Comparative Immunomic Analysis of Schistosoma japonicum Soluble Egg Antigens Reacting with Patient Sera Before and after Praziquantel Treatment

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Abstract: Schistosomiasis ranks as the second most serious parasitic disease worldwide after malaria. More than 250 million people are infected with schistosomes in the tropics or subtropics. The treatment and control of schistosomiasis which is a major neglected tropical parasitic disease, depends almost exclusively on chemotherapy with Praziquantel (PZQ). Current serologic diagnostic assays have shown that schistosome specific antibodies in human serum may remain for at least 1 year after cure. Repeated administration of PZQ for a long time might induce drug resistance to the parasite which is a big challenge for strategizing for the prevention and control of schistosomiasis. As schistosome eggs represent the most pathogenic form causing the disease, it is essential to determine if and how the level of antibodies against schistosome Soluble Egg Antigens (SEA) is affected by PZQ treatment. In this study, researchers carried out an immunomic analysis to profile Schistosoma japonicum SEA reacting with pooled human serum samples of pre and post treatment with PZQ by two dimensional electrophoresis combined with Western blotting. A total of 67 protein spots that were serologically recognized by serum samples were successfully subjected to mass spectrometric analysis. Of them, 37 different characterized proteins were successfully identified. Furthermore, of 67 protein spots, the reactivity of 49 protein spots to sera was reduced 20 weeks after PZQ treatment whereas only 5 spots showed increases in the intensity of recognition by post treatment sera. The present study suggested that chemotherapy with PZQ mainly affects the intensity of serological recognition of S. japonicum SEA. The immunomic proteins that were identified may facilitate a better understanding of the egg induced pathogenesis of schistosomiasis and host-parasite interplay and may provide potential targets for the diagnosis and evaluation of treatment for the disease as well.

Key words: Schistosoma japonicum, immunomic analysis, soluble egg antigens, praziquantel, protein, China

INTRODUCTION

Schistosomiasis remains a major neglected tropical parasitic disease affecting >200 million people in 76 countries. About 120 million have symptoms and 20 million suffer severe illness (Chitsulo et al., 2000, Hotez et al., 2008). Currently due to the absence of vaccines, efficient vector controls and water sanitation, the treatment of schistosomiasis still depends almost exclusively on Praziquantel (PZQ) although several recent drug candidates have exhibited potential anti-schistosomal properties in the laboratory such as oxadiazoles (Sayed et al., 2008), cysteine protease inhibitor K11777 (Abdulla et al., 2007) and mefloquine (Keiser et al., 2009). PZQ was first synthesized in the 1970s (Seubert et al., 1977) and subsequently introduced for the treatment of schistosomiasis. Owing to its safety, broad anthelmintic spectrum and reasonable cost, >100 million people have been treated with PZQ in schistosomiasis endemic areas (Cioli, 2000). Because PZQ targets the adult worm but has only minor activity against the young developing stages (i.e., schistosomula)

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(Xiao et al., 1985, 1987), retreatment is recommended to kill
the parasites (Renganathan and Cioli, 1998). However, its
mechanism of action has yet to be fully elucidated
(Greenberg, 2003).

Diagnosis as well as chemotherapy is central to the
control of schistosomiasis. Although, stool examination
is the gold standard for diagnosing or monitoring the
response to treatment in schistosomiasis cases, it is labor
intensive and time-consuming. In addition, the sensitivity
of this method is poor in the endemic areas with low
prevalence. Immunodiagnostic techniques are highly
sensitive, easy to perform and are useful epidemiological
tools for the screening of target populations in endemic
areas. However, most immunodiagnostic assays currently
applied in China have indicated that the anti-schistosome
antibody level has remained high for at least 1 year after
cure and in many cases much longer (Zhu, 2005) which
leads to the problem that a positive sero-test may be
found to be fecal egg negative. Repeated administration
of PZQ for a long time might induce drug resistance to the
parasite (Doenhoff et al., 2002; Doenhoff and Mattei,
2006) which is a big challenge for strategizing for the
prevention and control of schistosomiasis. Therefore, it is
critical to identify the immunogenic antigens that could
distinguish the cured cases from those with an ongoing
infection.

