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Scanning Electron Microscopical Studies on *Schistosoma mansoni* cercariae Exposed to Ultraviolet Irradiation

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Abstract: In the present work, scanning electron microscopical studies on *Schistosoma mansoni* cercariae exposed to ultraviolet irradiation (UV-irradiation) were done to answer the question: Is the damage inflicted seen on the adult schistosome worms developed from irradiated cercariae, due to direct effect of UV-irradiation on cercariae or due to the host's immunogenicity induced by UV-irradiated cercariae? The present observations showed that there were no changes on the surface topography of *S. mansoni* cercariae exposed to UV-irradiation. Thus we can suggest that the changes seen in the adult worm developed from UV-irradiated cercariae may be due to the immune responses of the host induced by attenuated cercariae; or it may be due to the mutagenic effects of the UV-radiation which appeared in the adult stages. Detailed discussion on the scope of the recent knowledge about the immunological aspects due to irradiated cercariae was presented.

Key words: Schistosome, Scanning electron microscopy, Ultraviolet, Irradiation, Cercariae, Vaccine

INTRODUCTION

Human schistosomiasis, a devastating parasitic worm disease caused mainly by three species of the genus *Schistosoma: S. haematobium, S. japonicum, S. mansoni* (He *et al.* 2005). More than 600 million people are at risk with about 200 million actually infected in 74 countries mainly in the tropics and subtropics (Ruelas *et al.* 2006).

Despite the availability of affordable chemotherapy, drug treatment has not influence transmission of the infection. Moreover, there is a concern of drug resistance against chemotherapy of schistosomiasis of choice (e.g. Praziquantel). Therefore, a prophylactic or therapeutic vaccine to eliminate, or substantially reduce, the impact of schistosomiasis remains a highly desirable goal (Bergquist *et al.,* 2002).

Large body of vaccine-oriented researches has been carried out using cercariae exposed to various degrees of gamma or ultraviolet radiation and infected different experimental models (Tewari and Biswas 1972; Webbe *et al.* 1982; Moloney *et al.* 1985; McLaren *et al.* 1990; Osman *et al.* 1994; Mostafa 1995; Kumar and Ramaswamy 1999; el-Gawish and Moawad 2003; Kariuki *et al.* 2004; Zhu *et al.* 2005; Torben and Hailu 2007a,b). Moreover, it was found that vaccination with attenuated infective cercariae is generally more effective at inducing host protection against experimental infection with *S.mansoni* than immunization with defined antigens (Ganley-Leal *et al.,* 2005).

Scanning electron microscope is an important tool in studying the surface of the parasite when considering the host-parasite relationship (Hockley, 1968). A lot of work has been focused on schistosome normal larval stages. Stereoscan observations on *S. mansoni* cercariae and miracidia were done by Hockley, 1968, Race *et al.* 1971 and Miegeville *et al.* 1979. The ultrastructure of the ventral sucker of *S.mansoni* cercariawas studied by Cousin *et al.* 1995. Dorsey *et al.* (2002) described the ultrastructure of the cells that comprise the cercaria of *S. mansoni* at ultrastructure level.

However, very few investigations have been done on schistosome larval stages exposed to irradiation and adult worms developed from irradiated cercariae. Harrop and Wilson (1993) studied the morphological differences between normal and gamma irradiated larvae of *S. mansoni* at the lung stage of development by using scanning electron microscope. Mohamed (1999) studied the surface topography of adult *S. mansoni*

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originating from ultraviolet-irradiated cercariae. On the other hand, no attention has been paid to the effect of irradiation on the ultrastructure and morphology of irradiated schistosome cercariae, therefore the present work was aimed to study the surface topography of ultraviolet-irradiated *S. mansoni* cercariae in comparison with non-irradiated cercariae of the same species.

