV sterilization, and minimum access to unauthorized personnel is required to avoid contamination which is much more prevalent in the tropics. Reliable supply of media, immunochemicals, plasticware, etc., is a must for a hybridoma unit to be operational on a continuous basis. This is likely to require special logistic arrangements in Kenya as well as in many other LDC.
Project Activities Outputs: Results of this project were presented in the following meetings:

- The 3rd Mediterranean Conference for Parasitology (Jerusalem 1987)
- Meetings of participants of the Egypt/Israel/USD NIAID Regional Project of Vector-Borne Diseases in the Middle East (Tel Aviv 1988, Taba 1990).
- Seminar at the Biomedical Research Centre, Kenya Medical Research Institute (Nairobi, 1989).

Training was provided to one Kenyan Senior technologist on theory and practice of the hybridoma technology, and on antigenic analysis using monoclonal antibodies. It took place in Jerusalem for a 4 months period. A hybridoma unit was established at KEMRI (in the Biomedical Research Centre) and initial cultures and fusion experiments carried out. A contamination-free room is yet to be arranged for this unit and logistic problems of supplies are yet to be solved before it can be operational on a continuous basis.
This project resulted in 4 major publications and 5 abstracts as follows:

Major publications:


Abstracts


Copies of the major publications are herewith enclosed.
**Project Productivity:** The project accomplished all of the proposed goals.

**Future Work:** The project is expected to lead to future work on several lines.
1) Development of Mab and DNA probes for detecting snails infected with other human schistosomes.
2) Development of non-radioactive, cost effective procedures for detecting prepatent infection in snails infected with *S. mansoni*.
3) Elucidation of strain specificity of the DNA probe representing highly repeated sequences of *S. mansoni*. This study is expected to include elucidation of consensus sequences from various strains in search of markers for strain associated differences in pathogenicity, response to drugs and various aspects of host-parasite compatibility.
4) The use of the Mab developed in this project in search of putative receptors on snail hemocytes responsible for recognition of intramolluscan *S. mansoni* larvae and subsequent cytoadherence. This is part of an overall study for elucidating molecular aspects of *S. mansoni*-snail interactions.

**Literature Cited**


SCHISTOSOMA MANSONI ANTIGENS RECOGNIZED IN BIOMPHALARIA GLABRATA HEMOLYMPH BY MONOCLONAL ANTIBODIES

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Abstract. In order to identify and characterize Schistosoma mansoni antigens in Biomphalaria glabrata, we examined 19 murine monoclonal antibodies (Mabs) for specific binding to schistosome larvae. None of the murine Mabs induced by infection or by immunization with a crude cercarial antigen (CCA) served this purpose. Two Mabs out of 9 (KCSMe22-3 and KCSMe22-31) induced by soluble egg antigens reacted with CCA but not with normal snail (NSN) extract. We selected these 2 for studies on detection and characterization of schistosomal antigens in snails. When employed in an ELISA, they differentially detected schistosomal antigens in extracts and cell-free hemolymph (plasma) of infected snails. The selected Mabs bind to cercarial surface as demonstrated by the indirect fluorescent antibody technique (IFAT) with parafformaldehyde-fixed cercariae. The epitopes corresponded to the selected Mabs are periodate sensitive, suggesting the glycophorin nature of the antigens recognized. Immunoblotting analysis employing the selected Mab revealed 1 antigen in CCA (M. = 205 kDa) and 3 antigens in snail plasma (M. = 220 kDa, 130 kDa, and 135 kDa). Schistosomal antigens were first detectable in the snails’ plasma 2 weeks after snail infection, and their quantity increased afterwards.

The widespread antigenic cross-reactivity which exists between Schistosoma mansoni and its host Biomphalaria glabrata is possibly one way the parasites evade snail defenses. Parasite specific antigens, on the other hand, have been suggested as possible targets of specific recognition of the parasite as foreign by snail hemocytes, recognition being an important initial step in mobilization of snail defenses against schistosome infection.

Little is known of the identity and characteristics of schistosome specific antigens within their intermediate snail hosts, despite the importance of such knowledge for the elucidation of schistosome-snail interactions. Schistosome specific antigens have been detected in hemolymph and hemocytes of infected B. glabrata by employing suitably absorbed infected mouse serum as antigen detector. 1 but none have yet been defined. Segmental surface proteins of S. mansoni sporocysts have been analyzed by monoclonal antibodies (Mabs), taking into account their possible role as targets of snail defenses. 2 However, their specificity in relation to snail antigens has not been defined. The employment of Mabs for identification and characterization of schistosome specific antigens in infected B. glabrata is the subject of this paper.

MATERIALS AND METHODS

Parasite and hosts

Schistosoma mansoni (Egyptian strain) was maintained in outbred albino female mice and in snails (Biomphalaria glabrata) as previously described. 4 Snails were infected individually by exposure to 10 miracidia each.

Antigenic preparations

S. mansoni ova were harvested from intestines of infected mice by homogenization, trypsinization, and differential sieving. Soluble egg antigen (SEA) was prepared from the ova by homogenization and ultracentrifugation.

A crude cercarial antigen (CCA) was prepared by sonication of lyophilized cercariae in phosphate buffered saline (PBS), pH 7.2. Sonication was carried out with a Sonicator W-225 (Ultrasonic Inc.) in 5 rows of pulses -40 s period, 3 min each.

Extracts from normal or infected snails were prepared in a test tube by mechanical disruption of whole snails with motor driven wooden applicators at 1000 rpm for 1 min. Disruption of snails was followed by centrifugation at 9000
rpm (Mikrotek Hettich, West Germany) for 15 min.

Snail hemolymph was drawn out with a mouth operated suction tube to which a 25 G needle was attached. The snails were pierced near the innermost coil of the shell. Snail plasma was obtained by centrifuging the hemolymph for 5 min at 12,000 rpm (Mikrotek Hettich). Protein concentration of the various antigenic preparations was determined by the method of Lowry and others.

Monoclonal antibodies

Three series of Mabs, differing in the nature of antigenic stimulation given to the splenocytes donors (BALB c female mice, 6 weeks old), were prepared. For the first series, mice were infected by subcutaneous injection of 50 S. mansoni cercariae; spleen cells were taken for fusion 6 weeks later. For the second series, mice were immunized to CCA; an ip injection of 100 μg CCA incorporated in Freund's complete adjuvant was followed 2 weeks later by 50 μg CCA incorporated in Freund's incomplete adjuvant. The mice were then allowed to rest for at least 1 month; 4 days before splenocytes were taken for fusion, the mice received 50 μg CCA without adjuvant. For the third series, mice were immunized against egg antigens by a weekly ip injection of 5.000 live S. mansoni ova for 3 consecutive weeks. After a month's rest, the mice received an iv injection of 20 μg SEA 4 days before splenocytes were taken for fusion.

Mabs were prepared according to established protocols.** Myeloma cells, line 165,74,79, were fused with splenocytes at a ratio of 1:5 with polyethylene glycol (M. 3,000). Subcloning was carried out by limiting dilution, and ascitic fluids were prepared by ip injection of hybridoma cells into pristane pretreated mice.

Enzyme linked immunosorbent assay

An ELISA was carried out for several purposes: the routine screening of antibody activity to homologous antigens in corresponding hybridoma supernatants and ascitic fluids, for secondary screening of Mabs in order to select those potentially suitable for specific detection of schistosomal antigens in infected snails, for the titration of selected Mabs, for the determination of the Ig isotype of selected Mabs, and for the identification of schistosomal antigens in extracts and hemolymph (or plasma) of infected snails.

Established ELISA procedures10 were employed, with slight modifications. Briefly, wells of flexible microtiter plates (Falcon, Becton-Dickinson Labware, Oxnard, CA) were coated overnight with 65 μg well antigen diluted in PBS to 10 μg/ml, unless otherwise indicated; 5% bovine serum albumin (BSA) was employed for the blocking of non-specific sites by incubation for 1 hr at 37°C. Aliquots (50 μl well) of tested culture supernatants or ascitic fluids, diluted when necessary with 5% fetal calf serum, were incubated in the antigen coated wells for 2 hr at 37°C and then for 1 hr at 4°C. The wells were washed with PBS plus 0.1% Tween 20 (PBS-T), and probing for antibody binding was routinely carried out with goat anti-mouse Ig (polyvalent) conjugated to alkaline phosphatase (Sigma Chemicals, St. Louis, MO). For determining Ig isotypes goat anti-mouse IgG (Sigma), and rabbit anti-mouse IgG,, IgG,, IgG,, and IgG, (Litton-Bionetics) were employed. Following incubation at the described conditions, the wells were washed with PBS-T and the substrate p-nitrophenyl disodium (Sigma) was added. The color reaction was read by an ELISA reader (Titrocheck Multiscan, Flow Laboratories, Scotland).