Schistosome eggs are the primary cause of
pathogenesis in schistosomiasis and egg antigens play a
critical role in regulating host immune responses to the
helminth (Pearce and MacDonald, 2002). In addition, there
is evidence that treatment with PZQ could lead to changes
in the Soluble Egg Antigen (SEA)-induced immune
responses (Martins-Leite et al., 2008) and variations in
specific anti-egg antibody levels which could reflect the
current antigen levels (Mutapi et al., 1998). Also, the
average number of eggs produced by Schistosoma
japonicum per day is much more than other schistosome
species (Moore and Sandground, 1956). Hence,
knowledge of the effects of PZQ on the level of antibodies
against S. japonicum SEAs is essential for evaluating
chemotherapy efficacy which may also be a useful
complementary way to carry out surveillance of
schistosomiasis in epidemic areas (Wang et al., 2008).
Furthermore, such knowledge is able to facilitate an
understanding of egg induced pathology during
schistosomiasis.

Crude antigens are valuable for detecting general
patterns of immune responses in infected populations.
However, single antigens permit a better definition of
humoral and cellular responses under investigation
(Mutapi, 2001). The advent of the omics era has yielded
an avalanche of helminth omics data that provide valuable
resources for defining those immunogenic proteins and
investigating the intriguing biology of helminth parasites
(Ju et al., 2010). A traditional expression library screening
method has its limitations in the detection of those
proteins with post translation modifications but a
combination of 2-Dimensional Electrophoresis (2DE) and
Western Blotting (2D-WB) can circumvent this
problem and cover a wider range of antigenic targets
(Wilson et al., 2004). A number of S. haematobium worm
antigenic proteins recognized by patient serum samples
before and after curative PZQ treatment were identified by
2DE, Western blotting and Mass Spectrometry (MS)
(Mutapi et al., 2005). The combination of proteomic and
serological techniques was subsequently used to analyze
the components of immunogenic proteins from the
tegument and excretory-secretory products of adult
S. bovis (Perez-Sanchez et al., 2006). More recently,
several proteomic investigations have addressed
schistosomal egg-associated proteins including SEA
(Curwen et al., 2004), Egg Secreted Proteins (ESP)
(Cass et al., 2007) and the soluble constituents in
developed and undeveloped eggs (Mathieson and
Wilson, 2010). In addition, the identification of
schistosome specific proteins will also profit from
comprehensive genome analysis of the blood
flukes S. japonicum (Liu et al., 2009b) and S. mansoni
(Berriman et al., 2009) such as an investigation of vaccine
candidate immunomics screening with S. mansoni protein
microarrays (Driuguez et al., 2010). To date, there have
been no reports determining the effect of treatment with
PZQ on S. japonicum egg immunogenic antigen profiles,
however such investigations would be valuable for a
better understanding of the egg induced pathogenesis of
schistosomiasis and host parasite interplays, provide
potential targets for diagnosis and evaluation of treatment
for the disease as well.

To identify and characterize the immunogenic proteins of S. japonicum, researchers combined 2DE and
Western blot analysis to determine the proteins of SEA
that were recognized by pooled serum samples of patients
with S. japonicum before and after PZQ treatment and
researchers compared the difference in serological
recognition between them.

MATERIALS AND METHODS

Ethics statement: Ethical clearance was granted by the
Ethics Committee of the National Institute of Parasitic
Diseases, Chinese Center for Disease Control and
Prevention in Shanghai, P.R. China (Ref No.: 20100525-1).
The objectives, procedures and potential risks were orally
explained to all participants. A signed written consent
form was also obtained from each participant or their proxy. All experimental uses of animals for the studies described in this study were also approved by the Institutional Animal Care and Use Committee.