MATERIAL AND METHODS

Cercariae Production:

Ten mice of CD1 strain were exposed to 100±10 cercariae of Egyptian strain of *S. mansoni* per mouse by the tail immersion method, modified by Oliver and Stirewalt (1952). Mice and cercariae were purchased from the Schistosome Biological Supply Program (SBSP) unit, Theodore Bilharz Research Institute, Giza, Egypt. Six weeks post-exposure, mice were sacrificed and dissected to obtain the liver and intestine. Hepatic and intestinal tissues were cut into pieces, placed in 0.85% saline solution and homogenized. The suspension was poured into a column of sieves arranged in descending order of mesh opening (420 µm, 177 µm, 105 µm and 45 µm). The eggs were collected from the bottom sieve, suspended in dechlorinated tap water and exposed to light to stimulate hatching of miracidia. One hundred Lab-bred *Biomphalaria alexandrina* (4-5 mm in shell diameter) were individually placed in wells of a 24-well plate, along with freshly hatching five to seven miracidia and 0.5 ml dechlorinated tap water. The snails were left overnight at room temperature to ensure maximum penetration. Then snails were removed from the wells and reared in culture. Snails were placed in plastic trays with a holding capacity of 1.5 liters of water. Each tray contained 25 snails at a time in dechlorinated water and was supplied with lettuce leaves (fresh or boiled and dried) for snail feeding. Trays were loosely covered with a glass plate to reduce evaporation, and maintained at room temperature 27-29°C. Water in the trays was changed every two days. At the 5th week post-exposure, snails were examined for cercarial shedding by exposing to strong light and the cercariae released were isolated and used in the present study.

UV Irradiation:

An S-68 mineral-light lamp, rated to deliver 95% of its output at 254 nM, was used as source of ultraviolet radiation. Thousands of cercariae were exposed to ultraviolet radiation for two and three minutes.

Samples Preparation and Stereoscan Examination:

The cercariae were fixed in 4% gluteraldehyde in cacodylate buffer for two hours, washed in the same buffer at pH 7.4, post-fixed in 2% osmic acid, washed in cacodylate buffer, finally dehydrated with ethanol. Specimens were mounted on stubs, coated with carbon and gold, examined with a Joel JEM-1200 EXII electron microscope.

Observations:

The cercariae of *S. mansoni* have an oval body or head and a long cylindrical tail which is divided into two furculae at the posterior extremity (Fig.1). The most anterior part of the head is provided with triangular slit surrounded by three spiny tegumental folds (Fig.2). The head of *S. mansoni* cercariae is covered with numerous spines which are posteriorly directed. The ventral sucker is well developed and provided with numerous, large and sharp spines directed backwardly (Figs. 3& 4). The posterior end of the head is tapered into a spiny collar-like folding over the narrow connection between the head and tail. This area represented the detachment site between the head and tail during penetration (Figs. 5& 8). The tail of *S. mansoni* has larger and sharper spines than that of the body. They are concentrated on the dorsal and ventral surface, and they are much fewer on the lateral surface. The ventral surface of the tail furculae has few short and pointed spines. The excretory pore is found on the tip of the tail furculae (Figs. 5, 6 & 7). The surface topography of *S. mansoni* cercariae exposed to 2 and 3 minutes ultraviolet radiation was more or less similar to those of non-irradiated cercariae, since there was no strike differences observed (Figs. 9, 10, 11&12).

Discussion:

In the present observation on non-irradiated *S. mansoni* cercariae, the construction between the most anterior part of the head and its middle part was not deep. This observation was agreed with that of Hockley (1968) on his work on *S. mansoni* cercariae. However, Sobhon *et al.* (1988) demonstrated that the most anterior part or the head of *S. japonicum* cercariae is conical and clearly demarcated from the middle section by a deep construction. The body and tail of *S. mansoni* cercariae were separated by a collar which was

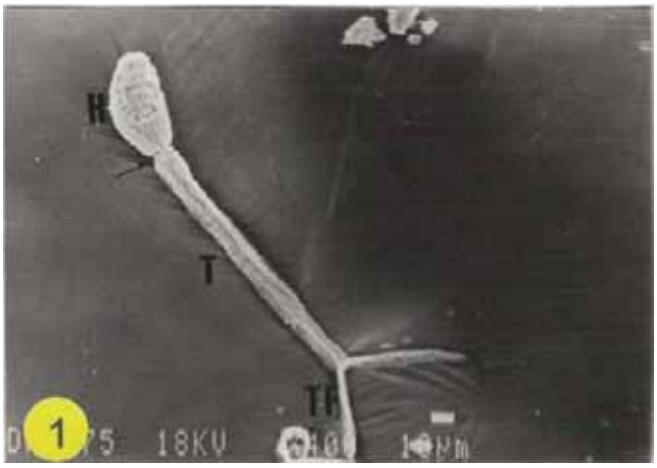

Fig. 1: Low power scanning electron micrograph of non-irradiated *S. mansoni* cercaria showing head (H), collar-like structure (arrow), tail (T) and tail furculae (TF).