Immunochromical characterization

To determine whether epitopes corresponding to the selected Mab were carbohydrate or polypeptide in nature, we absorbed infected snail (SN) plasma diluted 1:10 into 50 μg well BSA (65 ng well), and SEA (65 ng well) onto a microtiter plate. Treatment with various concentrations of periodate followed, and then an ELISA was performed as previously described. BSA anti-BSA (rabbit) served as a control for periodate insensitive epitopes. A Mab (KCSmegI) induced by the major egg glycoprotein of S. mansoni was in our laboratory and found to be directed against a carbohydrate epitope (unpublished results). KCSmegI was employed as a control for detecting periodate sensitive epitopes.

Indirect fluorescent antibody test

Binding of Mab to cercarial surface was investigated by an indirect fluorescent antibody test (IFAT). Cercariae were collected from infected snails.
snails and washed 3 times with 100 ml PBS in a filtration apparatus (Sartorius, West Germany) holding a 47 mm diameter filter with a pore size of 5 Î¼m. Washed cercariae were fixed for 15 min with 2.5% (final concentration) of neutralized paraformaldehyde in PBS. They were subsequently washed 3 times with PBS and incubated for 20 min with 0.5% BSA in PBS in order to bind any unreacted aldehyde groups. Mab diluted 1:2000 in 0.5% BSA-PBS was incubated, for 20 min at 37°C, with 100 cercariae in a total volume of 200 µl. This was followed by 3 washes in PBS and incubation as described above with 200 µl of fluorescein-conjugated rabbit antimouse IgG (H+L, Bioreyda, Rehovot, Israel) diluted 1:50 with 0.01% Evans blue in PBS. Following 3 washes with PBS, the cercariae were suspended in PBS gycerol 20% and examined under a fluorescent microscope. Photomicrographs were taken under 400 x magnification. Controls with no Mab and with irrelevant Mab were prepared and examined in parallel.

Polyacrylamide gel electrophoresis

The antigens SEA and CCA and ISN and normal snail (NSN) plasma were tested by polyacrylamide gel electrophoresis (SDS-PAGE). The various antigens were dialyzed against phosphate buffer (0.01 M, pH 7.4), and electrophoresis was carried out according to Laemmlli22 in 10% acrylamide slab gels (0.6 x 17 cm). About 40 µg of each antigen lane were run at 40 V overnight. Molecular weight markers MW-SDS 200K and MW-SDS 70K Sigma were run in parallel.

Western blotting and immunodetection

Proteins resolved by SDS-PAGE were transferred electrophoretically onto nitrocellulose paper (NCP) by established procedures.24 After the transfer, a portion of the NCP containing a set of resolved antigens and molecular weight markers was stained with 0.1% amido black in a mixture of H2O-acetic acid-methanol (4:1:5). A parallel set of resolved antigens underwent immunodetection as follows: nonspecific sites were blocked for 2 h at 5°C by a blocking solution (5% NP40, 5% P.I. Triton X-100, and 5% milk powder in PBS). The same solution was used for washing the NCP and for diluting the reagents. Mabs diluted 1:2,000 were reacted with the NCP at room temperature for 2 hr while shaking. The NCP was then washed 3 times and incubated under similar conditions with affinity-purified and alkaline phosphatase-conjugated goat antibody to mouse Fab (Bioreyda, Rehovot, Israel) diluted 1:1,000. After further washings, a color reaction was induced with a substrate mixture consisting of 40 mg naphtol phosphate disodium (Sigma) and 100 mg Fast Red TR (Sigma) in 100 ml of 50 mM Tris, pH 9.3. The color reaction was stopped by transferring the NCP into a mixture of H2O-acetic acid-methanol (5:1:5) and the NCP was then washed with water and dried.

RESULTS

Screening of monoclonal antibodies

A total of 19 Mabs were obtained. They were further screened by ELISA in order to select Mabs reactive with CCA but non-reactive with NSN extract. Such Mabs were considered potentially suitable for detecting schistosomal antigens in ISN extract. Of the 6 Mabs induced by infection, 4 reacted with both CCA and NSN extract and 2 reacted with neither of those antigens. Thus, none of these Mabs were suitable for our purposes. The 4 Mabs induced by CCA reacted with both CCA and NSN extract and were also unsuitable for our purposes. Of 9 Mabs induced by SEA, 2 reacted with both CCA and NSN. 4 reacted with neither of these antigens, and 2 (KCSme22-2 and KCSme22-4) reacted with CCA but not with NSN extract. These last 2 were selected for detecting schistosomal antigens in infected snails. Both these Mabs were IgM.

Quantitative aspects

Mab-antigen interactions

Binding of different dilutions of Mabs to wells exposed to different concentrations of antigens was examined by ELISA. Preliminary titration of both selected Mabs (in ascitic fluids) was carried out by ELISA with 150 µg well CCA. A titre of 1:6,000 was exhibited by both, subsequently, antibody activity of both Mabs at dilutions of 1:1,000, 1:500, 1:250, and 1:125 was determined with CCA at concentrations of 5-7 μg well and with snail extracts (not centrifuged) at concentrations of 5-7 μg well. Similar results were obtained with all Mab dilutions employed, and representative results with a 1:1,000 dilution of KCSme22-2 are presented in Figure.
1. KCSme22-3 showed similar binding activity (results not shown). Mab binding to CCA and to a pool of ISN extract rose with the rise in antigen concentration (3.50-7.50 ng. well). These concentrations are about 10 times higher than those yielding maximum binding with a variety of other antigens. Given this finding and taking into account that schistosomal antigens constitute only a fraction of the overall constituents in infected snail extract, these results seem to suggest that the relevant schistosomal antigens were predominantly bound to the plate. Calculations of CCA equivalents OD with ISN OD with CCA at ISN antigen concentrations of 3.5-50 ng well indicated that, on the basis of protein concentration, only about 3 times more ISN extract was required to give antibody binding equivalent to that attained with CCA. These results suggest the abundance of the corresponding epitopes in infected snails and, or a differentially high binding of these epitopes to the solid phase, as mentioned above.

Binding of the selected Mab to a pooled uncentrifuged extract of ISN was usually low. However, when high concentrations of pooled ISN extracts were employed, ELISA readings could occasionally reach a value several times higher than baseline values obtained with an irrelevant Mab (Fig. 1). Furthermore, when extracts of individual SNs were tested, several of them exhibited high binding (Fig. 2). This was attributed to the nonspecific binding of the Mab to nontoxic material present in the extract. Indeed, removal of particulate material by centrifugation abolished the binding (Fig. 2). Also, since centrifugation did not significantly change the binding of the Mab to the ISN extract (Fig. 2), it was assumed that the bulk of the corresponding antigens were soluble. This was supported by the finding that plasma (cell-free hemolymph) from infected snails is readily reactive with both selected Mabs.

Plasma from 5 ISN (shedding cercariae) and 5 NSN was applied to the microtiter plate at double dilutions of 1:10-1:5120 (corresponding to protein concentrations of 51.52 ± 16.6-0.09 mg well for ISN plasma and 62.39 ± 0.8-0.012 mg well for NSN plasma). The results of this test employing KCSme22-3 are presented in Figure 3A. Similar results were obtained with KCSme22-4 (results not shown). Mab binding to NSN plasma was invariably very low. High reactivity was exhibited with plasma from 3 of 5 ISN at all dilutions, and a prozone effect was observed. Plasma from the other 2 ISN in this group exhibited lower reactivity. Plasma dilutions of 1:160, corresponding to an average protein content of ≈3.250 ng well, gave the maximal average binding of Mab. Since binding maxima were obtained with pooled ISN extracts of a closely similar concentration (3.50 ng well) (Fig. 1), these results again suggest that a substantial proportion of the schistosomal epitopes detected in ISN by the selected Mab are present in the plasma.