**Serum samples:** Human serum samples were obtained from the residents living in Miaoling village in Anhui province of China where *S. japonicum* is endemic. Diagnoses were confirmed by the improved Kato-Katz stool examination (Katz et al., 1972) in which two fresh stool samples collected from each subject on two consecutive days were used to prepare six slides, coupled with a hatching test (Kassim and Gibertson, 1976). Following the initial examinations, all infected residents who had never been treated for any helminth infection agreed to receive chemotherapy with PZQ at the standard dose of 40 mg kg⁻¹ body weight. Serumological samples were collected from the residents that were confirmed as egg negative 20 weeks after treatment. Of these, 10 residents that donated both pre and post treatment sera were selected for the study. The sex ratio (males vs. females) of these compliant participants (19-54 years old) was approximately 1.5. Sera from persons free of *S. japonicum* from non endemic areas were included as normal controls. Almost no cross reaction with *Clonorchis sinensis* and *Fasciola buski* were found in all the studied serum samples. The treatment was performed in the schistosomiasis non-transmission season to avoid re-infection (Table 1).

**Preparation of soluble egg antigens:** The Anhui strain of *S. japonicum*, maintained in *Oncomelania hupensis* snails at the National Institute of Parasitic Diseases (Shanghai, China) was used for the experiments. Schistosome eggs were collected from the livers of New Zealand white rabbits that had each been percutaneously exposed to 800-1000 *S. japonicum* cercariae 42-45 days earlier using a method to isolate schistosome eggs free of host cells and debris (Dalton et al., 1997). Eggs were ground in a mortar and then sonicated (Boros and Warren, 1970) 8 times for 5 sec in lysis buffer (9.5 M urea, 65 mM DTT, 4% CHAPS and 0.2% IPG buffer) containing protease inhibitors, followed by centrifugation for 45 min at 12,000 g and 4°C. The SEA were produced from the supernatant and protein concentrations were determined using the Bradford assay. The samples were then stored at -80°C until needed.

**2-Dimensional Electrophoresis (2-DE):** 2-dimensional separation gels with SEA were performed in parallel, 100 and 800 µg SEA were loaded onto analytical and preparative gels, respectively. Isoelectric Focusing (IEF) was performed in 13 cm IPG strips (Amersham) with a range of pH 3-10. The IEF was accomplished using the Ettan IPGphor Isoelectric Focusing System (Amersham). The soluble antigens were mixed with 250 µL rehydration solution containing 8 M urea, 2% CHAPS, 0.5% IPG buffer and 18 mM DTT. After passive rehydration for 12 h, IEF was performed at a step voltage of 500 V for 1 h, 1000 V for 1 h and 8000 V for 5 h. Following IEF separation, the gel strips were equilibrated for 2-15 min in equilibration buffer containing 50 mM Tris-HCl, pH 8.8, 6 M urea, 2% SDS and 30% glycerol. About 1% DTT was added to the first equilibration buffer and in the second equilibration buffer, DTT was replaced with 4% iodoacetamide. The 2D-dimensional electrophoresis was performed on 12.5% SDS-polyacrylamide gels with the SE 500 System (Amersham) at 30 mA per gel for 40 min and then at 60 mA until the bromophenol blue reached the end of the gel. Each sample was repeated three times. The analytical gels were stained with silver as previously described (Hochstrasser et al., 1988a, b) and the preparative gels were stained with coomassie blue.

**Immunoblotting:** Proteins from the same two gels were electrotransferred synchronously to two hybrid-C nitrocellulose membranes (Amersham Biosciences) using the Trans Blot cell (Bio-Rad) for 90 min at 0.22 A. Following the transfer, the membranes were stained with ponceau S solution (Ameresco) to check the transfer efficiency and then were incubated in blocking solution (1% BSA in 1 × PBS) overnight at room temperature. The two membranes were subjected to 3 separate 15 min washes with 0.05%-PBST after blocking. Then, one membrane was incubated at room temperature for 2 h in a pool of three PBST (3 × 15 mm). HRF conjugated goat anti-human IgG (1:2000 in 0.05% PBST, Sigma, USA) was