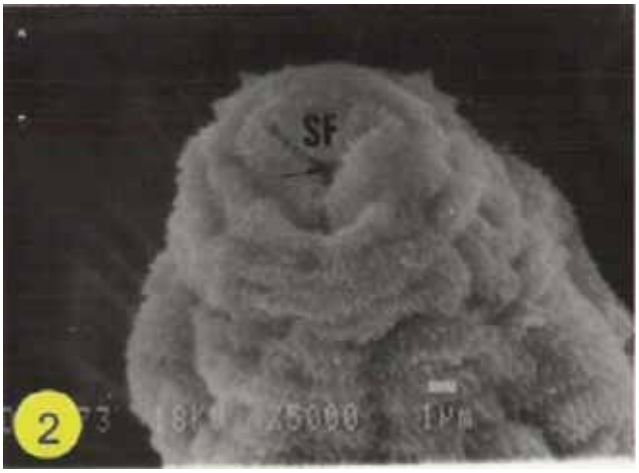

Fig. 2: Scanning electron micrograph of non-irradiated *S. mansoni* cercaria showing the most anterior part of the body provided with triangular slit (arrow) surrounded by three spiny folds (SF).

Fig. 3: Scanning electron micrograph of ventral surface of the head of non-irradiated *S. mansoni* cercaria showing the ventral sucker (VS).

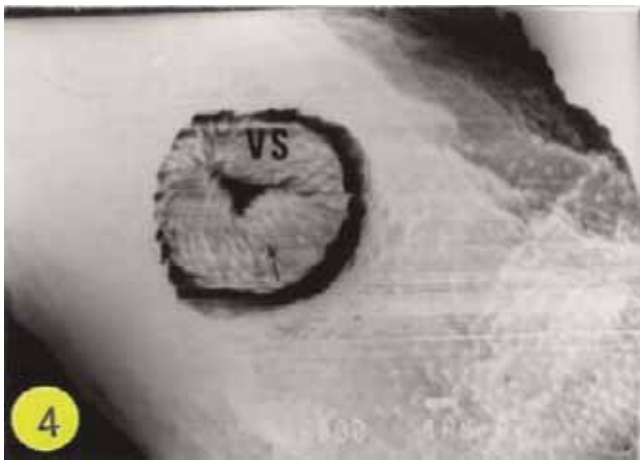

Fig. 4: Scanning electron micrograph of ventral surface of the head of non-irradiated *S. mansoni* cercaria showing the ventral sucker (VS) with backwardly directed numerous spines (arrow).

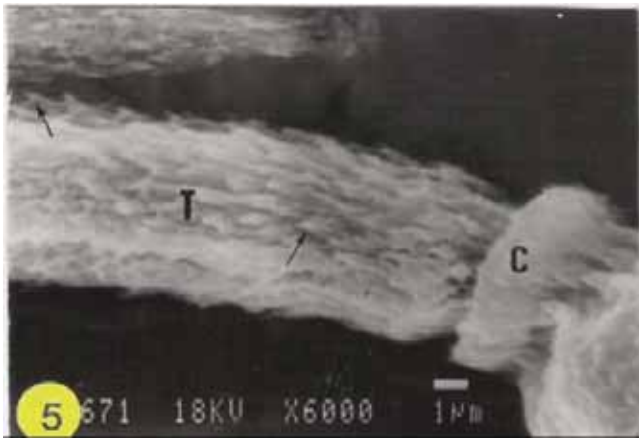

Fig. 5: Scanning electron micrograph of non-irradiated *S. mansoni* cercaria showing the collar-like structure (C) which connecting the head with the tail (T), the tail is provided with large spines directed posteriorly (arrows).

