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Immunological and molecular interaction between *Schistosoma mansoni* and its intermediate host

ABSTRACT

Interactions between snails and schistosomes are complex and there is a need to elucidate pathways involved in snail-parasite interactions and identify those factors involved in the intricate balance between the snail internal defense system and trematod infectivity mechanisms that determine the success or failure of an infection. The present work was evaluated to detect *Schistosoma mansoni* infection in the main host using antigens extracted from an intermediate snail host by Enzyme Linked Immunosorbent Assay (ELISA). Furthermore, the study was extended to detect the infection in the snail's tissue at prepatency and patency stages using polymerase chain reaction. The immunological results illustrated higher IgG reactivities with both snails' tissue homogenate and hemolymph of infected snails reflecting the presence of common antigens between the parasite and its snail intermediate host. PCR results showed 100% sensitivity and specificity of such technique to detect infection at early and late infection due to the presence of specific 338bp in all infected gDNA samples. The impact of such results on monitoring the transmission of *S. mansoni* infection in Egypt or any endemic area is discussed.

Keywords: PCR, ELIZA, *Schistosoma mansoni,* Biomphalaria snails

Abbreviations:
B. alexandrina: Biomphalaria alexandrina; B. glabrata: Biomphalaria glabrata; B. truncatus: Bulinus truncatus: CTAB: Hexadecyltrimethyl-ammonium bromide; ELISA: Enzyme linked Immunosorbent Assay; FCS: Fetal Calf Sera, gDNA: Genomic DNA; IgG: Immunoglobulin type G; IMP: Infected mice plasma: PCR: Polymerase chain reaction*; S. haematobium: Schistosoma heamatobium*; *S. mansoni: Schistosoma mansoni; S. japonicum: Schistosoma japonicum*; *T. ocellata: Trichobilharzia ocellata;* UIMP: Un-infected mice plasma.

INTRODUCTION

Common antigens between different species of *Schistosoma* and their intermediate hosts have been reported {1, 2, 3, 4}. Cross-reactive antigens may probably result from the adaptation of parasites to their invertebrate and vertebrate hosts. The parasite acquires snail molecules on its surface that would cover critical antigens, allowing its survival during skin penetration and the evasion from the protective immune mechanisms of definitive host during the first events of invasion. Furthermore, these molecules could be used in the diagnosis of schistosomiasis {5}. Diverse family of fibrinogen related proteins (FREPs) containing immunoglobulin-like domains has been discovered in *B. glabrata* and may play a role in snail defense {6}.

Bahgat et al., {7} recognized the tegument of developing cercariae from infected snails (*Biomphalaria*, *Bulinus* and *Lymnaea*) using infected sera. They also detected cross-reactivity of anti-sera extended to *T. ocellata*-infected *Lymnaea*, but not to *S. japonicum*-infected *Oncomelania*. Two carbohydrate epitopes were identified by monoclonal antibodies in hemolymph of infected snail vector and characterized with respect to their immunoreactivity, monosaccharide structure, and location {8}. In addition, the presence of antigenic carbohydrate epitopes shared by *B. glabrata* as well as by the sporocysts and miracidia of *S. mansoni* were reported {4}.

Specific immune response and protective effects against *S. mansoni* infection could be stimulated using hemolymph obtained from pre-treated *B. alexandrina* snails with some uracil compounds {9} and pyrimidene derivative {10}.The detection of *Biomphalaria* snails infected with *S. mansoni* is usually performed by cercariae shedding induced by artificial light exposure or by squeezing snails between two glass slides. However, these methods are not able to detect the parasite either in dead snails or in the pre-patent period. Thus, molecular methods are used to detect *S. mansoni* infection from pre-patent and patent periods {11, 12, 13, 14} or from traces of organic material from shells of the snails that are maintained in laboratory for ten years. Levels of prepatent *S. haematobium* infection were monitored in intermediate host snails (*B. truncatus*) using PCR {15}.