| Table 1: The information of sera samples in Miaoling village |
|-----------------|-----|-----|-----|
| ID              | Sex | Ages| Pre-Treatment | Post-Treatment |
| 400801          | Male| 19  | 4             | 0              |
| 806402          | Female| 35  | 16            | 0              |
| 702401          | Male| 28  | 96            | 0              |
| 703501          | Male| 47  | 8             | 0              |
| 704001          | Male| 54  | 12            | 0              |
| 705001          | Female| 33  | 28            | 0              |
| 702501          | Female| 21  | 80            | 0              |
| 703405          | Male| 36  | 8             | 0              |
| 703401          | Male| 44  | 16            | 0              |
| 702701          | Female| 29  | 4             | 0              |
added. The membrane was incubated at room temperature for 1 h and then washed 3 times in 0.05% PBST. The membrane was developed using H₂O₂ and 3,3'-Diaminobenzidine (DAB) in PBS. A pool of ten samples of normal human sera served as a control and was treated in the same way. The gel electrophoresis and Western blotting were repeated twice for all samples for confirmation.

**Image analysis and mass spectrometry:** Images from the immunoblot patterns were obtained by scanning with a Bio-Rad GS710 scanner and image analysis was accomplished using Image Master Software (Amersham) to match the reacted spots. Predicted matches were also visually verified. Protein spots on the coomassie blue stained gel that matched those on the Western blots were excised from the preparative gels and then destained with 100 mM NH₄HCO₃ in 30% ACN. After removing the destaining buffer, the gels pieces were lyophilized and rehydrated in 30 μL of 50 mM NH₄HCO₃ containing 50 ng trypsin (sequencing grade; Promega, Madison, WI, USA). After overnight digestion at 37°C, the peptides were extracted three times with 0.1% TFA in 60% ACN. Extracts were pooled together and lyophilized. The resulting lyophilized tryptic peptides were kept at -80°C until MS analysis. A protein-free gel piece was treated as above and used as a control to identify autolysis products derived from trypsin.

MS and MS/MS spectra were obtained using the ABI 4800 Proteomics Analyzer MALDI-TOF/TOF (Applied Biosystems, Foster city) operating in a result dependent acquisition mode. Peptide mass maps were acquired in a positive ion reflector mode (20 kV accelerating voltage) with 1000 laser shots per spectrum. Monoisotopic peak masses were automatically determined within the mass range 800-4000 Da with a signal to noise ratio minimum set to 10 and a local noise window width of 250 m/z. Up to five of the most intense ions with a minimum signal to noise ratio of 50 were selected as precursors for MS/MS acquisition, excluding common trypsin autolysis peaks and matrix ion signals. In the MS/MS positive ion mode, spectra were averaged, collision energy was 2 kV and the default calibration was set. Monoisotopic peak masses were automatically determined with a signal to noise ratio minimum set to 5 and a local noise window width of 250 m/z. The MS together with MS/MS spectra were searched against Schistosoma NCEI 2010-3-12 using the Software GPS Explorer 3.6 (Applied Biosystems) and MASCOT 2.1 (Matrix Science) with the following parameter settings: trypsin cleavage, one missed cleavage allowed, carbamidomethylation set as fixed modification, oxidation of methionine allowed as variable modification, peptide mass tolerance set to 100 ppm, fragment tolerance set to ±0.3 Da and the minimum ion score confidence interval for MS/MS data set to 95%.

**RESULTS AND DISCUSSION**

**2-DE:** In this study, researchers carried out 2-DE analysis of the proteins of SEAAs as described before. The total proteins from SEA were separated on 2-DE gels with a pH gradient from pH 3-10 (Fig. 1). The 2-DE gels were stained with coomassie blue and >300 discrete spots were displayed. Most of them were located between pH 4 and 8. Each sample was subjected to triplicate runs.

**Western blot analysis:** To investigate whether the components of *S. japonicum* SEA could be recognized by the pooled human serum samples collected before and after PZQ treatment, Western blot assays were performed on the basis of the 2D gels in the range pH 3-10. Compared with the hybridized membrane of normal human sera (Fig. 2), researchers observed that >80 strong

![Fig. 1: 2-dimensional image of SEAAs from *S. japonicum*. Coomassie blue stained gels with a range of pH 3-10. Molecular mass markers (kDa) are given on the left.](image1)

![Fig. 2: Normal control for *S. japonicum* SEA. Molecular mass markers (in kDa) are given on the left.](image2)
reactive spots displayed on the membranes hybridized with the pre treatment and post treatment human sera. There were differences between these two immunoblot patterns (Fig. 3A and B) while no significant immunoreactive spots were recognized by the normal human sera. Image analysis of 2D-WB showed that a total of 84 protein spots were recognized in the pre and post treatment sera.

