Protective Role of Purified Somatic, Excretory and Secretory Antigens from Adult *Echinostoma liei* Against Infection of *Schistosoma mansoni*

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**Abstract:** The development of a vaccine against schistosomiasis and also the availability of a more sensitive diagnosis test are important tools to help chemotherapy in controlling disease transmission. In this study, developing a novel immunization protocol against *Schistosoma mansoni* by using *Echinostoma liei* somatic -antigen (S-antigen) and excretory/secretory (E/S) products from the adult worms was evaluated on the basis of parasitological, histopathological and serological parameters. CFW1 SPE albino mice were deployed into four groups. The first group of mice was immunized with S-antigen, the initial dose was 100 µg /ml, followed two weeks later by 50 µg /ml dose and after one more week, they received another dose of 50 µg /ml. The second group of mice followed the same treatment schedule and doses but received S/E product instead of the S-antigen. Three days later, after the last dose administration, both groups of mice and the third one were all infected with 100 *S. mansoni* and sacrificed 8wk post-infection along with the normal non-infected group of mice. The data revealed a remarkably (p<0.001) reduction of the worm burden, egg loads and granuloma diameter in the tissue of the immunized groups of mice compared to their counterparts in the infected controls. However, the recorded significant change of worm burden, egg load and granuloma size was greater in the tissues of the mice immunized with purified excretory/secretory products than those of mice treated with S-antigen. On the other hand, detection of anti-SEA serum specific immunoglobulin G and M levels revealed a considerable elevation (p>0.05) compared to the IgG and IgM levels in the infected control mice. Likewise, a noteworthy surge (p<0.001) of anti-SEA specific immunoglobulin isotype IgG1 in in the sera of all immunized mice was demonstrated when compared to the IgG1 levels in the sera of the infected controls. To sum up, the multiple intraperitoneal immunization with S antigen or S/E products have been shown to engender protective and modulatory effects on murine schistosomiasis 8wks post-infection.

**Key words:** *Schistosoma Mansoni* • *Echinostoma liei* • Somatic Extract • Secertory/Execretory Products • Immunization

**INTRODUCTION**

Schistosomiasis is one of the prominent public health problems brought about infectious flukes (trematodes) of the genus Schistosoma. Three species produce the most frequent clinical diseases: *Schistosoma haematobium* (endemic in Africa and the Middle East), *S. mansoni* (in Egypt, northern and southern Africa, some West Indies islands, northern 2/3 of South America) and *S. japonicum* (in Japan, China, the Philippines, Celebes, Thailand, Laos) [1]. Merck Manual, 15th Ed). Schistosomiasis causes significant morbidity and mortality in the developing world with recent studies indicating that the geographic extent and burden of the disease exceeds official estimates [1-3]. Praziquantel-based chemotherapy has achieved some success in controlling the disease but is not an optimal strategy due to its inadequate impact on reducing long-term transmission [4], re-infection and prevalence [5].
Egypt, the MENA (Middle East North Africa) country, has more than 10 million persons infected with schistosome [5-7] and experiences the highest prevalence (~9% of the total population) of schistosomiasis [8] in MENA, in spite of using Praziquantel [9]. Consequently, there remains a critical need for the development of alternate approaches to control this crippling disease [9&10]. A schistosomiasis vaccine might effectively help encounter the disease, particularly, if it provides a potent, long-lasting immunity to the disease [2]. Homologous antigens have been used extensively in murine schistosomiasis to induce immunization, whereas, the heterologous antigens received less attention [11]. Antigens-induced immunization against schistosomiasis and fascioliasis was a promising approach as reflected by diminishing of the morbidity, mortality and transmission. In addition to protective effects with significant worm burden reductions, some vaccine candidates also have anti-fecundity, anti-pathology and anti-embryonation effects [11, 12].

Schistosomes and -Echinostomes are two groups of digenean trematodes that co-exist in snail populations [13, 14]. Despite the two parasites temporarily inhabit the snail intermediate host, schistosome complete their life cycle in the blood vessels of the definitive host (either birds or mammals) and echinostome requires one more snail intermediate host before infecting the definitive host (birds or mammals). It is worth noting that when a mutual snail host harbors the two parasites, echinostome typically dominates the interaction due to its larval attributes that are missed in the schistosomes [15-17]. The direct antagonism between the larval stages of these parasites has been well-established within the snail intermediate host [18-20], yet the effects of this conflict on other aspects of the interaction have received far less attention.