Herein, we used infected hemolymph and snail homogenate as antigens to detect the acute *Schistosoma mansoni* infection in mice using ELISA. Furthermore, the study was extended to evaluate PCR technique for detecting the infection in snail's tissues at both prepatency and patency stages to compare the sensitivity of the two different diagnostic tools in schistosomiasis diagnosis.

MATERIALS AND METHODS

I-*Serological analysis*

Collection of IMP to detect IgG reactivity against snail hemolymph and homogenate

IMPs were chosen from individuals at acute phase (27 days post infection) that were examined by their reactivity against different antigens to detect IgG level.

Collection of snails' hemolymph and preparation of snails' tissue homogenates:

After four weeks post exposure of individual snails to infection, hemolymph was collected from the snails by the head foot retraction method {16} and frozen at -80°C until use. Shells were broken between 2 glass slides, all debris was carefully removed, and tissues were washed and homogenized in 1 ml 0.01 M PBS. The homogenate was frozen and thawed 3 successive times, each for 5 min. then centrifuged at 16000 rpm for 5 min. The supernatant was collected, divided into aliquots, and frozen at -80°C until use.

Detection of IgG levels in plasma from infected mice against snails' hemolymph and tissue homogenates

ELISA plates were coated for 3 h at 37°C with 50µl/well of the appropriately diluted infected snails' hemolymph and tissue homogenates (1/1000) in coating buffer. Plates were washed 3 times with PBS-0.05% T20 and blocked against non-specific binding with 100µl/well of PBS-0.05% T-5% fetal calf sera (FCS) for 3 h at 37°C. After three washes, serially diluted individual plasma (1/250, 1/500, 1/1000) in the blocking buffer, 50 µl/well was applied and plates were incubated at 37ºC for 2 hours. After washing, diluted peroxidase labeled anti-mouse IgG (1/500) in PBS-0.05% T-5% FCS was applied (50 µl/well) and plates were incubated for 2 hours at 37ºC followed by 3 washes. For visualizing the antigen antibody binding, plates were dried and 50µl/well peroxidase specific colorimetric substrate O-phenylenediamine dihydrochloride (Sigma, St. Louis, Mo, USA) appropriately diluted in the substrate buffer in the presence of H_2O_2 was applied. IgG level was measured in ELISA reader at Optical density 490 nm {17}.

II- *Molecular analysis:*

gDNA extraction

DNA was extracted from the tissue of infected *B. alexandrina* snails using lysis buffer containing 2% hexadecyltrimethyl-ammonium bromide (CTAB) {18}.

Bioinformatics analysis of primers

The used forward (ACAGTGCGCGCGTCGTAAGC) and reverse (GAGATCAAGTGTGACAGTTTTGC) primers were previously reported to specifically amplify *S. mansoni* gDNA {19}. The sequences of both primers were compared by multiple alignments against the online available sequences in the GenBank (http://www.ncbi.nlm.nih.gov/) using the basic nucleotide BLAST analysis.

Amplification of gDNA

PCR reactions were carried out in a final volume of 50 µl. The amplification program included an initial denaturation at 94^{o}C for 5 min. followed by 35 cycles; each consisted of denaturation at 94^{o}C for 45 sec, annealing at 58^{o}C for 45 sec and extension at 72^{o}C for 60 sec. The program included a final extension step at 72^{o}C for 10 min. PCR products were resolved by electrophoresis on 1.5% agarose gel containing ethidium bromide {20} parallel to 100 bp DNA ladder (Promega, Germany). The amplified *S. mansoni* fragment was visualized using a UV-transilluminator and photographed using a gel documentation system.

RESULTS

Cross-reactivity of antigens prepared from snails with plasma and from infected mice:

Serial dilutions of IMP (1/250, 1/500 and 1/1000) showed higher IgG reactivities with both tissue homogenate and hemolymph of infected snails. Similar cross reaction was recorded by the same plasma at the same dilutions with tissue homogenate and hemolymph of clean snails reflecting the presence of common antigens between the parasite and its snail intermediate host (Figure 1). This was confirmed by the lower IgG reactivities of the UIMP with both snails' tissue homogenate and hemolymph (Figure 1) than the recorded reactivities in case of IMP. A representative Figure (2) at dilution (1/250) was used to illustrate the above mentioned results.