The appearance of schistosomal antigens in snails' plasma collected 1-6 weeks after exposure to miracidia was detected by ELISA. Detectable Mab
binding was exhibited starting at 2 weeks (results not shown). In our infected snails, this time coincides with the release and migration of daughter sporocysts. Binding curves obtained with plasma from 3 snails infected for 2 weeks are presented in Figure 3B. Prepatent infection plasma exhibited much lower Mab binding than did patent infection plasma (Fig. 3A). These results indicate that the quantity of the corresponding epitopes increases as infection progresses from prepatency to patenty.

Characterization of Schistosoma antigens detected by the selected Mabs

Periodate sensitivity of epitopes corresponding to KCSme22-3 and KCSme22-4 is presented in Table 1. Treatment of SEA, CCA, and ISN plasma with a suitable concentration of periodate (<0.1 mM for SEA, <0.5 mM for CCA, and 5–10 mM for ISN) abolished the binding of both selected Mabs to these antigens. The observed differences in periodate sensitivity may represent differences in consumption of periodate depending on the total concentration of the absorbed antigen and on differences in the density of periodate sensitive sites. Based on these results, we assume that antigens exposing epitopes corresponding to both selected Mabs are glycoproteins.

Western blot analysis of infected snail plasma constituents resolved by SDS-PAGE was carried out with both selected Mabs. Results obtained with KCSme22-3 are presented in Figure 4. Mul-
multiple plasma constituents with M, of > 200 kDa-35 kDa were resolved by SDS-PAGE (Fig. 4, lanes g, h). CCA was resolved into multiple constituents within 200 kDa-14 kDa (Fig. 3, lane f). Immunodetection with KCSme22-3 revealed 1 constituent at 205 kDa in CCA (Fig. 4, lane b) and 3 schistosomal constituents of 220 kDa, 150 kDa, and 135 kDa in the plasma of SN (Fig. 3, lane d). Plasma from NSN did not exhibit any binding of Mab as expected (Fig. 4, lane c). A similar pattern was obtained with KCSme22-4 results not shown.

Paraformaldehyde-fixed cercariae gave strong fluorescence on cercarial surface. Representative results with KCSme22-3 are presented in Figure 5.

**DISCUSSION**

In the present study, we have employed Mabs in search of parasite specific antigens in *B. glabrata* infected with *S. mansoni*. In view of the widespread cross-reactivity of schistosomal antigens with their snail host, it is not surprising that among a total of 15 Mabs which reacted with cercarial antigens (CCA), 1 also reacted with extracts from normal snails (NSN). Antigen sharing between snails and schistosome larvae is thought to be largely due to acquisition of small constituents by the parasites* and perhaps also by true antigen mimicry*. 1,3,4 The antigen sharing found between schistosome eggs and small antigens, as detected by 3 of 4 Mabs which were induced by SEA, cannot be explained as being due to acquisition, since schistosome eggs do not come in contact with snail tissues. Therefore, it may be assumed that the corresponding epitopes are synthesized by schistosome eggs. Such shared antigens, presumably synthesized by the parasite, are particularly interesting in the context of the molecular evolution of the host-parasite adaptation. 3

Cross-reactivity between egg antigens and cercarial antigens was exhibited by 5 of 4 Mabs induced by SEA. Among these, KCSme22-3 and KCSme22-4 were parasite specific and identified carbonate epitopes (Table 1) in schistosomal antigens of high relative molecular mass (Fig. 4). Both selected Mabs were IgM and gave similar results in all tests employed (ELISA, Western blotting, IFAT). Whether they are directed against the same epitope remains to be determined by
epitope-specificity analysis. Since the 3 antigens detected in the snails' plasma appear to be different in molecular mass from the 1 present in CCA (Fig. 4), it can be tentatively assumed that they are not derived from cercariae. This assumption is supported by the finding that these antigens first appear in the plasma 2 weeks after snail infection, before the development of cercariae in our infected snail colony. Since their quantity increases from prepatency to patency (Fig. 3), it can be assumed that they are released in a progressive manner as an outcome of the normal development of the parasite, perhaps during the release of larvae from mother and daughter sporocysts.

The parasite specificity of the schistosomal antigens detected in the snail suggests that they may be foreign to the snail, but whether they can be recognized as such by the snail remains to be determined. At present, these parasite specific epitopes and the corresponding Mabs can be considered as candidate reagents for identifying putative receptors mediating recognition of parasite antigens by snail hemocytes. Whether these epitopes play a role in the regulation of snail defenses is unclear. Since these epitopes are parasite specific, they cannot possibly contribute to immune-evasion mechanisms based on antigen sharing. On the other hand, since parasite-specific epitopes appear to be abundant in infected snail's plasma (Fig. 5), they may play a role in blocking hemocyte activity by saturating putative receptors.

From a practical point of view, several features of the Mabs and the immunoassay described for the detection of parasite specific antigens in infected snails suggest the feasibility of an immunodetection approach for identifying infected snails. The results of a preliminary evaluation of this approach for epidemiological purposes, where the identification of schistosome-infected snails was required, follow this article.7

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IDENTIFICATION OF SNAILS INFECTED WITH SCHISTOSOMES
BY ELISA EMPLOYING MONOCLONAL ANTIBODIES:
SCHISTOSOMA MANSONI IN LABORATORY SNAILS (BIOMPHALARIA GLABRA)
AND IN FIELD SNAILS (BIOMPHALARIA PFEIFFERI)
FROM KENYA

J. HAMBURGER, M. WEIL, T. TURETZKY, J. H. OLUMA, D. K. KOECH.
R. KLUMPP, T. K. A. SIONGOK, AND R. F. STURROCK

Abstract. An enzyme-linked immunosorbent assay (ELISA) employing monoclonal antibodies was used for detecting Schistosoma mansoni antigens in hemolymph of laboratory snails (Biomphalaria glabrata) and field snails (B. pfeifferi) from Kenya. Infection was demonstrated by ELISA from 100% of infected snails with 100% sensitivity and specificity. Prepatent infections were detected by ELISA in field snails 2 weeks after exposure to miracidia. Thus, ELISA revealed infection 3 weeks before maximal patency was reached (12 weeks post-exposure).

The transmission of schistosomiasis is affected by a variety of factors relating to human infection and behavior, environmental conditions, and snail monoclonal snails and infection. Since identification of snails is important for designing control projects and for evaluating the effect of their application, an increase in the use of antischistosomal drugs in control campaigns. Furthermore, the increased use of antischistosomal vaccine development lead to a reduction in the proportion of infected snails in a particular area. It is necessary to examine large numbers of snails from a variety of locations and all seasons. Snails infection is routinely monitored by examining each of the snails to see if it sheds cercariae. The shedding test, however, simple, does not identify snails harboring prepatent infections which may constitute a significant proportion within infected snail populations. The omission of prepatent infections from snail infection rate data results in an inaccurate evaluation of transmission and interferes with the application of mathematical models of transmission.

Prepatent infections in snails are currently not routinely monitored; the methods available for this purpose are not easily and accurately applicable for large numbers of snails. These methods involve microscopical examination of crushed snails for presence of larval stages, or serial shedding tests over several weeks in snails transmitted from the field to the laboratory. In both of these cases, removal of large numbers of snails from the transmission site is required. This may
alter transmission conditions in sites containing small snail populations, and interfere with longitudinal transmission studies. Therefore, a simple test which can identify snails infected prepatently as well as patently with human schistosomes should be useful for epidemiological studies. Such a test would have further advantages if the snails could be returned alive after testing to the site for longitudinal transmission studies.

We have developed monoclonal antibodies (MAb) which recognize, by an enzyme-linked immunosorbent assay (ELISA), Schistosoma mansoni antigens in the hemolymph of infected snails during prepatency and patency. Only minute amounts of hemolymph were required for this purpose, and many snails were left alive after bleeding. These findings suggest the feasibility of employing MAb for the identification of snails infected with *S. mansoni* by immunodetection of schistosomal antigens. The present report provides a preliminary evaluation of the immunodetection approach with regard to test sensitivity and specificity.

**Materials and Methods**

**Snails and parasites**

*S. mansoni* (Egyptian strain), originally obtained from Wellcome Laboratories, was maintained for 25 years at the Department of Parasitology, Hebrew University, *Biomphalaria glabrata* and in mice. A Kenyan strain of *S. mansoni* was maintained in *Biomphalaria pfeifferi* and in mice at the Kenya Medical Research Institute.