This research was designed to investigate the possible effect of vaccine-induced immunization against S. mansoni infection with purified somatic -antigen and excretory/secretory products from adult worms of Echinostoma liei.

MATERIALS AND METHODS

Schistosoma Mansoni Cercariae: S. mansoni cercariae were obtained from the Schistosome Biological Supply Program (SBSP) at TBRI; this strain has been passages through outbreed mice and Biomphalaria alexandrina snails, cared for and maintained at SBSP/TBRI.

Somatic Antigen Preparation: Adult worms were collected from the intestines of rats four weeks post-infection (wpi) with 100 metacercariae of E. liei. After thorough washings with phosphate buffered saline (PBS, pH 7.4), the worms were homogenized in culture medium of PBS containing 0.8 mM phenylmethylsulfonyl fluoride (Sigma), 100U penicillin (Sigma) and 100 µg/ml streptomycin (Sigma). After initial centrifugation at low speed to remove larger particles, the supernatant was centrifuged at 15000 g for 30 min at 4°C. The protein content was measured by the Bio-Rad protein assay and adjusted to 1 mg/ml [11].

Excretory/Secretory Products: Adult worms were collected as described above, yet the adult worms were maintained in the culture medium at concentration of 10worm/ml for 12 hrs at 37°C. The medium was collected and centrifuged as before and the supernatant was collected and concentrated to 1 mg/ml using an ultra filtration membrane (YM-3, Amicon). All prepared antigens were stored at –20°C for use [12].

Antigen Purification: The prepared S antigen and E/S products of E. liei were purified using cyanobromide-(CNBr-) activated sepharose column according to Axen et al. [21].

Animals: CFW1 SPE albino mice 6 weeks old, 18 – 20 g each, were bred at Theodor Bilharz Research Institute (TBRI), Cairo, Egypt and maintained under conventional conditions. Mice were deployed into four groups (10 in each). The first group of mice was primarily immunized with intraperitoneal dose (0.1 ml; 100µg /ml/mouse) of purified S- antigen of E. liei emulsified in a complete Freund’s adjuvant. This immunization was boosted twice with 50 µg/ml of S-antigen in incomplete Freund’s adjuvant such that the first boosting was two weeks after the initial administration and one week before the last boosting. The second group of mice was stimulated by the E/S products following the same schedule of time, dose and emulsification in the adjuvant. Three days later, these groups along with healthy mice were infected with 100 S. mansoni cercariae via tail immersion. A fourth group of healthy mice was maintained neither infected nor immunized and under the same laboratory conditions. All mice were sacrificed 8-weeks post infection for the parasitological, serological and histo-pathological studies.

Parasitological Criteria

Worm Burden: Hepatic and portomesenteric vessels were perfused [22] recover worms for subsequent counting.
**Tissue Egg Load:** The number of ova/gm hepatic or intestinal tissue was counted after digestion overnight in 5% KOH [23, 24].

**Percentage Egg Developmental Stages "Oogram Pattern":** The percentages of eggs at the developmental stages were examined in 3 samples/mouse and the mean of each stage/animal was obtained [25].

**Histopathological Parameters:** Livers of mice were fixed in 10% buffered formalin, processed into paraffin blocks, serially cut at 4µm thickness and stained with hematoxylin and eosin. Hepatic granuloma measurements were done according to Von Lichtenberg [26] using an ocular micrometer for those containing a central ovum only [26].