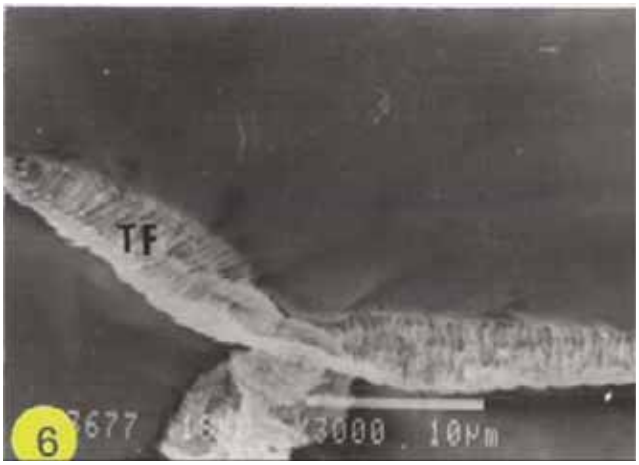

Fig. 6: Scanning electron micrograph of tail furculae (TF) of non-irradiated *S. mansoni* cercaria.

Fig. 7: Scanning electron micrograph of the ventral surface of tail furculae (TF) of non-irradiated *S. mansoni* cercaria showing short and pointed backwardly directed spines and the excretory pore on the tip of tail furculae (arrow).

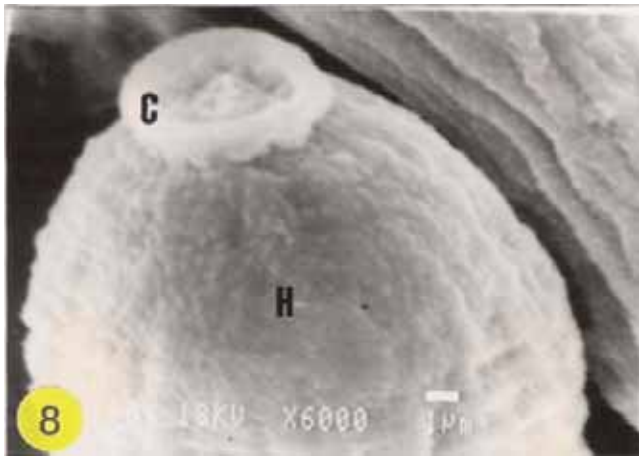

Fig. 8: Scanning electron micrograph of the head (H) of non-irradiated *S. mansoni* cercaria after detachment of the tail showing the collar-like structure(C).

Fig. 9: Scanning electron micrograph of 2 min UV-irradiated *S. mansoni* cercaria showing the anterior part of the body with three spiny folds (SF).

Fig. 10: Scanning electron micrograph of 3 min UV-irradiated *S. mansoni* cercaria showing the anterior part of the body with three spiny folds (SF).

Fig. 11: Scanning electron micrograph of the ventral surface of 3 min UV-irradiated *S. mansoni* cercaria showing the ventral sucker (VS).

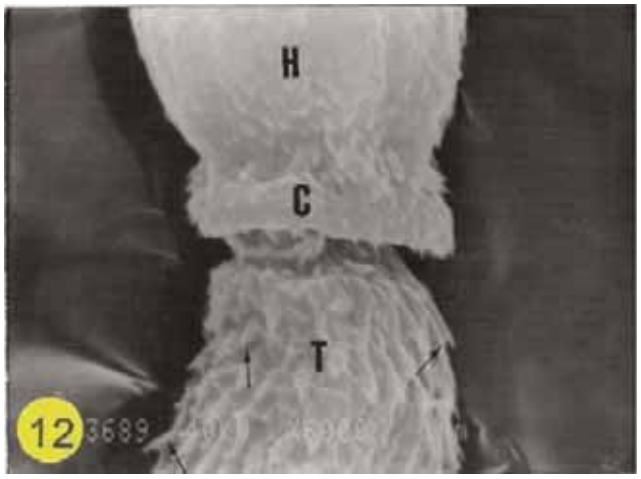

Fig. 12: Scanning electron micrograph of 3 min UV-irradiated *S. mansoni* cercaria showing the collar-like structure (C) and the proximal part of the tail (T) which provided with large spines directed posteriorly (arrows).

described by Hockley (1968) as smooth surface collar. In the present investigation the surface of the collar appeared spiny. Sobhon *et al.* (1988) on their investigations on *S. japonicum* cercariae described the collar region as a heavily spined tegumental area.

