**Gigantobilharzia Crude Antigen As Vaccine Against Schistosoma mansoni Infection**

By

Refaat, M.A. Khalifa*, Ahmed, K. Dyab*, Atef, A. Saad**
and Naglaa, M. Kamel**

*Department of Parasitology*, **Faculty of Medicine, Assiut University and**
**Department of Zoology, Faculty of Sciences (Aswan),**
South Valley University

**ABSTRACT**

Crude antigen prepared from adult *Gigantobilharzia* worms was used - for the first time - to immunize Balb/c mice against challenge infection with *Schistosoma mansoni*. Results of immunization were estimated by appearance of eggs in stool, antibodies in serum by Indirect Haemagglutination Test, counting the number of eggs and adults in liver and intestinal veins and tissues, estimation of the number and size of granulomas in the liver as well as description of adults deformities by ordinary and scanning electron microscopes. Obvious reduction in all these parameters was recorded in immunized mice. Further studies were recommended through the use of purified bird schistosomes antigens.

Key words: crude antigen - *Gigantobilharzia* - immunization - *Schistosoma mansoni*

**INTRODUCTION**

Schistosomiasis is the second most prevalent tropical disease in the world after malaria; with about 200 million adults and children infected in 74 countries. It is estimated that 20 million of them have a serious form of disease or related disability, and that 200,000 people die from the disease every year (WHO, 1996). The magnitude of the endemicity fully justifies the interest for the development of effective methodology for transmission control, based on an antischistosomiasis vaccine. In addition, reinfection after treatment, particularly in young inhabitants of endemic areas, makes chemotherapy expensive. The available drugs have no effect on already developed hepatosplenic manifestations and do not affect transmission rates.

Naturally acquired partial immunity is well demonstrated in endemic areas, with reduced intensity of infection in adolescents and older age groups as compared to children (Tendler et al., 2001).

Little attention has been given to heterologus immunity although epidemiological observations suggest that exposure to schistosome infections of animals may confer some degree of heterologous immunity in man. Le Roux (1961) suggested that cercariae from animals may immunize man against *S. mansoni* or *S. haemtobium*, and that the animals exposed to human infections may develop some immunity against their own species of schistosomes. This theory was further developed by Nelson et al. (1968) who noted that there were
many species of schistosomes in domestic and wild animals in Africa. Although most of these fail to reach maturity in man, yet they might be of considerable medical importance since constant exposure to their cercariae could interfere with the development of *S. mansoni* and *S. haematobium*.

Many laboratories now are attempting to identify candidate vaccines for schistosomiasis. Different candidate vaccines have been proposed for *Schistosoma* protection, including Sm 68 kDa (King et al., 1987), 38 kDa (Capron et al., 1984), 53 kDa (Smith and Clegg, 1985), 35-41 kDa (Hazadai et al., 1985), 97 kDa (Sher et al., 1986), and 200 kDa (Colley et al., 1991).

Despite the usage of different protocols in identifying these candidate vaccines, their protective levels range only from 25-60%. This achievement, which provides a relatively low level of protection, raised the need for a new approach in examining more suitable antigens for vaccination (Badary et al., 1997). This study was done to investigate the role of bird schistosomes antigen in protection of Balb/c mice against *S. mansoni*.

**MATERIAL & METHODS**

**Animals:** inbred Balb/c mice were obtained from Institute of Theodore Bilharz, Cairo, Egypt and kept in our animal facilities; Parasitology Dep., Faculty of Medicine, Assiut University.

**Antigen:** was prepared from *Gigantobilharzia* adults and sterilized and standardized as crude antigen dissolved in phosphate buffer, using the biological facility, Theodore Bilharz Institute, Cairo, Egypt.