**Serological Parameters**

**Determination of Serum-Specific Immunoglobulins:** Anti-SEA IgG, IgM, IgG1 and polyvalent mouse immunoglobulins were measured using indirect ELISA [27] based on the method of Engvall and Perlman [28]. To measure polyvalent immunoglobulins; IgG, IgM and IgG1; ELISA microtitre plates were coated with 250µg/well of 30 µg/ml of SEA and sera were diluted 1:100 for measuring the candidate Igs. The peroxidase conjugate, polyvalent rabbit anti-mouse Igs (Sigma) and monoclonal sheep anti-mouse IgG, IgM and IgG1 (Binding site, Birmingham, UK) were used at a dilution of 1/1000, 1/3000, 1/5000 & 1/500, respectively. The reactions were read at optical density (OD) values 492 nm using an ELISA reader (Bio-Rad Micoplate Reader, Richmond, CA, USA).

**Statistical Analysis:** The data are presented as mean ± standard error of mean (X ± SE). The means of the different groups were compared globally using the analysis of variance ANOVA. Data were considered significant if p values were less than 0.05.

**RESULTS**

**Parasitology:** Immunization with S antigen against *S. mansoni* infected mice showed a remarkable decline (31.4 % reduction) in the worm burden concomitant with higher percentage of reduction in the deposited intestinal and hepatic *S. mansoni* ova (69.7 % and 67.1 %, respectively), compared to the data collected from the infected controls. On the other hand, the worm burden evaluated in mice immunized with E/S products was significantly (p<0.001; 38.9%) lower than those of the S-antigen immunized mice and the infected controls. Moreover, the *S. mansoni* ova count in the intestine and liver was significantly (p<0.001) decreased (74.8 % and 70.8%) compared to that of the infected mice (Table 1) and still the % reduction of all parasitological parameters in E/S immunized mice is higher than those recorded for mice immunized with SE (Fig. 1).

**Histopathology:** As depicted in Table 2, the intraperitoneal immunization with S-antigen against *S. mansoni* infection mice had significantly (p<0.001) decreased (55.4%) the granuloma diameter (142.2 µm), yet, the E/S products reduced it (119.2µm) more (62.6 %) when compared to the mean granuloma diameter (318.8µm) of the infected controls (Fig. 2).

**Sera-Specific Immunoglobulins:** Table (2) shows that anti-SEA specific immunoglobulins Ig G and IgM considerably (p<0.05) increased in S-antigen- and E/S- immunized mice compared to the infected control group. Compared to the levels of Ig G1, the levels of the latter [26] based on the method of Engvall and Perlman [28]. were the highest in the sera of the mice immunized with S-antigen products against *S. mansoni* infection.

**DISCUSSION**

The helminthes genus *Echinostoma* has a large geographic distribution due to their ability to parasitize a variety of invertebrate and vertebrate hosts. Some species develop their larval stages in *B. alexandrina* snails, the most important intermediate host of *S. mansoni* [17, 27]. The interference effect of the larval stages of schistosoma and echinostome was studied intensively in the co-infected snails [15, 17, 27, 29, 30], however, a few investigations evaluated the mutual immune response and its outcome in murine models [31, 32]. The interactions between *E. caproni* and *S. mansoni* in the homologous or heterologous definitive hosts were investigated. Mice pre-infected by *E. caproni* showed an increase in its natural resistance concomitant with a decrease of the *S. mansoni* load [31, 33, 34]. Echinostomes have been proposed as potential bio-schistosomiasis-control agents due to the capability of *E. liei* to inhibit the infection of *B. alexandrina* by *Schistosoma* miracidia [17, 20, 31, 32]. These successfully promising approaches that explained the relationship between Echinostoma and Schistosoma have initiated our present study to investigate the potential efficicacy of purified antigens from *E. liei* against *S. mansoni* infection in a vaccine-induced immunization model system.
Table 1: Worm burden and tissue egg load in the experimental groups

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Worm Burden</th>
<th>Ova Count</th>
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<tbody>
<tr>
<td></td>
<td>X ± SD</td>
<td>% Reduction</td>
</tr>
<tr>
<td>Infected Control</td>
<td>29.6 ± 0.26</td>
<td>–</td>
</tr>
<tr>
<td>S-antigen-immunized mice</td>
<td>20.3 ± 0.31**</td>
<td>31.4%</td>
</tr>
<tr>
<td>E/S-immunized mice</td>
<td>18.1 ± 0.24***</td>
<td>38.9%</td>
</tr>
</tbody>
</table>

