Ultrastructural changes of Schistosoma mansoni worms associated with administration of its polyvalent vaccine

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Abstract

There is currently no effective vaccine against schistosomiasis, so long term effective vaccine can be beneficial in case of long term chronic disease. This experiment was carried out on 90 BALB/c mice which divided into 6 groups, 1st group (control negative), 2nd group (infected with 50 ± 5 of cercaria), 3rd group (vaccinated with CAP stimulus dose + SEA + SWAP), 4th group (vaccinated with SEA + SWAP), 5th group (vaccinated with CAP), 6th group (vaccinated with CAP + SEA + SWAP). Changes of tegument distortion, erosion & sloughing, edema & blebbing, constricted suckers, spine blunting, atrophy tubercles, swelling & dimple appearance and peeling reported in different groups with a varying degree of severity. The severest changes were evaluated in the group (6) with administration of polyvalent vaccines (CAP + SEA + SWAP). We can conclude that the polyvalent vaccination program had a great effect against challenge of Schistosoma mansoni.

Keywords: CAP, SEA, SWAP, Vaccination, Schistosoma, Tegument

1. Introduction

Schistosomiasis is a water-borne disease that caused by dioecious (separate sex) trematodes, of the genus schistosoma that considered the most important human helminth infection in many areas of the developing world [1, 2]. Currently, there is no effective vaccine against schistosomiasis, so there is an innumerable effort for development of vaccine in the last twenty-five years [3]. The control programs of schistosomiasis have the fact that mass treatment does not prevent reinfection [4]. Long-term effective disease control will be benefit from the combination of vaccination beside chemotherapy, plus sanitation and public health control measures [5]. The target of vaccine is the prevention of infection and/or the reduction of parasite fecundity [4]. Many authors suggested that the possibility of development of a vaccine program against the different species of schistosomes [6, 7]. Administration of one or more booster immunizations enhanced the level of protection but never produced complete resistance to cercarial challenge of S. mansoni under the experimental conditions [8].

Most investigators studied the potential of schistosomal vaccine antigens individually rather than in combinations. It is unlikely single antigens which give the required protective results due to the complex structure of the different stages in the schistosomal life cycle besides the multiple immune responses involved [3]. Several antigens from S. mansoni can constitute the basis of a protective vaccine.
The comprehensive knowledge of tegumental components would be helpful in the development of new drugs [9]. So, the importance of studying the tegument of schistosomes arises because it acts as an interface between the parasite and its environment in the host which used to evade the immune responses of the host [10].

Soluble egg antigen (SEA) originating from the eggs of Schistosoma species, it is potent enough to evoke pro-inflammatory responses by recruiting macrophages into the liver, which then initiate granuloma formation to limit the immune responses against SEA to the location of the trapped egg in the liver [11, 12, 13].

SWAP is a soluble adult worm antigen preparation which used by immunologists to probe host responses to schistosome infections [14]. Curwen et. al. [15] provided a comparison of soluble preparations from four different life-cycle stages, among them adult worms. The adult worm membrane antigens induced antibodies capable of killing the schistosomulum in vitro [16] and partial protection in vivo [17].

Cercariae are covered by a thick glycocalyx that activates complement by the alternative pathway and is recognized by antibodies in the serum of infected animals [18, 19]. It contains antigens reactive with protective monoclonal antibodies [20, 21]. We recently investigated the potential effect of different types of the three Schistosomal antigens against challenge of Schistosoma in murine model. One of the main targets of this study is the efficiency of a fusion one or more of schistosomal antigens on ultrastructure of the tegument for adult worms.

2. Materials and methods

2.1 Experimental animals and design

Ninety laboratory Swiss albino mice weighing 20 to 25 g were obtained from Theodor Bilharz Research Institute (SBSP/TBRI). They were maintained on standard diet 24% protein content. Mice were divided in to 6 groups each one consists of 15 mice as following in Table (1). Mice were scarified after 10 weeks post infection in all groups. These mice put under observation for recording any clinical signs appear on them.

Table 1: The experimental design of the different groups with the time of infection and vaccination.