**Studies on laboratory snails**

Normal snails (*B. glabrata*) were kept in breeding aquaria at 25°C in dechlorinated tap water. They were fed romaine lettuce and were exposed to artificial light 12 hr daily. Snails measuring 5-8 mm were infected by exposing them individually to 10 miracidia snail. Infected snails were kept in separate containers in the dark. At peak patency, the infection rate in the *B. glabrata* colony was 50% as determined by shedding tests (Table 1).

The sensitivity and specificity of the immunodetection test were determined by testing snails shedding cercariae together with normal controls. For immunodetection of prepatent infections, individual snails were randomly picked from batches exposed to miracidia 1-6 weeks earlier. We also examined a group of snails infected for more than 11 weeks. A proportion of these snails had stopped shedding cercariae spontaneously; therefore, we were interested in knowing whether schistosomal antigens also disappeared under these circumstances.

**Studies on field collected snails**

The objective was to make snails shedding schistosome cercariae (presumably *S. mansoni*), snails shedding non-human cercariae, and snails which did not shed any cercariae at all available for the immunodetection studies. In addition, snails infected prepatently with *S. mansoni* were required for our study. It was anticipated that such snails should be found in sites where high transmission occurs.

Snails (*Biomphalaria pfeifferi*) were collected from 3 sites in Machakos District, Kenya. The sites were located in the study areas previously surveyed by Siengok and others and by Butterworth and others. Snails were initially collected from a site on the Kakoi River, chosen for its remoteness from human habitation: they were therefore not expected to be infected with *S. mansoni*. Additional snails were collected from 2 separate sites on the Kinyui stream. Both were proven by previous surveys to be active transmission sites of *S. mansoni*. In each case, the snails were hand collected, cleaned of detritus, and shipped to a field laboratory near the village of Tetum, for immediate shedding tests. Each snail was tested for cercarial shedding after exposure to daylight in individual glass vials filled with borehole water. Cercariae were examined by a magnifying glass. Snails shedding human cercariae (HC), non-human cercariae (NHC), and snails not shedding (NEG) were pooled separately and shipped to the central laboratory in Nairobi. The next day the snails were bled and their hemolymph tested for presence of schistosomal antigens.

**Collection of hemolymph for immunodetection**

The snail of each snail was cleaned by water and 70% ethanol using a swab and then dried. The snail was punctured near the innermost con
of the shell and hemolymph was drawn out by a mouth operated suction tube to which a 25 G needle was attached. About 10 µl hemolymph were taken from each snail and introduced directly into wells of a microtiter plate. Each snail was bled with a separate needle. After bleeding, the hole in the shell was covered with plasticine and the snail returned to its container. Percent viability was determined immediately after bleeding and a week later. Normal safety precautions were taken when handling the snails to prevent infection.

**Immunodetection**

Immunodetection was carried out by ELISA: ¹ Snail hemolymph (~10 µl) was mixed with 120 ml BPS in a well of a microtiter plate and 85 µl of the mixture was then transferred to an adorning well as a duplicate. Adsorption was carried out by overnight incubation in a humid chamber. ELISA was carried out with Mab KCSme 22-3. ² The Mab, in murine ascitic fluid, exhibited an original titer of 1:51,200 against SEA and was employed for ELISA at 1:5,000 dilution in PBS with 5% fetal calf serum. Controls, included in each plate, consisted of pooled hemolymph from infected and normal snails. These were tested with both relevant and irrelevant Mab.

**RESULTS**

**Detection of S. mansoni infection in laboratory snails**

The results are summarized in Table 1. Snails preselected for patency were initially tested. A total of 83 snails shedding cercariae and 117 normal control snails were included in this group. ELISA OD readings were considered positive if they were at least twice the maximal value obtained with matching normal controls. All the snails which shed cercariae gave positive results by ELISA, with an average OD of 1.37 ± 0.560 for 83 infected snails and 0.16 ± 0.504 for 117 normal snails. The maximal value obtained with normal controls was 0.16 ± 0.488. Thus, the average infected snails gave ELISA OD readings which were higher than the controls by a factor of 11.5. Infected snails were differentially identified from uninfected snails with 100% sensitivity. Percent ELISA positives among cercariae

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sheding snails) and specificity (percent ELISA negatives among uninfected snails). Such a complete match between ELISA positivity and cercarial shedding capacity was also evident in snails which were maintained for more than 11 weeks after exposure (Table 1). Only about a third of these snails maintained cercarial shedding capacity (20.3%) and only those shedding remained ELISA positive, suggesting that the relevant antigens disappear from the hemolymph quickly after cessation of cercarial shedding.

We then tested hemolymph taken from snails randomly picked from batches of snails exposed to miracidia 1–6 weeks earlier. As seen in Table 1, cercarial shedding was not observed 1, 2, and 3 weeks post-exposure; by 4 weeks post-exposure, 10% of the snails tested ELISA negative, with only 22.5% of the snails remaining positive. The maximal percent patency was reached at 6 weeks post-exposure, at which time only 17.5% of the exposed snails shed cercariae. Since comparison of detection of infection by ELISA preceded maximal patency by about 3 weeks, ELISA positivity was first observed at 2 weeks post-exposure. Percent ELISA positivity at this time was 11%, a little lower than maximal percent patency (18.5%). This down-regulation may be the outcome of sub-detectable amounts of the relevant antigens in the hemolymph of some of the snails during early prepatency. [4, Hamburger, personal communication]. By 3 and 4 weeks post-exposure, percent ELISA positivity was higher than maximal percent patency, reaching 34% and 57.5% respectively (Table 1). These results suggest that not all ELISA positive, prepatently infected snails eventually reach patency. This is in line with the existence of resistance in a proportion of the snails with the resulting destruction of parasites before onset of patency.

Immediately after bleeding for hemolymph, all snails remained alive. Snail viability a week after bleeding was 50–70%.

Detection of S. mansoni infection in field collected snails

These experiments were carried out in Kenya on B. pfefferi. Results of initial experiments with laboratory-infected B. pfefferi were similar to those obtained in the laboratory with B. glabrata. In both cases, a complete differentiation between infected and uninfected snails was obtained by ELISA (Table 1). Subsequently, studies on field collected snails provided an opportunity to examine the suitability of ELISA for differentially identifying snails infected with S. mansoni from snails infected with other trematodes.

About 300 snails were collected from a site on Kakoi River where S. mansoni transmission does not occur (Table 2). Of these, 50 shed non-human cercariae (NHC) and none shed schistosome cercariae. We tested by ELISA 138 which shed NHC and found them all negative. Microscopical examination of microtiter wells containing hemolymph from these snails revealed rediae and or NHC. Of the several hundred not shedding in this lot, 143 were tested by ELISA; all were found to be ELISA negative, although 33 (23%) of them exhibited rediae and or NHC by microscopical examination of the hemolymph. The non-human trematodes were identified as echinostomes or strengtids but species identification was not done. These results indicate that B. pfefferi infected in nature with S mansoni can be differentiated by ELISA, with 100% sensitivity and specificity, from non-infected snails and from snails infected with non-human trematodes common in the study area.

About 120 snails were collected from a known transmission site on Kinyui stream (Table 2). Of these, 5 shed schistosome cercariae and also presented schistosome cercariae or germ balls in their hemolymph. All of these 5 snails, patently infected by schistosomes, were positive by ELISA. A single case of mixed infection (S mansoni with NHC) was found among the snails in this group (data not shown). This case of mixed infection was identified as positive by ELISA, indicating that the superimposed infection with non-human trematodes did not interfere with the expression of schistosomal antigens in the hemolymph during patency. Whether such interference does occur during prepatent mixed infection is not known.