Molecular analysis:

Using the basic nucleotide blast application, results showed 100% homology to the highlighted termini of the used primers suggesting their exact annealing positions on the parasite template that yields amplification products of molecular size 338bp. This confirmed the specificity of the used primers to *S. mansoni* as they did not show any extent of homology towards other existing human parasites in Egypt (Fig. 3).

PCR amplification of the *S. mansoni* sequence from *gDNA* extracted from infected *B. alexandrina* homogenate:

Electrophoresis of amplification products from gDNA of exposed infected *B. alexandrina* homogenate at 18th (lanes 3, 4, 5, 6, 7 & 8) and shedding snails at 30th (lanes 9, 10, 11, 12, 13 & 14) days post infection were subjected to electrophoresis on 1.5% agarose gel containing ethidium bromide parallel to 100 bp DNA ladder (lanes 1 & 17) products from a negative control (lane 16) and control of non-infected snails (lanes 2 &15). All gDNA preparations from both exposed and cercariae-shedders *B. alexandrina* snails were uniformly PCR positive for the 338 bp fragment that were not evidenced in both control and negative control lanes (Fig. 4).

DISCUSSION

The sharing of molecules that is able to elicit immune responses between parasites and their vectors and among different species of parasites from various genera, families, or phyla, is known as antigenic community, which is responsible for antigenic cross-reactivity {8}. The advantage of antigenic community is that drugs or vaccines can be simultaneously effective against different species or genera of parasites. However, the non-specific reactions are a disadvantage for immunodiagnostic tests {21}.

In the present study, high extents of IgG cross-reactivity were recorded between plasma from *S. mansoni* infected mice with homogenates and hemolymph of clean snails. On the other hand, a weak reactivity was seen by using UIMP.

These observations reflected the presence of common antigens between the parasite and its snail intermediate host. This experiment was primarily conducted to detect parasite antigens within the snail tissues using sera from infected mice as a source of anti-parasite antibodies but it failed due to a high degree of cross-reaction between the parasite and its intermediate host. This produced clear evidence that snail tissue cannot be a source of diagnostic antigens against infection .

On the contrary, protective value of extracted nucleoproteins from snail tissues against *S. mansoni* challenge infection has been reported {22}. Previous studies {1, 2, 3} reported presence of common antigens between different species of *Schistosoma* and their intermediate hosts. Schmitt et al., {8} identified *S. mansoni* and *S. haematobium* carbohydrate epitopes in haemolymph of infected vector snails using specific monoclonal antibodies. Common carbohydrate determinants between *B. glabrata* as well as *S. mansoni* sporocysts and miracidia were identified {4, 23}.

Another major issue concerning the study of schistosomiasis intermediate hosts is the detection of infected snails. The routinely diagnostic methods are ruled out when sporocysts are undergoing early stages in the cephalopodal region, or when field collected snails are infected with early stages of different trematode species. Added to this is the fact that field collected snails may reach the laboratory dead. Thus, molecular-based techniques should be used for further diagnosis of infected mollusks {24, 25}. Furthermore, the application to the detection of *S. mansoni* is rare, despite a recommendation of the World Health Organization that a major focus of research on schistosomiasis should be on the development and evaluation of new strategies and tools for the control of the disease {26}. In this context, few studies have been published for the detection of the parasite in snails {11, 12, 13, 14} or monitoring of cercariae in water bodies {27}.