**MS/MS analysis:** After matching the immunoblots with their homologous coomassie blue stained gels of SEA with Image Mater 2D gel software (Amersham), researchers localized the spots that corresponded to the antigenic proteins in the 2D gels. In total, 84 matched spots were excised from the coomassie blue stained gels, digested by trypsin and were further analyzed by LC/MS-MS (Fig. 4). The MS/MS raw data were used to search *S. japonicum* and *S. mansoni* protein public databases with the SEQUEST search program. Finally, 67 spots were successfully identified. The identified spots with their summary protein scores, isoelectric points (pI) and molecular mass (kDa) are shown in Table 2. MS/MS analysis suggested that some different spots were derived from the same proteins. For example, spots 48, 49, 50, 51, 56 and 57 all referred to Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) as these spots corresponded to the same proteins but with different NCBI accession numbers.

For the reason above, the 67 identified protein spots were grouped into 37 different proteins by molecular functions which included chaperone proteins, motor proteins, catabolic activity, binding proteins and others (Table 3). Furthermore, 1 unknown protein and 3 hypothetical proteins without functional annotation are also shown in Table 3. The raw data of peptide mass fingerprints and secondary MS are shown in supplementary 1 and 2. The peptide data (sequence, ion score and C. I. %) are listed in the supplementary 3.

**The effect of PZQ treatment on serological recognition:** Comparative immunomic analysis of *S. japonicum* SEA recognized by the patient sera of pre and post treatment with PZQ was performed to investigate how chemotherapy altered responses to the antigens. The results suggested that treatment with PZQ mainly affected the intensity of serological recognition of *S. japonicum* SEA. The reactivity of most protein spots to sera was reduced by chemotherapy with PZQ after 20 weeks (Table 2). A total of 49 spots showed reduced reactivity. The most apparent spots included spots 25-27 (ATP synthase alpha subunit mitochondrial and 2 hypothetical proteins), 34-37 (branched chain amino acid aminotransferase, lactate dehydrogenase A and heterogeneous nuclear ribonucleoprotein A2 homolog 1) and 44-58 (actin, glyceraldehyde-3-phosphate dehydrogenase, troponin T, major egg antigen P40, phosphatase 2A inhibitor I2PP2A, tubulin beta 2, ester hydrolase C11orf 54 homolog and an unknown protein). However, only five spots (1, 3, 17, 18 and 19) showed increases in the intensity of recognition after treatment. They were the dentin sialophosphoprotein precursor, KH type splicing regulatory protein and dihydropyrimidinase like 2.

![Image](image.png)

Fig. 3: Immunoblot patterns of *S. japonicum* SEAs recognized by serum samples. A: Spots reacting with pre treatment sera; B: Spots reacting with post treatment sera. Red circles represent the different areas between pre treatment and post treatment. Molecular mass markers (kDa) are given on the left.
Fig. 4: Coomassie blue stained 2-D gel of *S. japonicum* SEAs showing spots matched to immunoblots. Molecular mass markers (kDa) are given on the left.