A second known transmission site on Kinyui stream (near Kangundo) was characterized by a high rate of S. mansoni infection (6.41%) or 9.3%) and by the absence of infection with non-human trematodes (Table 2). The high rate of patent infections in this site suggested the likelihood of a high rate of prepatent infections. Also, the absence of non-human trematodes precluded the possibility of mixed infections, the effect of which on the expression of schistosomal antigens in the hemolymph during patency is not known. Among the total of 251 snails collected in this site, 6 (13.1%) were not shedding but were ELISA positive. They represent 14.5% (8 55) of the total
number of those not shedding. Of these 3 snails, 4 exhibited schistosome cercariae or germ balls in the hemolymph. Judging from the high degree of specificity of the test, as seen in the preceding studies with laboratory and field collected snails, these 3 snails probably harbored prepatent S. mansoni infections. In this particular case, the infection rate by the shedding test alone was 9.8%, while the combined prepatent and patent infection rate (13.1 plus 9.8%) was 22.9%. These results demonstrate a case where prepatent infections constitute a substantial proportion within populations of infected snails.

**DISCUSSION**

We employed a Mab which recognized S. mansoni antigens in the hemolymph of the intermediate snail host for a preliminary evaluation of the epidemiological usefulness of immunodetection by ELISA of S. mansoni infection in Biomphalaria.

Infected laboratory snails were differentiated at all stages of patency from uninfected snails with 100% sensitivity and specificity (Table 1), and the clear-cut differentiation enabled visual reading of ELISA results for qualitative determination. Studies with field collected snails (B. pfeifferi) demonstrated the same high degree of specificity and sensitivity in differentially detecting schistosome infected snails among a mixed population containing uninfected snails and snails infected with non-human trematodes (schistosomes and digeneans). Further evaluation of the specificity of the test should include testing of a wide variety of non-human trematodes capable of naturally infecting Biomphalaria as possible. In addition, since Biomphalaria also serves as a natural host for S. mansoni, it remains to be determined whether the test can differentiate S. mansoni from S. mansoni. With these reservations in mind, the suitability of the Mab employed here for immunodetection of S. mansoni infection in field collected Biomphalaria is indicated. It is important to find out whether the same Mab can be employed for differential identification of S. haematobium in its intermediate snail host, Bulinus. Immunodetection can be very helpful if found suitable for species differentiation in this case, since morphological differentiation of S. haematobium cercariae from those of the several animal schistosomes naturally infecting Bulinus is impossible.

In practice, it is not expected that the immunodetection test, even if made inexpensive and usable by community health workers in endemic areas, will replace shedding tests for large-scale identification of snails patently infected with S. mansoni. It may, however, be useful in those areas for periodic quality control of shedding tests, in particular where routine differentiation of S. mansoni cercariae from a large variety of NHC is difficult. Our results with laboratory snails (Ta-
Table 1) suggest the potential of the test for identifying prepatent infections and providing more accurate snail infection rate data. Findings with field collected snails from a site with high S. mansoni transmission further support this suggestion by showing a case where infection rate by shedding test alone was 9.8%, whereas the combined figure of patent and prepatent infection rates was 32.9% (Table 2).

The inability of the test to identify early prepatent (1 week) infections (Table 1) is problematic in the sense that the full extent of the parasite flux from the definitive host to the snail cannot be evaluated. However, very early detection of snail infection may not be completely relevant for evaluating prospective cercarial shedding capacity, since resistance (or incompatibility) of a proportion of snails in natural populations of Biomphalaria may exist, resulting in destruction of the parasites at very early prepatency. Since only 79.6% of the exposed laboratory snails eventually reached patency (Table 1) and assuming that practically all the young snails exposed individually to 10 miracidia were penetrated by miracidia (an assumption consistent with the known high infection rate of susceptible B. glabrata), it indeed appears that a portion of the snails destroyed the parasites sometime between miracidial penetration and maximal expression of patency. The finding that percent ELISA positivity at 3 and 4 weeks post-exposure (Table 1) is higher than maximal percent patency (at 5-6 weeks) tends to support this assumption. Moreover, loss of infection appears to be a continuous process in our system since it proceeded after maximal patency had been reached. With regard to old infections, it is important to note that ELISA positivity was proof of patency and not of residual schistosomal antigens in hemolymph after cessation of cercarial shedding.

When taken together, the results seem to suggest that percent ELISA positivity during prepatency is a more reliable estimate of prospective cercarial shedding capacity than is the miracidial penetration rate. This assumption is supported by the timing of antigen release into the hemolymph, which parallels active development and release of larvae from sporocysts. Whether the amount of schistosomal antigens in the hemolymph correlates with the number of cercariae released totally or at any one point in time during patency remains to be determined.

The results justify further development of the immunooassay to provide a simple, cost-effective tool suitable for routine monitoring of prepatent S. mansoni infection in Biomphalaria. In combination with the conventional shedding test, this should provide more accurate measurements of snail infection rates and enable the determination of prepatency/patency ratios under various transmission conditions and following the application of control measures.

Another related subject which warrants attention is snail survival after bleeding for hemolymph. This subject was not addressed beyond the observation that 50-70% of the snails remained alive 1 week after bleeding. Long-term post-bleeding survival of the snails after reintroduction into the transmission site should enable the determination of the kinetics of prepatency/patency in field collected snails under various natural conditions and after application of control measures.

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following chemotherapy of Schistosoma mansoni in St. Lucia, West Indies. Trans R Soc Trop Med Hyg 75: 713-714. UI:812130103
Detection of *Schistosoma mansoni* DNA in extracts of whole individual snails by dot hybridization*

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The potential usefulness of DNA-DNA hybridization as a diagnostic tool has been demonstrated for a number of parasitic infections (Gonzales et al. 1984; McLaughlin et al. 1985; Pollack et al. 1985; Sim et al. 1986; Wirth and McMahon Pratt 1982) and has also been proposed for identification of schistosomiasis in snails (Rollinson et al. 1986). Monitoring schistosomiasis infection in snails is regularly required to elucidate the dynamics of transmission and the effect of control measures (Haisston 1973; Christie and Uthmath 1977; Barnish 1982), both requiring examination of a large number of snails. Thus, a rapid and accurate detection method will aid parasitologists particularly since detection of prepatent infection is not straightforward and its omission leaves data on snail infection rates incomplete (Sturrock et al. 1979). Cloned DNA probes containing *S. mansoni* ribosomal RNA gene sequences were recently prepared (McCutchan et al. 1984) and used for schistosomiasis species identification by Southern blot analysis, and for detection of infected snails by dot blot hybridization with DNA from infected snail hepatopancreas (Rollinson et al. 1986). However, any attempt to employ dot hybridization for mass screening of snail infection would require a simple procedure for treating whole individual snails for analysis. Such a procedure is described in the present report.

Snails (*B. glabrata*) were infected by exposure to 10 miracidia of *S. mansoni*. Infected and normal snails were prepared for dot hybridization. Whole snails (up to 12 mm in diameter) were each titrated in 0.3 ml 1 M NaOH containing 1% Triton X-100. Titration was performed with a glass rod having a bulged and indented bottom, in a round-bottomed plastic test-tube. The titrated snails were left in the NaOH-detergent solution at room temperature for up to 4 days. Just before dot blotting, concentrated HCl was added to neutralize the samples which were then heated to 50°C for 5 min, cooled quickly, and debris removed by centrifugation at 12,000 × g for 10 min. Snail extracts were individually spotted onto nitrocellulose paper (NCP), presoaked in 10 × SSC (10 × SSC is 0.15 M NaCl, 0.015 M sodium citrate) using a manifold microfiltration apparatus (Biorad Laboratories Richmond, CA). Conventional procedures for preparing extracts for immobilization on NCP such as proteinase K digestion and the use of SDS, resulted in an extract that was not completel, filterable. Similar difficulties were encountered when the neutralization and/or heating steps were omitted.

After blotting, the NCP was washed with 10 × SSC, air-dried, baked under vacuum at 80°C for 2 h, and incubated for 4 h at 42°C in prehybridization solution containing 50% formamide, 5 × SSC, 0.05 M sodium phosphate pH 6.5, 75 μg/ml Salmon sperm DNA and 0.02% each of polyvinylpyrrolidone, BSA, and Ficoll. The NCP sheet was then hybridized for 10–15 h at 42°C in a solution containing 4.5 volumes of prehybridization solution, 1/5 volumes of 50% dextran sulphate, and 7 ng/ml (about 2 × 10⁶ cpm ml) heatdenatured ³²P-labelled total *S. mansoni* DNA prepared from adult worms (see below). The NCP was subsequently washed once for 20 min at room temperature with a solution containing 50% formamide, 2 × SSC, and 0.1% SDS. Then followed three washes, 20 min each at room temperature, in 2 × SSC containing 0.1% SDS, and finally two washes at 50°C for 15 min each in 0.1 × SSC containing 0.1% SDS. The NCP was air-dried and...
adult worm DNA, infected snail DNA, uninfected snail DNA, and whole cercariae (extracted by the procedure described above for whole snails), underwent hybridization under similar conditions.