<table>
<thead>
<tr>
<th>Experimental design</th>
<th>Mice without infection</th>
<th>Mice infected with 50 ± 5 cercaria</th>
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<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; group (control negative group)</td>
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<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; group (control positive group)</td>
<td>Mice without infection</td>
<td>Mice infected with 50 ± 5 cercaria</td>
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<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; group (combined vaccination post infection, CAP +SEA+SWAP)</td>
<td>Mice infected with 50 ± 5 cercaria, two doses of CAP antigen (25 µg protein of each dose at 3, 6 days post infection) will be injected. SEA and SWAP antibodies injection began at 7 days post infection, followed by three doses at 10, 20 and 30 days post infection. Three excessive doses of SWAP antigen at 33, 35 and 37 days post infection. Total doses of SEA and SWAP antigens was 100 µg and 175 µg protein respectively</td>
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<td>4&lt;sup&gt;th&lt;/sup&gt; group (vaccinated with combination of SEA and SWAP antigens 100 µg and 175 µg protein respectively)</td>
<td>Mice infected with 50 ± 5 cercaria, initial dose will be injected after 3 days post infection (25 µg protein), stimulating doses at 3, 11 and 25 days after the initial vaccination, excessive doses of SWAP antigen will be given at 28, 30 and 37 days post initial vaccination.</td>
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<td>5&lt;sup&gt;th&lt;/sup&gt; group (CAP vaccinated group)</td>
<td>Total dose 100µg protein of CAP antigen, initial dose 25 µg followed by three stimulating doses at 3, 11 and 25 days after initial dose, in day 33, mice will be infected with 50 ± 5 cercaria</td>
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<td>6&lt;sup&gt;th&lt;/sup&gt; group (combined vaccination pre- and post-infection,CAP, SEA and SWAP antigens with total, 100 µg,100 µg and 175µg protein respectively)</td>
<td>Mice prevaccinated with CAP antigen with same schedule in third group, after 33 days post vaccination, infected with 50 ± 5 cercaria, then vaccinated with a combination of SEA and SWAP antigens with the same dose and same schedule as above (4&lt;sup&gt;th&lt;/sup&gt; group).</td>
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CAP: cercarial antigen preparation; SEA: soluble egg antigen; SWAP: soluble worm antigen preparation

2.2 Schistosomal antigens preparation:

Cercarial antigen preparation (CAP), soluble worm antigen preparation (SWAP) and soluble egg antigen (SEA) were prepared at the Schistosoma Biological Supply Program (SBSP) at Theodor Bilharz Research Institute (TBRI). CAP was prepared according to the method of [22], SWAP was prepared according to the method of [23] and SEA was prepared according to [24].

2.3 Adjuvant preparation

Freund’s adjuvant (Adj) {Freund’s complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA)} was obtained from Sigma Chemical Co., St Louis, Mo.
USA and emulsified in phosphate buffered saline (PBS) at a ratio of 2:1 (v/v).

2.4 Scanning Electron Microscope (SEM)
Adult worms were recovered from unvaccinated and vaccinated groups after ten weeks post infection by Hepatic and porto-mesenteric vessels were perfused using previously reported techniques [25]. Worms collected in the perfusates were washed several times with saline and fixed in 5% glutaraldehyde in 100 mM phosphate buffer (pH 7.4, 4 °C) for 24 hours. They were post fixed with 1.5 osmium tetraoxide for 2 hours and washed four times with 100 mM phosphate buffer (pH 7.4). After slowly dehydrating with an ethanol series, the samples were dried at 30-40 °C, and then glued to stubs coated with 20 nm of gold and viewed with Scanning Electron Microscope (JSM 5400 LV) at 15 Kv at Electron Microscope Center, Assuit University, Egypt.

3. Results:
3.1 Ultrastructure malformation in male worms
In the male of *Schistosoma mansoni*, the dorsolateral surfaces are ventrally curved posterior to the ventral sucker, forming the gynecophoric canal. The normal configuration of the normal worms characterized by configured oral and ventral suckers which were covered with many directed spines (Fig.1A). Tegmental structures were appeared with supported spines on the dorsal surface behind the region of the gynecophoric canal (Fig. 1B). The dorsolateral surface of the mid-body was covered with tubercles which uniform in size and distribution and interspaced with tegumental ridges and ciliated sensory papillae (Fig. 1C). The substantial changes in the male worms of *Schistosoma mansoni* in different groups were summarized in Table (2).