**Vaccination and assessment of protection:**

**Vaccination:** three groups of male Balb/c mice (each group consists of 10 mice) at the age of 6-8 weeks were used. In group A, no vaccination was given, and was used as a control. In group B, each animal was injected subcutaneously with 100 ul Complete Freunds Adjuvant (CFA) at the base of the tail. In group C, each mouse was injected with CFA (100 ul) and crude antigen of *Gigantobilharzia* (100 ul of 1 mg/ml) at the base of the tail. After 2 weeks, all the 3 groups of mice were infected by wading in water containing 200 cercariae of *S. mansoni* (Watson and Abdel-Azim, 1949).

**Assessment of protection:**

i. **Stool examination:** 8 weeks after infection with cercariae stools of the three groups of mice were examined using Kato smear method for the presence of *S. mansoni* eggs.

ii. **Sera from vaccinated mice were collected from the three groups of mice and assessed for the presence of anti- *Gigantobilharzia* antibodies using Indirect Haemagglutination Test (IHA).**
Pefusion technique (Smithers and Terry, 1965):

By this technique, the number of worms recovered from the liver & intestinal veins were calculated for each mouse in each group. The liver and intestine of mice were removed for egg counting. The recovered male and female adult worms from the vaccinated mice and the control groups were examined for any morphological changes.

Scanning electron microscopy:

Was done by washing the sample with sodium cacodylate buffer, fixing in 3% (V/V) glutaraldhyde in 0.1 M of the buffer, then the sample was allowed to air-dry and transferred into stubs and then sputter coated with gold. The preparation was viewed by a Jeol 100 Cx-11 SEM operated at 40 KV.

Pathological examination:

Samples from each liver lobule of all groups of mice were obtained, formalin fixed, paraffin processed and 5 serial sections of 5-6 um thickness each were stained by routine Haemtoxylin and Eosin (H&E) stain.

RESULTS

I- Number and morphological changes of the recovered worms:

Immunization of Balb/c mice with crude antigen of adult worms of *Gigantobilharzia*, resulted in a significant reduction in the total number of adult worms (p<0.001) in comparison to control group or the group of mice vaccinated with CFA alone (table 1). Additionally, most of reduction was directed towards the adult male worms. The morphological features of the adult male were noticed to be affected. Microscopic examination of the adult males recovered from immunized Balb/c mice revealed decrease in the number and size of surface tubercles on the worm cuticle. Moreover, the elongated spines along the margins of gynaecophoric groove were decreased in number and size.

Table (1) The effect of immunization with crude *Gigantobilharzia* antigen on the number of recovered worms from Balb/c mice after infection with 200 cercariae of *S.mansoni*.

<table>
<thead>
<tr>
<th>Worm</th>
<th>Group A Unvaccinated</th>
<th>Group B Immunized with CFA</th>
<th>Group C Immunized with <em>Gigantobilharzia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Mean number of recovered worms (Mean ± SD)</td>
<td>Mean number of recovered worms (Mean ± SD)</td>
<td>Mean number of recovered worms (Mean ± SD)</td>
</tr>
<tr>
<td>Male</td>
<td>85 ± 10.6</td>
<td>68 ± 8.5</td>
<td>54 ± 6.7</td>
</tr>
<tr>
<td>Female</td>
<td>67 ± 8.5</td>
<td>43 ± 5.4</td>
<td>39 ± 4.8</td>
</tr>
<tr>
<td>Total</td>
<td>152 ± 19</td>
<td>111 ± 13.9</td>
<td>93 ± 11.6</td>
</tr>
</tbody>
</table>
II- Number of recovered eggs:
The average number of eggs in the liver, intestine and eggs passed in the mice faeces (per gram faeces) showed marked reduction in crude antigen of *Gigantobilharzia* vaccinated group (Table 2).

Table (2) The effect of immunization with crude *Gigantobilharzia* antigen on the number of recovered eggs from Balb/c mice after infection with 200 cercariae of *S.mansoni*.