DNA from *S. mansoni* adult worms and from infected and uninfected snails was prepared as follows. Worms (usually 1 ml packed) were washed with ice-cold TE (10 mM Tris-Cl pH 8.0, 1 mM EDTA) and titered by repeated passages through a syringe. Snails (usually 15) were removed from their shell, washed in TE, and cut into small pieces with a scalpel. Then they were repeatedly passed through a syringe. Five volumes of lysis buffer (10 mM Tris-HCl pH 8.0, 50 mM EDTA, 5% SDS, and 250 μg/ml proteinase K) were added and the mixture incubated for 2 h at 37°C. Phenol-chloroform extraction of DNA and alcohol precipitation were carried out by standard procedures (Maniatis et al. 1982). Alcohol-precipitated DNA pellets were vacuum-dried and treated with 100 μg/ml RNAase in TE, for 3 h at 37°C, after which the extraction and precipitation steps were repeated. Total *S. mansoni* adult worm DNA was labelled with 32P-dCTP (Amersham Radiochemicals, 3000 Ci mmol⁻¹) by nick translation (Pollack et al. 1982), and used as a probe in hybridization experiments.

Figure 1 shows the results of hybridization of total DNA probe with *S. mansoni* adult worm DNA, infected snail DNA and uninfected snail DNA. As little as 25 pg *S. mansoni* DNA, and not less than 5 ng infected snail DNA gave positive signals. By comparison, about 4 × 10³ times higher quantities (> 20 μg) of non-infected snail DNA did not yield positive signals. We have not tested the effect of excess snail DNA on the sensitivity of detection of schistosomal DNA. The specific signal, however, was strong enough to differentiate between infected and non-infected snail DNA.

Figure 2 shows the results of hybridization with infected and non-infected snails directly extracted on NCP without prior isolation of DNA. Non-infected snails did not show positive DNA, while all 9 infected snails gave positive signals.

Figure 3 shows that early prepatent as well as patent infection can be detected. In addition, intensification of positive signals can be demonstrated during the course of development of *S. mansoni* in the snail. Thus, while at 1 and 2 weeks of infection, when only mother sporocysts are present, the signal is weak. At 3 weeks, when daughter sporocysts are expected to be present, the signal
INFECTED SNAILS

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Fig. 3. Hybridization of total schistosomal DNA probe with extracts from infected snails at varying times after infection, and with cercarial extracts.

After infection, strenghts and it becomes clearly stronger at 4 and 6 weeks of infection in parallel with the expected cercarial development. Extracts of 10, 100, and 1000 whole cercariae all gave signals with intensities directly correlated to cercarial numbers.

While the use of a total DNA probe appears sensitive enough for detecting infection in snails without prior laborious isolation of their DNA, it is not likely to constitute a specific tool for species differentiation in cases of mixed infections. Such differentiation was accomplished by the use of cloned S. mansoni ribosomal RNA gene in Southern blot analysis of different species of schistosomes. However, when used in dot hybridization it could identify infection in only some of the snails, and as much as 0.2 μg of DNA from infected snail hepatopancreas were required (Rollinson et al. 1986), suggesting a relatively low sensitivity. Moreover, since this probe gives positive signals (although with different banding) in Southern blot analysis with different schistosome species, it is not useful as a species-specific probe in dot hybridization. We have also found in preliminary experiments that a cloned S. mansoni DNA probe prepared from extranuclear schistosomal DNA (including mtDNA), although capable of strain differentiation in Southern blot analysis, could not detect schistosomal sequences in as much as 20 ng infected snail DNA by dot hybridization (Pollack and Hamburger 1986). Thus, it appears that a probe or a mixture of probes providing both sensitivity and specificity sufficient for mass species identification of schistosome infection in snails by dot hybridization is still unavailable.

Our present work demonstrates the feasibility of detecting patent and prepatent infections in snails by employing a simple procedure for preparing snails followed by hybridization with a total schistosomal DNA probe. However, several issues have to be taken into account before considering application of the approach for field epidemiology studies. Firstly, the detection of patent infection by shedding of cercariae is simple and suitable for mass screening. Secondly, the utility of the hybridization approach should be evaluated vis-à-vis crushing and microscopic examination for detecting prepatent infections. Thirdly, the degree of cross hybridization with DNA from other trematodes, commonly infecting Biomphalaria in nature, should be determined.

Our results suggest that quantitation of schistosomal DNA in snails may be possible by DNA hybridization followed by suitable measurements of sign intensity (Figs. 1, 3). This, however, would require standardization and calibration of the detection system. Since changes in sign intensity parallel the course of development of S. mansoni in the snail (Fig. 3), it may be worthwhile to evaluate the suitability of the detection approach for determining the effect of various factors (physical, chemical, and biological) on the development of S. mansoni in laboratory-infected snails.

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Highly repeated short DNA sequences in the genome of *Schistosoma mansoni* recognized by a species-specific probe

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A cloned 0.64 kb DNA sequence of *Schistosoma mansoni* contains 120-bp tandem repeats and comprises at least 12% of the schistosomal genome of both sexes. It exhibits a high degree of species specificity upon hybridization with *Schistosoma haematobium* and *Schistosoma mekongi* DNA, and could detect with high sensitivity schistosomal DNA sequences in infected snails.

Key words: *Schistosoma mansoni*; Repeated DNA; DNA probe; Species identification

Introduction

There are 5 schistosome species which regularly infect man. Of these, the 3 major species of human schistosomes afflict more than 200 million people around the globe in tropical and subtropical regions [1]. About a dozen other mammalian schistosome species exist in the same geographic regions, and these may infect the same freshwater snails which serve as intermediate hosts for human schistosomes. This presents a diagnostic problem, as the various schistosome species cannot be differentiated by the morphology of the larval stages developing in snails. Thus, since snail infection rates are routinely determined by identifying snails shedding schistosome larvae (cercariae) [2], it is impossible to obtain separate data on snails infected with human schistosomes in areas where human and animal schistosomes coexist. Also, since direct identification of schistosome species developing in humans is based on egg morphology alone, identification presents a problem if infection with animal schistosomes exists in the same geographic regions. This presents a diagnostic problem if infection with animal schistosomes exhibiting similar egg morphology, or interspecific hybridization, is suspected [3,4]. It is therefore desirable to develop alternative means of diagnosis that will enable differentiation between species and strains of schistosomes.

Previous studies have described rDNA probes capable of differential identification of schistosome species based on restriction fragment length heterogeneity of the corresponding sequences [5,6]. Schistosome species identification was also accomplished by dot hybridization, employing as a probe a degenerate repeat present only in the genome of female *Schistosoma mansoni* [7]. In these cases the corresponding sequences are repeated approximately 100 and 75 times per haploid genome. In the present report we describe the cloning and preliminary characterization of highly repeated DNA sequences present in the genome of both male and female *S. mansoni* and exhibiting species specificity.
Materials and Methods

Parasites. S. mansoni (Egyptian strain) was originally obtained from Wellcome Laboratories about 30 years ago. It has since been maintained by passages through outbred albino mice and in snail hosts (Fiona phalacraciensis). Mice were infected by subcutaneous injection of 350 cercariae. Cercariae were shed under fluorescent light from snails infected 6-10 weeks previously by individual exposure to 5-10 miracidia per snail. Miracidia were induced to hatch from ova in liver homogenates of mice infected 9 weeks previously. Adult worms were collected from livers of infected mice by perfusion [8], washed once with phosphate-buffered saline (PBS), excess liquid removed, and the packed worms quickly frozen in liquid nitrogen and stored at -70°C.