Table 2: The substantial changes in the male worms of *Schistosomamansoni* in different groups

<table>
<thead>
<tr>
<th>group</th>
<th>Tegmental distortio n</th>
<th>Erosion/ sloughing</th>
<th>Edema/ blebbing</th>
<th>Constricted suckers</th>
<th>Spine blunting</th>
<th>Atrophied tubercles</th>
<th>Swelling with dimple appearance</th>
<th>Peeling appearence</th>
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<td>Group (2)</td>
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<td>Group (3)</td>
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<td>Group (4)</td>
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<td>Group (5)</td>
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<td>Group (6)</td>
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Fig 1: Scanning electron micrograph of adult *S. mansoni* male worms showing: (A) Adult worms recovered from control infected group with normal configuration and typical oral and ventral suckers; (B) The plain structure of middle dorso-lateral area of tegument showing numerous large tubercles with spines; (C) Higher magnification of tegument showing structure of tubercles which bearing numerous spines, intertubular ridges and ciliated sensory papillae are evident; (D) Micrograph of adult males recovered from 2nd vaccinated infected group showing widening of the gynecophoric canal with distortion of the tegument; (E) The dorsal tegument surface of male worms recovered from 2nd vaccinated infected group showing focal sloughing with occasional eruption of some blistered tubercles, an evident dimple is clear as a result of edema and blebbing; (F) The dorsal tegument surface of male worms recovered from 2nd vaccinated infected group showing erosion and loss of tubercles in some areas.
Fig 2: Scanning electron micrograph of adult *S.mansoni* male worms recovered from 3rd vaccinated infected group showing: (A) Adult worms showing oral and ventral suckers with constrictions in the ventral sucker; (B) Tegumental distortion in large area of dorsal surface with tubercular and intertubercular coarse bumpy with irregularities and surface erosion; (C) Higher magnification of tegument showing protruding in ridged area, erosion, restricted spine loss and tegumental tear were appeared; (D) Prominent reduction in the numerical and volume density of tubercules with extensive distortion and retracted tubercles; (E) Abnormal wrinkle male surface with atrophied tubercles and swelling alterations with furrows, dimples and bumpy appearance; (F) Higher magnification of tegumental tubercles showing bumpy surface with dwindle tubercles.
Fig 3: Scanning electron micrograph of adult *S.mansoni* male worms showing: (A) Adult worms recovered from 4th vaccinated infected group showing swollen oral and ventral suckers with edema and blebbing; (B) Tegmental surface exhibited severe damage and edema with eruption in soma blistered tubercles; (C) Dorsal surface with shrinkage and flattened of tubercles; (D) Prominent flattening of dorsal tubercles with shortened spines, and loss of them in some areas; (E) SEM micrograph recovered from 5th combined vaccinated infected group showing normal oral and ventral suckers and severe damage of the surface of female worms; (F) Higher magnification of male tegumental surface showing completely distortion of surface tubercles with spine blunting. Focal sloughing of the tegument with peeling of the surface.

The CAP vaccinated group (group 5) and combined group (group 6) showed the most intrinsic results on recovered adult male worms. SEM examinations showed marked extensive tegumental alterations. These were demonstrated by swelling, oedema and blebbling by swollen with edema and blebbing of the tegument (Fig. 3B, F). Worms exhibited distortion, erosion and eruption of blistered tubercles (Fig. 3C, D, F) which irregularly positioned or even lost in some areas (Fig. 3D, F). Worms recovered from CAP vaccinated group (group 5) showed shrinking and flattening of the tubercles with accumulation of the secretion which are aggregated on the surface. Eruptions and atrophied tubercles and tegument which were associated with dimple appearance (Fig. 3B, C, D). In the combined group (group 6), the alterations were more extensive. The dorsal surface revealed peeling and focal erosion with sloughing of their tubercles with cracked surface (Fig. 3F). The representative SEM results in other vaccinated groups showed less pronounced lesions on the tegument. The second vaccinated group with SEA and SWAP and stimulus CAP, showed distortion and retracted tubercles with sloughing erosion in some areas (Fig. 1E). Occasional eruption of some blistered tubercles with focal areas of edema and blebbing (Fig. 1F). The third vaccinated group with SEA and SWAP exhibited constrictions and edema of the ventral sucker. Surface
erosion with tegumental tear was appearing (Fig. 2B, C). The distortions of the tubercles were the dominant alteration which associated with swelling dimples and bumpy appearance on the surface. Loss of tubercles was restricted with shortened spines (Fig. 2E, F). The widening of the gynacophoric canal was mutual in the second and third vaccinated groups (Fig. 2D and 3B).

3.2 Ultrastructure malformation in female worms

Adult female worms of S. mansoni were more resistant to antigens than male worms. Fig (5 and 6) showed representative SEM images of female worms recovered from control unvaccinated mice and vaccinated with different combinations of antigens. The female worms which are recovered from the unvaccinated mice, showing a normal body structure with distinct oral and ventral suckers (Fig. 4A) with fine circular ridges interspaced with regular clefts and was carrying conspicuous sensory bulbs on the dorsal surface (Fig. 4B). The alterations in the female worms obtained in the front area or on tegument. On group (3), suckers showed variable increase in size with edema on ventral sucker. Spines lining the suckers were absent (Fig. 4C). The tegument of group (3) and group (6) showed severe damage compared to other groups.