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein name</th>
<th>Species</th>
<th>NCBI Accession No.</th>
<th>Protein score</th>
<th>PI</th>
<th>Daltons</th>
<th>Trend</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>Dentin sialophosphoprotein precursor</td>
<td><em>S. japonicum</em></td>
<td>gi</td>
<td>226485495</td>
<td>116</td>
<td>4.13</td>
<td>51762.9</td>
</tr>
<tr>
<td>2</td>
<td>Actin</td>
<td><em>S. mansoni</em></td>
<td>gi</td>
<td>256079407</td>
<td>95</td>
<td>5.30</td>
<td>41704.8</td>
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<tr>
<td>3</td>
<td>Dentin sialophosphoprotein precursor</td>
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<td>218</td>
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<td>4</td>
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<td>5.14</td>
<td>90445.1</td>
</tr>
<tr>
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<td>Myosin heavy chain</td>
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<td>83</td>
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<td>Rootletin (Ciliary rootlet coiled coil protein)</td>
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<td>gi</td>
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<td>71</td>
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During the complex life stages of schistosomes, the egg is the key agent responsible for both the transmission and pathogenesis of schistosomiasis. Immunopathology in schistosomiasis is due to granulomatous inflammation around parasite eggs in the host liver and intestines which may result in scarring, portal hypertension, haemorrhage and death. The treatment and control of schistosomiasis relies virtually on a single drug, PZQ. However, chemotherapy with PZQ causes a series of problems for serological diagnosis of schistosomiasis (Pearce and MacDonald, 2002; Mutapi et al., 1998; Martins-Leite et al., 2008). Characterization of the SEA immunome may provide clues for potential diagnosis markers and for broader understanding of the egg’s interaction with the mammalian host. In this study, a combination of 2-dimensional electrophoresis and Western blotting was employed to profile *S. japonicum* SEAs with pooled human serum of pre and post treatment by PZQ.

A total of 84 proteins spots recognized by human infection sera were subjected to MS analysis and 37 different characterized proteins were successfully identified, four of which have no functional annotation. Moreover of these, the reactivity of 49 protein spots (30 characterized proteins) to sera was reduced after 20 weeks by chemotherapy with PZQ whereas only five spots (3 different characterized proteins) showed increases in the intensity of recognition after treatment.

Of the 37 different immunogenic proteins identified, proteins related to catabolic activity were the most abundant, forming the largest functional category in SEA. This is probably because eggs are rich in carbohydrates and polysaccharide antigens and tend to stimulate an IgM response (Woolhouse and Hagan, 1999). Consequently, numerous enzymes might be involved in PZQ chemotherapy. For instance, one of the most abundant proteins with catabolic activity, glyceraldehyde-3-phosphate dehydrogenase (GAPDH; spots 48, 49, 50, 51,
56 and 57) which was considered as a putative vaccine candidate against *S. japonicum* showed a correlation of antibody titer with resistance to reinfection (Waine et al., 1993; Argiro et al., 2000). Its abundant expression across developmental stages of *S. mansoni* (Curwen et al., 2004) indicated that GAPDH is associated with the growth and development of schistosomes. Furthermore, MS/MS analysis identified a glycolytic and gluconeogenic enzyme i.e., enolase which is also found in excretory/secretory proteins of schistosome adult worms with high expression (Liu et al., 2009a). Many studies have shown that enolase can be found on the surface of several eukaryotes and prokaryotes including schistosomes in which it acts as a plasminogen binding receptor (Ramojo-Hernandez et al., 2007; Seweryn et al., 2007). It is well known that energy acquisition by schistosomes relies mainly on the glycolytic pathway and enolase is a key enzyme in this pathway. Enolase exhibited only poor protection against *S. japonicum* schistosomulum infection (Yang et al., 2010) but assessment of its potential as a diagnostic target has yet to be reported.

The chaperone group such as Heat Shock Proteins (HSP) was another abundantly expressed group of immunogenic proteins in *S. japonicum* SEA. Both HSP60 and HSP70 were identified in this investigation. Serological evaluation of HSP60 demonstrated that the sensitivity of this antigen was >90% with ELISA. HSP70 is considered the predominant HSP family and plays a key regulatory role in parasite development and pathogenesis. A DNA vaccine of SjGST combined with SjMLP/HSP70 could induce a 31.31% reduction of the worm burden and a 58.59% reduction of the egg burden in the intestinal tissue of immunized mice (He et al., 2010). Another typical immunogen chaperone protein is a major egg antigen, P-40. The pathogenesis of *S. mansoni* infection depends largely on host T-cell mediated immune responses such as the granulomatous response to tissue deposited eggs and subsequent fibrosis (Pearce and MacDonald, 2002). The major egg antigens have a valuable role in desensitizing the CD4+ Th cells that mediate granuloma formation which may prevent or ameliorate clinical signs of schistosomiasis (Abouel-Nour et al., 2006). There were significant differences after Smp40 stimulation in IL-5 and 10 levels compared with the control group. There is no information available on the changes in reactivity of this antigen following chemotherapy before this study. Among the recognized proteins, motor and binding proteins are the other two groups of immunogens. The former group might be involved in the formation and development of eggs. It is generally believed that Ca²⁺ channels are the molecular targets of PZQ action (Greenberg, 2005). Interestingly, the latter group comprises components of Ca²⁺ ion signaling pathways such as calreticulin and calcium binding protein. In addition, these immunogens also include some *S. japonicum* egg-specific proteins such as troponin T and their biological characterization needs to be further explored.