** Statistically significant difference at p< 0.01 compared to infected control group
*** Statistically significant difference at p< 0.001 compared to infected control group

Table 2: Effect of immunization with somatic -antigen and excretory/secretory products from adult worms antigen on hepatic granuloma diameter of mice infected with S. mansoni

<table>
<thead>
<tr>
<th>Group Name</th>
<th>Ø GD ± SE</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected Control</td>
<td>318.8 µm ± 26.3</td>
<td>-</td>
</tr>
<tr>
<td>Group I</td>
<td>142.2 µm ± 25.1</td>
<td>***</td>
</tr>
<tr>
<td>Group II</td>
<td>119.2 µm ± 29.5</td>
<td>55.4% ***</td>
</tr>
</tbody>
</table>

*** Statistically significant difference at p< 0.001, compared to the controls

Table 3: Determination of serum-specific immunoglobulins

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Ø O.D± SE Poly.Igs</th>
<th>Ø O.D± SE IgG</th>
<th>Ø O.D± SE IgG1</th>
<th>Ø O.D± SE IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.309±0.12***</td>
<td>0.289±0.23***</td>
<td>0.200±0.178***</td>
<td>0.259±0.217</td>
</tr>
<tr>
<td>Infected control</td>
<td>1.19±0.126</td>
<td>0.598±0.211*</td>
<td>0.377±0.123**</td>
<td>0.409±0.190*</td>
</tr>
<tr>
<td>Group I</td>
<td>1.42±0.222</td>
<td>0.908±0.188</td>
<td>0.877±0.219</td>
<td>0.601±0.198</td>
</tr>
<tr>
<td>Group II</td>
<td>1.66±0.143*</td>
<td>1.28±0.222**</td>
<td>0.903±0.190**</td>
<td>0.877±0.232***</td>
</tr>
</tbody>
</table>

Ø O.D: Mean readings were conducted at 492 nm.
SE: Standard Error.
* Statistically significant difference at p< 0.01 compared to infected control group
** Statistically significant difference at p< 0.05 compared to infected control group
*** Statistically significant difference at p< 0.001 compared to infected control group

Fig. 1: Shows the efficacy of vaccine-induced immunization on the worm burden and egg count in the experimental groups

Somatic -antigen and excretory/secretory -antigen of the adult worms of E. liei were the candidate antigens used in this experiment in combination with Freund's adjuvant to induce immunization against the S. mansoni infection in mice. The intraperitoneal injection of S-antigen and E/S products from E. liei in the infected mice yielded remarkable reduction in the worm burden, ova count and hepatic granuloma diameter when compared to the infected controls and the impact was more potent in the mice immunized with E/S. Likewise, using complete Freund's adjuvant and BCG with S. mansoni antigens reduced the number and size of the granulomas [33-36], which might boost the immunization effect in the both models. It's noteworthy that the prominent mechanism of BCG is to modulate the response of the T cells and the parasite damage by the lymphokine-activated macrophages [37, 39]. This kind of interaction upon exposure to the joint S. mansoni antigen and BCG could be also the same for E. liei S-antigen or E/S in combination with the Freund's adjuvant.

The decline of the parasites burden was concurrently associated with changes in the dynamics of specific immunoglobulins; the levels of all specific immunoglobulins increased in the infected mice sera compared to those of the uninfected controls, however, the significant increase was in favor of the total specific polyclonal Igs which may be attributed to the increase of other Igs as well as IgE. It had been reported that
antibodies IgM, IgG and IgA classes are produced in response to worm antigens, even though the most significant immunoglobulin class involved in resistance to parasite worms is IgE [39]. The recorded surge of specific antibodies IgM, IgG and IgG1 was associated with a great drop in ova count and worm burden. Likely, the B cell response was brought about in immunization models used S. mansoni soluble egg antigen [41, 42].

In essence, the current paper research might shed some of the light on the capability of E. liei antigens to antagonize the immune response to S. mansoni infection. Yet, we still need to investigate the T cell response and the cytokines secreted due to the immunization with S-antigen and E/S extracts from E. liei against S. mansoni infection.

REFERENCES