<table>
<thead>
<tr>
<th>Sites of recovered eggs</th>
<th>Mean number of recovered eggs</th>
<th>Mean (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A Unvaccinated</td>
<td>Group B Immunized with CFA</td>
</tr>
<tr>
<td>Stool</td>
<td>39.39</td>
<td>18.25</td>
</tr>
<tr>
<td>Intestine</td>
<td>285 ± 16</td>
<td>213 ± 6.2</td>
</tr>
<tr>
<td>Liver</td>
<td>398 ± 17</td>
<td>172 ± 7.8</td>
</tr>
</tbody>
</table>

III- Scanning electron microscopy of normal and treated *S.mansoni*:

At low magnification (Fig. 1a, x 75) the tegument of male worms of (group A) is uniformly roughed and relatively has no pitted rims, and the general body form has no distortions. At a magnification (Fig. 2a, x 350) the tegument of the lateral and ventral surfaces of the anterior area are devoid from papillae, oral sucker rounded and opened. The ventral sucker, rounded, peduncluted (partially invaginated) with oval aperture. At higher magnification (Fig. 3a, x 750) very few bosses are seen on the outer rim of oral sucker also, very minute papillae were seen in the inner surface of the oral sucker and directed inwards to oesophagus.

Tegment of the lateral and dorsal areas is highly folded (Fig. 4a, x500). These folds are uniform in shape and size with papillae provided with several tapered, broader bases spines (Fig. 5a, x2000 & Fig. 6a, x7500) which are distributed in the inner, upper and outer sides of folds.

In group B (Fig. 1b, x50) which was injected with only 100 ul CFA worms showed some distortion in their morphology as the gynaecophoric canal became wider and opened. Oral and ventral suckers exhibited a transverse shape with narrower openings and became more contracted through their margins (Fig. 2b, x350) also, genital opening became smaller and contracted. (Fig. 3b, x750) showed some wrinkles and pores in the oral sucker.
The tegument of lateral and dorsal area is affected due to CFA treatment. It has distortion of the structure and size of tegumental folds (Fig. 4b, x500) that disappeared in several regions and became wider and lost their uniformity in other regions. There are several swellings with pores on each swelling (cratered papillae) (Fig. 5b, x2000). Also, some papillae of the lateral and dorsal sides are affected through partial disappearance of their spines. Sometimes, papillae became completely naked or have one digitate process-like spine (Fig. 6b, x7500).

In group C which was injected with CFA and the crude antigen of *Gigantobilharzia sp.*:

At low magnification (Fig. 1c, x50) males showed distortion in their morphology as they became flabby, gynaecophoric canal became more wider and several areas were devoid of papillae in the tegument. The oral sucker is affected due to the antigen through the more contraction of its margins and the oral opening became nearly closed, also some pores appeared around the sucker. Several swellings appeared especially at the lower margin of the sucker (Fig. 2c & 3c, x350 & x750). Ventral sucker exhibited a transverse shape and become completely outside its normal position (partially invaginated). It has several pores in its margins.

The tegument of dorsolateral and lateral areas: the tegument of those areas is highly affected due to adminstration of antigen through erosion of several papillae, highly disrupted wrinkled areas of tegument with several pores (Fig. 4c, x500). In addition to that the interpapillar space is heavily disrupted and lost its original unifomity. Some cratered flabby papillae appeared (Fig. 5c, x2000). At higher magnification (Fig. 6c, x7500) papillae became flabby and completely or partially naked from spines, others with shorter flabby spines. Interpapillar spines disappeared.

**IV- Histopathological examination of the liver:**

Grossly, the livers of infected non-vaccinated animals showed enlargement and dark coloration with scattered numerous small white spots representing granulomas in comparison to the vaccinated mice. Microscopically, the liver of the infected mice showed many cellular granulomas. The size and number of the granulomas were markedly reduced in vaccinated group (Table 3 and fig. 7,8).

Table (3): The effect of immunization with crude *Gigantobilharzia* antigen on the number and size of granulomas in Balb/c mice after infection with 200 cercariae of *S.mansonii*.

<table>
<thead>
<tr>
<th>Size and mean no of granulomas / section</th>
<th>Group A Unvaccinated</th>
<th>Group B Immunized with CFA</th>
<th>Group C Immunized with crude <em>Gigantobilharzia</em> antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granuloma size*</td>
<td>++++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Granuloma / section</td>
<td>14</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>
* An estimated size compared to the transverse section of *Schistosoma* eggs in the tissue section.