Preparation of DNA. About 1 ml packed frozen worms (about 1000 worms) were thawed and washed in cold extraction buffer (50 mM Tris-HCl, pH 7.5/10 mM EDTA/100 mM NaCl), then homogenized, mixed with sodium dodecyl sulfate (SDS) to 10 μg ml⁻¹ and incubated at 50°C for 2 h. Nucleic acids were extracted twice with phenol, once with phenol/chloroform (1:1) and once with chloroform, then dialyzed and precipitated in ethanol. Treatment with RNase A (100 μg ml⁻¹) for 3 h at 37°C then followed, and the DNA was extracted as above, dialyzed, alcohol-precipitated, dissolved in TE (10 mM Tris-HCl pH 7.5/1 mM EDTA) and stored at -20°C. DnaA of S. haematobium and S. magrebowiei was kindly supplied by Yaakov Pollack, Ben Gurion University, Beer-Sheva, Israel.

Construction of S. mansoni genomic Sau3A fragment library in the BamH1 site of pUC18. DNA from S. mansoni adult worms was partially digested with Sau3A and ligated with plasmid DNA which was predigested with BamH1, and dephosphorylated with calf intestinal alkaline phosphatase [9]. Several ligation reactions were carried out in parallel at various weight ratios of genomic/plasmid DNA (1:5:1, 3:1, 9:1 and 15:1), and subsequently pooled and used directly to transform competent cells of E. coli strain F15 by established protocols [9]. Transformants were plated out on LB agar plates containing ampicillin, IPTG and X-Gal were included in the final plating mixture.

Screening of library. Screening for cloned highly repeated sequences was carried out by colony hybridization. The colonies were transferred to nitrocellulose membranes, then treated with 10% SDS, and the DNA denatured in 0.5 M NaCl for 5 min and neutralized in 0.5 M Tris-HCl pH 7.5/1.5 M NaCl. Following a wash in 2x SSPE [9] the membranes were dried and baked at 80°C for 2 h. Total genomic DNA from S. mansoni, 32P-labeled by nick translation [10], was used as a probe. Colonies exhibiting a strong hybridization signal were selected for further screening. Plasmids from these clones were harvested by a rapid plasmid purification procedure [11], restricted with EcoRI and PstI according to Maniatis et al. [9], then separated by agarose gel electrophoresis and underwent Southern blotting [12] and hybridization with a 32P-labeled hamster rDNA probe under low stringency hybridization conditions (see below). Cloned repeated sequences unrelated to rDNA were subsequently selected.

Preparation of probes. A recombinant cosmid p11-a2 [13] which contains a hamster ribosomal gene (inserted into the EcoRI site of the cosmid vector Mua-3) was harvested [11], restricted with EcoRI and fragments separated by agarose gel electrophoresis [14]. The rDNA fragment was isolated from the agarose str.p by electrolyelution of the model U.A.E. Unidirectional Electrolute (International Biotechnologies, Inc. New Haven, CT, U.S.A.) according to the manufacturer's instructions. It was then labeled with 32P by nick translation [10] and employed for screening to identify clones containing rDNA sequences. A recombinant plasmid, pSm1-7, containing a 640-bp insert of S. mansoni DNA, comprising repeated sequences unrelated to rDNA, was selected (see below). The insert was released by digestion with EcoRI and PstI or with Smal and XbaI and separated by agarose gel electrophoresis and electroelution as described above. Fragments of the pSm1-7 insert were produced by Sau3A digestion. This was followed by electrophoresis in 5% polyacrylamide gel [14], and elution from the
polyacrylamide gel strips by diffusion in 500 mM 
\( \text{NH}_4\text{Ac} \), 1 mM EDTA. DNA was subsequently ex-
tacted and dissolved in TE. The pSml-7 insert and its fragments were \( ^{32}\text{P} \)-labeled [10] and used as probes.

**Dot blot analysis.** A nitrocellulose membrane was pretreated by soaking in water and washing once with 20 \( \times \) SSC (1 \( \times \) SSC is 0.15 M NaCl/0.015 M Na-citrate) for 30 min. DNA to be dotted was diluted as desired in 0.3 M NaCl then denatured by boiling for 10 min and kept on ice. Immediately before dotting the DNA was diluted with 2 M cold ammonium acetate then filtered through the membrane in a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories, U.K.). The membrane was subsequently washed in 1 M cold ammonium acetate and dried, and the DNA underwent denaturation, neutralization and baking as described above for colony hybridization.

**Filter hybridization.** Standard high-stringency hybridization involved prehybridization for at least 4 h at 42°C in 50% formamide, 5 \( \times \) SSPE [9], 5 \( \times \) Denhardt's solution [9], 1% glycine and 200 
\( \mu \text{g ml}^{-1} \) denatured salmon sperm DNA. This was followed by overnight hybridization at 42°C with with heat denatured and \( ^{32}\text{P} \)-labeled probe (at least 1.5 \( \times \) \( 10^6 \) cpm \( \text{ml}^{-1} \)) in hybridization solution consisting of 50% formamide, 5 \( \times \) SSPE, 1 \( \times \) Denhardt's solution, 0.3% SDS and 120 \( \mu \text{g ml}^{-1} \) denatured salmon sperm DNA. Hybridized membranes were washed twice with each of the following solutions: 2 \( \times \) SSPE for 5 min at room temperature (RT); 2 \( \times \) SSPE/0.1% SDS for 30 min at 53°C; 0.5 \( \times \) SSPE/0.1% SDS for 30 min at 53°C; 0.1 \( \times \) SSPE/0.1% SDS for 30 min at 53°C; and finally with 2 \( \times \) SSPE at RT for 5 min. The membranes were then air-dried and subjected to autoradiography (see below). Densitometry of dot blot autoradiograms was carried out using the 'Quick-Scan R&D Densitometer' (Helena Laboratories, Beaumont, TX, U.S.A.). Hybridization at low stringency with the \( ^{32}\text{P} \)-labeled hamster rDNA probe p11-a2 [13] involved prehybridization in 1 \( \times \) Denhardt's solution for 4 h at 60°C. This was followed by hybridization at 37°C with the heat-denatured radioactive rDNA probe in 1.0 M NaCl/10 Tris-HCl, pH 7.4, 10 \( \times \) Denhardt's solution and 10% formamide. The membrane was subsequently washed five times each for 30 min at 42°C with 6 \( \times \) SSC (1 \( \times \) SSC is 0.15 M NaCl/0.015 M Na-citrate), air-dried, and subjected to autoradiography using a Kodak X-ray film, and a 'Carnex' intensifying screen.

**DNA sequencing.** The nucleotide sequence of the schistosomal DNA inserted in pSml-7 was determined using Sanger's dideoxy chain termination technique [15] as modified by Haltori and Sakaki [16] for double-stranded sequencing, using denatured plasmid template. The 'Sequenase' kit (Stratagene, La Jolla, CA) and \( ^{35}\text{S} \)-labeled dATP were employed.

**Results**

**Identification of clones containing species specific DNA sequences unrelated to rDNA.** About 5000 recombinant colonies representing a partial genomic library of Sau3A-digested S. mansoni DNA in the BamHI site of pUC18 underwent hybridization with total \( ^{32}\text{P} \)-labeled genomic DNA of S. mansoni. A total of 140 colonies exhibiting strong hybridization signals were identified, amplified and cryopreserved. Each of these clones was digested with EcoRI and PstI to release the inserted S. mansoni DNA fragments and underwent agarose gel electrophoresis, Southern blotting and hybridization with \( ^{32}\text{P} \)-labeled hamster rDNA isolated from p11-a2 [13]. About 40 clones which did not react with the hamster rDNA were selected. A preliminary study on species specificity of these 40 clones was carried out by colony hybridization with \( ^{32}\text{P} \)-labeled DNA from S. mansoni, S. haematobium and S. magrebowiei. Five clones exhibiting strong hybridization signals with S. mansoni DNA, and none with S. haematobium or S. magrebowiei were identified, and shown to cross-hybridize with each other. One of these clones, pSml-7, containing a 640-bp insert of S. mansoni DNA was taken for further analysis.