**Fig 4:** Scanning electron micrograph of adult *S.mansoni* female worms showing: (A) Adult worms recovered from control infected group with normal body structure and distinct oral and ventral suckers; (B) Mid-dorsal surface of control female showing fine circular ridges interspaced with regular clefts and carrying conspicuous sensory bulbs; (C) Micrograph of adult females recovered from 2\textsuperscript{nd} vaccinated infected group showing oral and ventral suckers with variable increase in size; (D) Micrograph of adult females recovered from 2\textsuperscript{nd} vaccinated infected group showing strangulation associated with blebbing and dimples; (E) Eminent invagination surface of a females recovered from 2\textsuperscript{nd} vaccinated infected group exhibited complete destruction of external surface of the female body; (F) The tegument surface of female worms recovered from 2\textsuperscript{nd} vaccinated infected group exhibiting swelling surface alternating with bumpy, grooves, gapping and pucker surface.
Fig 5: Scanning electron micrograph of adult *S. mansoni* female worms showing: (A) Dorsal surface of worms recovered from 3\textsuperscript{rd} vaccinated infected group exhibited disorganized circles with dimples; (B) SEM micrograph of females recovered from 4\textsuperscript{th} vaccinated infected group showing the anterior end with distinct atrophied sucker; (C) Dorsal surface of females recovered from 4\textsuperscript{th} vaccinated infected group showing absence of regular circulation with wrinkling and dimple appearance; (D) Adult female worms recovered from 5\textsuperscript{th} vaccinated infected group showing anterior region with distinguished suckers, host leucocytes attachment on the worm surface; (E) Higher magnification of female worms recovered from 5\textsuperscript{th} vaccinated infected group showing the absence of linear transverse tegumental circles; (F) Higher magnification of tegument surface of female worms recovered from 5\textsuperscript{th} vaccinated infected group exhibiting strangulation with completely loss of mid-dorsal circular ridges.

In group (3), the alterations occurred in the form of extensive swelling with bumpy, grooves, gapping and pucker surface (Fig. 4D, E). Strangulation of the body circles was present with blebbing expression. Destruction of the external surface occurred with eminent invagination of the body (Fig. 4F).

In group (4), females were convoluted and expressing irregularly constrictions and disorganized circles with dimples in various areas of their bodies (Fig. 5A).

The suckers of group (5) exhibited atrophy and swelling, depressions and wrinkling (Fig. 5B). The transverse tegumental circles of the surface of the worm’s body were not observable (Fig. 5C).

The female worms of group (6) showed high alterations in the body surface, which loss its transverse encircling appeared, being replaced by swelling induced loosing of the clefts, forming dimples, gapping, blebbing with kinky surface (Fig. 5E, F).

4. Discussion
The alterations in the morphological structure of adult worms give a strong indication of immune responses
elicited by tested vaccines. There are few reviewed articles about ultrastructure changes of Schistosoma mansoni adult worms under combined antigens. This work was carried out to assess the potential effect of three schistosomal antigens with different combinations on the ultrastructure of the adult worms of Schistosoma mansoni which recovered from immunizing animals.

The ultrastructure detail can improve the understanding of the host-parasite relationship and the effect of different treatments. The ultrastructure of the cuticle of S. mansoni showed an outer syncytial portion of a tegumental unit which is likely that of all other described trematodes [26]. The male of S. mansoni has spines all over the body and is covered with tubercles except for a part of the posterior end [27, 28, 29]. The ventral sucker of S. mansoni has spines which smaller than those on the oral sucker [27, 28]. The inner surface of the gynacophoric canal is covered with small spines arranged very irregularly [30]. The tegument of the female is smoother than that of the male and covered with spines, but there is a heavy covering of anteriorly directed spines on the posterior part of the body [28]. These tegumentally structures interpreted as sensory receptors which are present all over the male and bodies, and are more numerous especially at the anterior end of the gynecophoric canal [30].

The tegument of S. mansoni worms has many functions and features which raised the importance to study it. It is an interface between the parasite and its environment in the host [31]. The ultrastructure of the tegument of S. mansoni at all stages of the life cycle was discussed by [28, 29, 32]. Destruction of it is a response to the effect s of hypo- and hyper tonic media, drugs and host immunity [29].