**V-** Detection of antibody in mice vaccinated with crude *Gigantobilharzia* antigen is shown in table (4).

Table (4): Titre of antibodies detected in mice immunized by crude *Gigantobilharzia* antigen through IHA.

<table>
<thead>
<tr>
<th></th>
<th>Group A Unvaccinated</th>
<th>Group B Immunized with CFA</th>
<th>Group C Immunized with crude <em>Gigantobilharzia</em> antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titre</td>
<td>&gt; 1/40</td>
<td>1/320 or more</td>
<td>1/160</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Rifaat et al. (1971) used bird schistosome cercariae obtained from *Melania tuberculata* and *Biomphalaria alexandrina* snails to identify antibilharzial antibodies in human sera by the fluorescent antibody technique, but they failed; suggesting no heterologous reactions. However, Hsu and Hsu (1961) succeeded to get a strong protection against human strain of *S.japonicum* through inoculation of rhesus monkies with cercariae of non-human strain.

Hunter et al. (1962) evaluated the importance of some variables in the production of acquired resistance to *S.mansonii* in albino mice. Their results appeared to be consistent with the hypothesis that maturity of the initial infections with egg deposition is necessary before ‘immunity’ to a challenge exposure with *S.mansonii* can be demonstrated.

Alteration in host resistance to helminth infection induced by infection with related and unrelated parasites is well established, although the mechanisms involved are incompletely understood (Crandall et al., 1966). Hunter et al. (1967) even showed that immunization by infective larvae of *Nippostrongylus brasiliensis* and a challenge by *S.mansonii* cercariae resulted in a significantly lower recovery of worms when compared with controls. Immuno-electrophoresis showed that these 2 helminth species shared at least one antigenic component. Previously, Jachoski and Bingham (1961) found also that the nematode *Trichinella spiralis* gave partial protection against a challenge by *S.mansonii* cercariae.

Nelson et al. (1968) and Amin and Nelson (1969) indicated that heterologous immunity resulting from bovine and other animal schistosomes might cause ‘zooprophylaxis’ against human schistosomes. The most potent immunizing effect was produced by *S.bovis* and *S.mathheei* in mice which resulted in a reduction in the expected *S.mansonii* egg load of 74% and 85.7% respectively. However, Eveland et al. (1969) failed to get cross-protection in
monkeys immunized with *S.bovis* and challenged with *S.japonicum*, suggesting that cross-protection may be correlated with phylogenetic relationships between schistosome species. Moreover, Massoud and Nelson (1972) proved that immunization with *Ornithobilharzia turkestanicum* from cattle produced a considerable degree of immunity in mice against a challenge with *S.bovis, S.mansoni* and *S.haematobium*.

El-Azzouni (1988) used heterophyid antigens in mice and found that the highest reduction in *S.mansoni* worm load was obtained one month after heterophyid infection (58.66%). Khalil (1991) suggested the possible use of *Echinostoma caproni* to immunize against *S.mansoni*.

It seems, therefore, that the present study is the first trial to use bird schistosome antigens for immunization against *S.mansoni*. The reduction in a challenge was demonstrated by significant reduction in egg output, number and morphology of adults (particularly males), number and size of liver granulomas, antibodies production as well as the SEM picture of adult. Further studies are recommended through the use of purified bird schistosome antigens which are expected to give better results. The assessment of the role of heterologous reactions in relation to the epidemiology of human infections has been generally neglected. This is surprising since the first effective vaccine used in human medicine was the result of Jenner’s observation on natural heterologous immunity between cowpox and small pox.

**LEGEND OF FIGURES:**

Figure (1-6): Showing SEM pictures of morphological characters of male *S. mansoni* in the three groups.

Figure (7-8): Showing number and size of liver granulomas in the 3 groups (x 200 and 400 respectively).

**REFERENCES**