**Abundance and arrangement of the repeated sequence.** Preliminary information on the abundance of the repeat was obtained from quantitative dot-blot analysis. Equal quantities of \( ^{32}\text{P} \)-labeled pSml-7 insert were hybridized with various con-
probes:

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<th>Quantity of DNA (ng)</th>
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Fig. 1. Autoradiogram of different quantities of total *S. mansoni* DNA and pSm1-7 recombinant plasmid DNA dotted on NCP (Fig. 1). Similar hybridization signals were obtained in both cases with DNA quantities ranging between 100 pg and 1 ng. This similarity is further demonstrated in Fig. 2 which presents the results of densitometric analysis for determining the relation between DNA quantity and intensity of the hybridization signal. Given the similarity of the hybridization signals as seen in the lower section of the curve (DNA quantities ranging between 100 pg and 1 ng) and since the schistosomal insert DNA constitutes only about 1/6 of total pSm1-7 DNA it can be estimated that the insert represents about 17% of the total schistosomal genome. In a reciprocal experiment total 32P-labeled *S. mansoni* DNA was used as a probe in a quantitative dot hybridization with total *S. mansoni* DNA and pSm1-7 (Fig. 1). The signals obtained with 5 ng and 10 ng pSm1-7 were roughly similar by visual examination to 0.1 ng and 0.25 ng total *S. mansoni* DNA, respectively (Fig. 1). The visual similarity corresponds to at least 80% similarity by densitometric analysis (not shown). Calculation of the ratio of signal strength (by weight of peak area on paper) to DNA quantity gave average values of 23.4, 8.0 ng total *S. mansoni* DNA and 4.6 for 5 ng and 10 ng pSm1-7. The value for pSm1-7 is thus 1.96% of the total DNA. However, considering that the schistosomal 0.64-kb sequence constitutes about 1/6 of pSm1-7, it can be estimated that this pSm1-7 insert constitutes about 12% of the schistosomal genome.

DNA from *S. mansoni* males and females and from mixed sexes was partially digested with *Sau3A*, and underwent electrophoresis and Southern blot analysis [12] with pSm1-7 schistosomal DNA insert. A ladder of bands was observed (Fig. 3), each band varying in length by about 120 bp. This pattern indicates that the schistosomal DNA contains a tandemly repeated sequence about 120 bp long, and that this sequence is present in pSm1-7. This tandem repeat is present in both female and male *S. mansoni* adult worms. Partial sequence analysis of pSm1-7 insert revealed the presence of a dimer of a 120 bp sequence and a start of a third such repeat. This sequence is presented in Fig. 4. A complete *Sau3A* digestion of the 640-bp insert yielded fragments 240 bp, 120 bp and 80 bp long (Fig. 5). These fragments were isolated, labeled with 32P and used as probes at similar radioactivity/size ratios in dot blot hybridization against various quantities of total *S. mansoni* DNA. The pSm1-7 insert fragments exhibited similar detection sensitivities, namely between 100 and 250 pg (Fig. 5). These results indicate that all parts of the pSm1-7 insert are equally highly represented in the schistosomal genome.
Fig. 3. Southern blot analysis of *S. mansoni* DNA (M, adult males; F, females; F + M mixed sexes) digested with *Sau3A* and probed with 32P-labeled *pSm1*-7 insert. The position of molecular weight markers is indicated.

Fig. 4. *S. mansoni* 120-bp repeat sequence.

Upon dot-blot hybridization (results not shown) the 120 bp fragment hybridized with both the 240-bp and the 80-bp fragments, suggesting that the 640-bp insert in *pSm1*-7 is composed entirely of sequences closely homologous to the 120-bp repeat units. The intensity of staining of individual bands (Fig. 5) suggest that the 640-bp insert is composed of one 240-bp fragment, two 120-bp fragments and one 80-bp fragment. The 80-bp unaccounted for may be the result of generation of very small fragments. Minor changes in nucleotide sequence may be present in the distal, yet unsequenced, part of the 640-bp sequence to create additional *Sau3A* restriction sites that may account for the generation of very small fragments.

**Sensitivity and specificity of the pSm1-7 insert for detecting schistosomal DNA sequences.** Analysis by quantitative dot hybridization demonstrated
SNAIL DNA

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Fig. 7. Detection of S. mansoni DNA sequences in DNA from infected snails (I) and from normal controls (N) by dot blot hybridization with 32P-labeled pSml-7 insert.

that 32P-labeled pSml-7 insert can detect as little as 100 pg schistosomal DNA (Fig. 1). The pSml-7 insert was then used as a probe to detect schistosomal DNA sequences in infected snails by dot hybridization (Fig. 7). This probe revealed schistosomal sequences in as little as 1 ng DNA from infected snails, while as much as 50 μg of normal snail DNA gave negative results.

Clone pSml-7 was originally selected for species specificity by colony hybridization with total 32P-labeled DNA from S. mansoni, S. haematobium, and S. magrebowiei. Quantitative dot hybridization was subsequently carried out. S. haematobium and S. magrebowiei DNA (1-1000 ng), and S. mansoni (1-5 ng) were dotted on NCF probe with 32P-labeled pSml-7 insert (Fig. 8). S. haematobium DNA at 500 ng and 1000 ng gave positive signals, but none was detected with any of S. magrebowiei DNA quantities tested. Judging from the maximal sensitivity (100 pg) of S. mansoni DNA detection by pSml-7 insert (Fig. 1) it appears that the S. mansoni specificity of the pSml-7 insert is >1000 vis à vis S. haematobium, and >>1000 vis à vis S. magrebowiei. Since only two heterologous schistosome species were available for the test, the evidence for the species specificity of the pSml-7 insert is considered partial.

Discussion

We have cloned and characterized a short, tandemly repeated DNA sequence which is highly represented in the genomes of both male and female S. mansoni. Repeated DNA sequences have been used by other investigators as probes for detecting parasites and for identifying their taxon [15,17,18,19]. Small, highly repeated sequences can be considered highly suitable probes for such purposes. Firstly, because their high representation obviates a high detection sensitivity, and secondly, because such sequences are most likely non-coding and therefore evolve more rapidly than the rest of the genome. Species identification of schistosomes has so far been accomplished by employing ribosomal gene probes in Southern blot analysis to demonstrate species specific restriction fragments [5]. However, such a test cannot be considered suitable for multiple assays where simplicity and low cost are crucial. Also since the ribosomal gene is represented only in about 100 copies/haploid genome [6], the detection sensitivity of the rDNA probe is relatively low.

A DNA probe from S. mansoni which represents a tandemly repeated 0.4-kb degenerative sequence, present approximately at 75 copies per adult female genome [7] was recently described.
This probe allows determination of sex in larval parasites. It is specie-specific vis à vis a number of schistosoma species tested allowing species identification by simple dot hybridization, but the degree of specificity has not been addressed. Clone pSm1-7 described here contains a 0.64-kb schistosomal sequence which constitutes at least 12% of the total S. mansoni genome. Further analysis with a DNA probe of known size and copy number is required in order to exactly determine the abundance of the cloned pSm1-7 repeat sequence within the schistosomal genome. However, the results so far (Fig. 1) clearly indicate its high representation and correspondingly high detection sensitivity. A repetitive sequence of such a high representation has not been yet described in the schistosomal genome. Whether this may be ascribed to strain differences remains to be examined. Analysis of the species specificity of pSm1-7 is yet only partial, but the degree of specificity detected with S. mansoni is at least 3 orders of magnitude higher than with S. haematobium and S. magribowiti. Elucidation of the full differentiation potential of this probe requires quantitative dot blot analysis with a wide spectrum of schistosome species and strains. The cross-reactivity demonstrated with S. haematobium (Fig. 8) suggests that a similar repeat is present in S. haematobium. It is therefore expected that the pSm1-7 probe should be suitable for identifying partially cross-hybridizing sequences from the heterologous schistosoma species (under suitable hybridization conditions). From a practical point of view the differential identification of S. haematobium from other schistosomes developing in Bulinus should provide more accurate snail infection rate data, and may also enable differentiation between species of terminal-spined schistosomes developing in the vertebrate. Comparative sequence analysis may be helpful for elucidating evolutionary relations between schistosome species and strains, and for constructing highly specific oligonucleotide probes as has been demonstrated for filariae [19].

The pSm1-7 probe could detect schistosomal DNA in 1 ng infected snail DNA (Fig. 7) confirming the high detection sensitivity and indicating that the repeat is highly represented in DNA from S. mansoni intramolluscan larvae. The high detection sensitivity should allow rapid detection of individual infected snails without prior purification of DNA from the snails. A procedure for this purpose was previously described by us [20].

Acknowledgements

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We are grateful for the advice of Dr. Silvia LeBlancq. The assistance of Mr. Miguel Weil in maintaining the S. mansoni life cycle is grateful acknowledged.

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