Herein, using specific *S. mansoni* primers, we could detect the infection in the intermediate host homogenate with 100% sensitivity at both acute and late stages post infection. Our results agree with the previous reports that were adopted for detecting *S. mansoni* DNA in *Biomphalaria* species very early after infection {12, 13}, *S. haematobium* in *B. truncatus* and *S. mansoni* cercariae in water {25}. Furthermore, Multiplex-PCR was capable of providing specific identification of *Biomphalaria* snails and diagnosis of infection with *S. mansoni* simultaneously {28}. Real time PCR was also evaluated to monitor the potential of infection of transmission sites by estimating the intensity of infestation of water bodies and intensity of infection in *Biomphalaria* snails {29}.

CONCLUSION

From our recorded data, we could conclude that there is a homology between the antigens derived from the snail intermediate host and the parasite that makes immunodiagnosis against these antigens difficult due to high cross-reactivity. Furthermore, we believe that molecular-based methodologies may be very useful for detecting *S. mansoni* infection in both early and late stages post infection in the intermediate snail host *B. alexandrina* that enhances biological control of the parasite.

Fig.1. Serial dilutions of IMP (1/250, 1/500 and 1/1000) showed higher IgG reactivities with both snails' tissue homogenate and hemolymph of infected *B. alexandrina* snails.

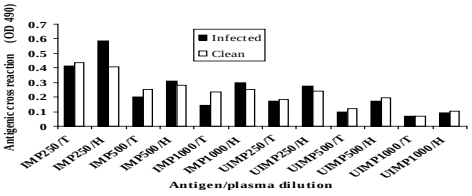


Fig.2. Representative figure was used to illustrate higher IgG reactivities with both snails' tissue homogenate and hemolymph of infected snails reflecting the presence of common antigens between the parasite and its snail intermediate host at dilution (1/250).

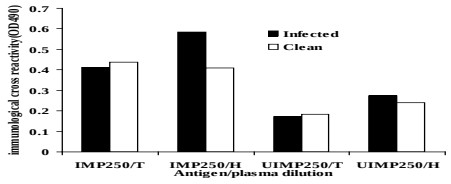
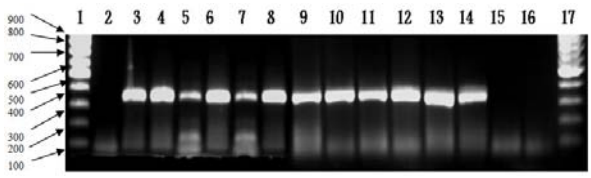


Fig.3. Deduce target *S. mansoni* sequence for the PCR amplification using the previously published primers. Sequences of both the forward and reverse primers were compared with the published *Schistosome* sequences on the GenBank (http://www.ncbi.nlm.nih.gov) using the basic nucleotide blast application. Results showed 100% homology to the highlighted termini of the below mentioned previously *S. mansoni* sequence suggesting their exact annealing positions on the parasite template.

GAGATCAAGTGTGACAGTTTTGCTCTGAGCTACCCTTGGAGTCGGGTTG
TTTGTGAATGCAGCCCAAAGTGGGTGGTAAACTCCATCAGGCTAAATAC
TTACACGAGTCCGATAGCAAACAAGTACCGGGAAAGTTGAAAAGTACTT
TGAAGAGAGAGTAAACAGTGCGTGAACCGCTAAAGGTAAACGGGTGGA
GTTGAACTGCAAGCTCTGGGAATTCAGCTGATGAGTGTGATTTGTACTT
GGGCATACTGGCCGCCTTCAGTGTCTGTTTAACCGCAGGTGCCTTCCTTT
TTGGTGGGTATGTGTGAATCGTTTGCTTACGACGCGCGCACTGT

Fig.4. Electrophoresis of amplification products from gDNA of exposed infected *B. alexandrina* homogenate at 18th (lanes 3, 4, 5, 6, 7 & 8) and shedding snails at 30th(lanes 9, 10, 11, 12, 13 & 14) days post infection were subjected to electrophoresis on 1.5% agarose gel containing ethidium bromide parallel to 100 bp DNA ladder (lanes 1 & 17), and products from a negative control (lane 16) and control of non-infected snails (lanes 2 &15) where 338 bp specific amplification products were visualized in all samples that were not evidenced in both control and negative control lanes.



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