In this study, there was a significant malformation in the tegument of male and female worms in different vaccinated groups comparing to infected control group. The degree of effect was different in male worms than females, and it determined on the basis of duration of immunization and types of antigens. These factors are a probable diminished fecundity of the worm pairs and increased rate of egg excretion due to the egg death [33]. The most dominant damage observed in this study was in the tegument of vaccinated treated worms in the form of distortion, erosion and sloughing of the tegument tubercles. This confirms the disappearance of the immunological disguise of the worm [31]. These observations confirmed the differential adverse effect of different types of schistosomal antigens on adult worms. Abnormal morphological changes were first observed in the tegument of male worms, this is due to the soft tissue alterations are more pronounced in males than in females [34].

The severity and variability of different antigens induced structural changes between male and female worms were seen. This could reflect differences in tegumental functioning associated with the presence of a female with in the gynecophoric canal. Disruption of the male tegument became the severe damage which cause broke down of tegument leaving the underlying layer exposed to the exterior [35]. Adherence of the host cells to such an immunological unprotected surface cause abnormal structural changes and hepatic shift of worms which was responsible for their final elimination [35]. Kohn et. al. [36] described extracellular edema which gave rise to the swelling of whole segments of some male worms. Such lesions were observed in some groups of the present work, in our work the male worms are more susceptible to the effect of schistosomal agents than females. These agreements with many authors under the effect of different schistosomicides [37, 38, 39, 40] but disagree with Webbe and James [41] and Utzinger et al. [42].

The phenomenon of surface blebbing and the presence of membranous bodies in the schistosome tegument represent a response to mild forms of surface attack [33]. Indeed, the results discussed from vaccinated-damaged worms in the present investigation supports such a proposal. However, in our study the different vaccinated antigens caused a lethal damage and the disruption of the tegument was clearly irreversible.

Moreover, the immunization with combined antigens had the most severe effect on the ultrastructure of adult worms. We recently reported the efficiency of using polyvalent vaccines against S. mansoni and we suggested that these antigens were good representative for S. mansoni infection. The development of novel cocktail vaccine formulations is necessary to enhance protection level which ranges from 70 to 80% [43].

The results of our work were closely related to Ismail [44] which reported that the reduction in worm burden was 88% in mice vaccinated with combined antigens (CAP+SEA+SWAP) compared to non-vaccinated infected group. Etewa et al., [45] agreement with that with percentage 90.28% in the worm burden, and tissue egg load (liver and intestine: 93.67 and 93.98%) respectively. In contrary, our results demonstrated that CAP antigen may give a high percentage of protection if it pre-vaccinated for 33 days before infection. This results conflict with that reported about the lower antigenicity of CAP antigen [46, 47, 48]. More recently, authors studied ultrastructure malformation under different types of antigens. Mossallam [3] discussed the
morphological ultrastructure with vaccination with afusion of schistosomal antigen, Sm14 and Sm29 which observed similar tegumental damage and ultrastructural changes in S. mansoni worms recovered from infected mice.

5. Conclusion
In this study, administration of a single or combined antigens pre and post infection resulted in severe damage in the tegument of S. mansoni adult worms with variable degrees. This is could be give a powerful force in research of vaccination against Schistosomiasis. As a conclusion the severest damage to the tegument was reported in the group administrated the three vaccines, this donate the synergistic and cooperative between the three vaccines. The above mentioned fact directed our attention to go in the direction of using the combined effect of the three types of S. mansoni vaccine.

6. References
4. Pinheiro CS et al. A multivalent chimeric vaccine composed of Schistosoma mansoni SmTSP-2 and Sm29 was able to induce protection against infection in mice. Parasite immunology. 2014; 36(7): 303-312.
19. Samuelson JC, Caulfield JP. The cercarial glycoalyx of Schistosoma mansoni. The
34. Mostafa OMS, Soliman MI. Ultrastructure alterations of adult male *Schistosoma mansoni* harbored in albino mice treated with Sirid honey and/or Nigella sativa oil. Journal of King Saud University-Science. 2010; 22(3): 111-121.
41. Webbe G, James C. A comparison of the susceptibility to praziquantel of *Schistosoma haematobium*, *S. japonicum*, *S. mansoni*, *S. intercalatum* and *S. mattheei* in hamsters. Zeit
44. Ismail OA. Study of the efficacy of adult worm, cercarial and egg antigens in protection against experimental intestinal schistosomiasis. 2005. MD. Thesis. Faculty of Medicine, Suez Canal University.
48. Pearce EJ, Basch PF, Sher A. Evidence that the reduced surface antigenicity of developing Schistosoma mansoni schistosomula is due to antigen shedding rather than host molecule acquisition. Parasite immunology. 1986; 8(1): 79-94.