The opening of the penetration gland ducts are situated at the anterior tip of the head region in specific pattern in *S. japonicum* and *S. mansoni* cercariae (Robson and Erasmus, 1970, Short and Cartrett, 1973 and Sobhon *et al.,* 1988). However, the ducts opening of penetration glands were not observed in the present investigation since the anterior tip of the body is provided with three spiny tegumental folds. It is possible that such tegumental folds covered the ducts opening of penetration glands. The ducts opening of penetration glands were not seen by Hockley (1968) on his work on *S. mansoni* cercariae. The present observations showed that there were no changes on the surface topography of *S. mansoni* cercariae exposed to UV-radiation.

The radiation-attenuated schistosome vaccine induces a high level of protective immunity in rodents, and > 50% protection to a challenge with normal larvae has been achieved in primates (Yole *et al.,* 1996). Generally, it has been found that immunization with defined antigens is generally less effective at inducing host protection against experimental infection with *S. mansoni* than vaccination with attenuated infective cercariae (Ganley-Leal *et al.,* 2005). Li *et al.* (2002) made a preliminary study on *S.japonicum* cercariae antigens before and after ultraviolet irradiation; they were revealed novel antigens on UV irradiated cercariae. In addition, the protection with irradiated cercariae led to decreased pathology or reduced development of granulomas in the liver and increases the level of the inflammatory tumor necrosis factor- α (TNF-α) that could enhance inflammatory reactions due to the activation effects on macrophages, eosinophils and lymphocytes (Joseph *et al.,* 2004, Torben and Hailu, 2007b).

Several possibilities for explanation of the increased immunogenicity of irradiated larvae were summarized by Hewitson *et al.,* 2005. One of them is their delayed and truncated pattern of migration as they move slowly through the skin, skin draining lymph nodes, and are delayed in their arrival in the lungs where migration is truncated. This means there is greater opportunity for interaction of parasite antigen with immune cells at these sites, which may in turn prim of a protective response. In addition, irradiation may alter the antigens present on the surface of the parasite as it may convert a poorly immunogenic surface molecule into one that is highly immunogenic. Irradiation may also result in the greater exposure of certain cryptic antigens that are highly protective but ordinarily only exposed to a limited extent (i.e. by normal larvae). It is also possible that irradiation affects some non-protein component of the parasite in the tegument as normal and irradiated parasites have different lectin-binding properties, indicating differences in their surface carbohydrates.

Harrop and Wilson (1993) on their stereoscan study on gamma irradiated larvae of *S. mansoni* at the lung stage of development reported that irradiation of cercariae impairs neuromuscular function in developing schistosomula, since the irradiated larvae exhibited random constrictions, probably resulting from contraction of circular muscle fibers, at intervals along the length of the body and showed subtle differences in motility. They suggested that these abnormalities account for persistence of attenuated larvae in the skin-draining lymph nodes and lungs, 2 events that are instrumental to the induction of protective immunity in this vaccine model.

DNA is often damaged by many environmental agents (natural solar irradiation for example) that lead to the up-regulation of several genes involved in different repair mechanisms. Recently, studies showed that a gene called nucleotide excision repair gene (NER) is involved in mechanism for removing a broad spectrum of different DNA damages. It was revealed that the correlation of these genes with their analogues in other eukaryotes (Silva *et al.,* 2007). It seems that the *S.mansoni* irradiated cercariae may be has a damage repair genes to some extent as Mohamed (1999) studied the surface ultrastructure of adult *S. mansoni* worms developed from ultraviolet-irradiated cercariae, she observed a generalized deformities in the different regions of male and female worms originating from irradiate cercariae. These observations suggest that the repairing genes are important repair pathway during the complex life cycle of *S.mansoni.*

Finally, we can suggest that the changes seen in the adult worms developed from UV-irradiated cercariae may be due to the immune responses of the host induced by attenuated cercariae Also; it may be due to the mutagenic effects of the UV-radiation which appeared in the adult stages. Further investigations on the DNA of cercariae exposed to UV-radiation must be done.

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