Researchers found that some relatively heavily stained gel spots failed to be recognized by serum samples which is consistent with the results for *S. haematobium* Soluble Worm Antigens (SWA) (Mutapi et al., 2005). Mature schistosomes have evolved highly effective mechanisms for evading the consequences of the cellular and humoral immune responses that they provoke (Pearce and Sher, 1987; Pearce and MacDonald, 2002). Perhaps these unrecognized SEA components also play a vital role in host immune evasion or modulation and hence deserve attention and further evaluation.

Comparative analysis on the serological recognition of SEA revealed that chemotherapy with PZQ suppressed the reactivity of most of SEA spots to human sera. On the
contrary, PZQ could increase the intensity of recognition for some SWA spots after treatment (Mutapi et al., 2005). As a single drug for the treatment of schistosomiasis, it is well established that PZQ exerts different actions on mature or immature adult worms and eggs. PZQ kills adult worms (Andrews, 1985) and mature eggs (Richards et al., 1989) but does not significantly affect immature worms (Sabah et al., 1986). PZQ can damage the tegument of adult worms and exposes more parasite antigens to the host immune system compared with natural infection (Mutapi, 2001). The changes in serological recognition of SEA might be directly ascribed to the reduction of egg burden because of PZQ-induced worm death. Moreover, although PZQ has no effect on S. japonicum eggs trapped in tissues, previous research showed that PZQ can restrain schistosomal ovum granulomas (Xu et al., 2007). Therefore, researchers presume that the decreases in serological recognition of SEA were probably related to the change of ovum granuloma after treatment. PZQ treatment resulted in marked changes in S. haematobium specific antibody responses directed against SEA which persisted from 18 weeks to at least 36 weeks following chemotherapy (Mutapi et al., 1998). This reminds us that extension of the sera samples collection time after chemotherapy may be able to reflect the dynamic changes of the immunogenic antigens researchers identified.

It is well known that antibodies of high affinity are more efficient than lower affinity antibodies in a wide range of biological reactions. Antibodies are traditionally considered to engage antigens in a mono-specific bivalent manner which enhances apparent affinity by decreasing the rate of dissociation from the ligand. Heterologous could improve antibody affinity when homotypic bivalent binding was not possible during the immune response to human immunodeficiency virus (Mouquet et al., 2010). Patients who developed AIDS either lost or failed to develop high affinity antibodies early in the infection. The antibody affinity values declined with time in some AIDS patients who remained asymptomatic (Chargelegue et al., 1995). Chemotherapy with PZQ caused changes in the amount of antigens directly into the bloodstream, thus stimulating different antigen presenting cells which resulted in different interactions between the antigens and the immune system when compared with natural infection (Mutapi, 2001). During this process, the alteration of SEA reactivity to sera after treatment may be associated with the change in the affinity of specific antibodies against SEA.

CONCLUSION

The present study suggested that chemotherapy with PZQ mainly affects the intensity of serological recognition of S. japonicum SEA. The immunomic proteins that were identified may facilitate a better understanding of the egg-induced pathogenesis of schistosomiasis and host-parasite interplay and may provide potential targets for the diagnosis and evaluation of treatment for the disease as well.

ACKNOWLEDGEMENTS

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REFERENCES


