Isolation and characterization of *Schistosoma mansoni* gene coding for antigenic protein from UV irradiated cercariae cDNA expression Library

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Abstract: The attenuated cercariae model is such a good one for facilitating the identification of antigens capable of evoking the protective host immune response which may be the first step towards the production of an effective anti-schistosomal vaccine. Although it is well known that irradiated cercariae is an effective vaccine against schistosomes in the experimental model, the actual effect of irradiation on DNA, RNA and protein to produce this protection is unknown. In this work in the schistosomiasis research we have try to isolate post-irradiation gene transcripts encoding antigens with immunogenic potentiality. SM21.7 was considered to be such a larval stage antigen and its localization in the tegument and subtegument layers confirmed its importance to be used as a target vaccine candidate in case of hosts challenged with *S. mansoni.* The present study was to clone some gene(s) encoding antigen(s) isolated from UV irradiated *S. mansoni* expression library that might be vaccine candidates. The antigens were recognized by protective antibodies (IgG fraction) separated from rabbits vaccinated with irradiated cercariae. We have screened UV irradiated *S. mansoni* cercariae cDNA expression library constructed in lammda ZAPlI, using IgG fraction taken from rabbits vaccinated with irradiated cercariae. The immunoscreening resulted in eight clones that may code for antigenic proteins. Two highly positive clones were isolated and designated UV-Irradiated *S. mansoni* cercariae 1 and 2 (UVIRSmC1) and UV-IRSmC2. The (UVIRSmC2) clone was determined by restriction enzymes digestion and sequence analysis. The gene has a total size 1088 bp and showed a high degree of identity with differences at 5' end the amino acid level with previously reported *S. mansoni* antigens: SM21.7 and SMS01. The gene encodes a 21.7 kDa antigen with the highest point of hydrophilicity from amino acid 33 to amino acid 38. The significance of the changes that occurred at the noncoding region sequences of the gene compared to the sequences of SM21.7 and SMS01may have a role in the deference of response to normal and UV irradiated cercariae. [The Journal Of American Science. 2007;3(4):99-112]. (ISSN: 1545-1003).

Keywords: *Schistosma mansoni*, Irradiated cercariae, Sm21.7, SMS01, EF-hand, Ca^{2+} binding motif

1. Introduction

Schistosomiasis (also known as Bilharzia), infection with the helminthes parasites in the genus *Schistosoma,* remains an important infection in many tropical areas, especially Africa. More than 200 million people have schistosomiasis, with 20 million exhibiting severe symptoms (Zhang *et al.,* 2007). Recent analyses suggest that the morbidity due to schistosomiasis is grossly underestimated (King *et al.,* 2005), resulting in an estimated 280,000 deaths annually in sub-Saharan Africa alone (Hotez *et al.,* 2006). Since the mid-1980s praziquantel has been the drug of choice for schistosomiasis; effectively it is currently the only choice available. Artemether has shown promise as a new drug for schistosomiasis, targeting larval parasites more effectively than praziquantel, which is primarily effective against adult parasites (Utzinger *et al.,* 2003).

A schistosome vaccine would provide a useful tool for the control of *S. mansoni*. In spite of several decades of research an effective vaccine remains elusive but the successful induction of high levels of protective immunity in laboratory hosts, by radiation-attenuated (RA) cercariae, nevertheless gives hope that it is feasible (Coulson, 1997).

Current advances in post-genomic techniques are providing new avenues to identify the secreted and surface-exposed antigens that mediate protection (Curwen *et al.,* 2004, Dillon *et al.*, 2006) and should eventually lead to replacement of the RA vaccine with recombinant protein formulations. Once their protective potential has been established in laboratory models, human vaccine trials will be required. Such trials will inevitably be undertaken in endemic areas where many people receiving the vaccine will either harbor a schistosome infection or have previously been infected and had curative chemotherapy. Given the fact that, schistosome infections rapidly down modulate from the acute to the chronic stage (King, 2001).

The protective activity induced by vaccination with live attenuated cercariae was found to be directed against early larval stages and associated mainly with IgM and IgG antibodies (Jwo and LoVerde, l989; Mangold and Dean, 1992). The mechanism of protection induced by radiation-attenuated larvae is not completely understood and the molecular effects of radiation or the larval stages that elicit this protection have not yet been recognized. Therefore the aim of the present study was to clone some genes encoding proteins (antigens) isolated from UV irradiated *S. mansoni* expression library that might be vaccine candidates. The antigens were recognized by protective antinodes (IgG fraction) separated from rabbits vaccinated with irradiated cercariae. Also, the effects of UV irradiation, if any, on the genetic material of the parasite and consequently on the released antigens were investigated.

2. Materials and Methods

2.1. Parasites and animals

An Egyptian strain of *S. mansoni* cercariae and the NewZealand rabbits (1-1.2 Kg) were supplied by Schistosome Biological Supply Programm (SBSP) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Fecal screening and serological tests (indirect haemo-agglutination, circum oval precipitin test) showed that all were negative for *S. mansoni* eggs and antibodies.

2.2. UV irradiation of *S. mansoni* cercariae

UV irradiation of *S. mansoni* cercariae was carried out according to Dean *et al.,* (1983). Briefly, the source of UV irradiation was An S-68 Mineral Light Lamp (Ultraviolet. Products, Inc.) , rated deliver 95% of its output at 254 nm. The cercarial suspension was placed in a clear flat-bottomed glass dish; the cercarial suspension depth was adjusted to 1.2 cm. The tubular UV bulb was centered horizontally over the dish position, parallel to the long axis of the dish, at a distance 22.2 cm above the water surface. The UV lamp was suspended from the roof of a dark box inside which the dish was put to avoid the harmful effects of UV radiation on the eyes and skin. The UV intensity at the water surface, as measured by a J-255 short wave UV meter (Ultraviolet, Products, Inc.), was 110 microwatts/cm^2. Cercarial suspensions were exposed to UV radiation for 90 seconds and used in different ways. The first way was for animal infection and the second was for RNA preparation and finally for cercarial antigen preparation.

2.3. Preparation of Sera

Vaccinated and normal rabbit sera were obtained from NewZealand rabbits, immunized by exposure to UV-irradiated *S. mansoni* cercariae. Sera were prepared according to Jwo and LoVerde (1989). In Brief, *S. mansoni* cercariae were irradiated and used within 10-15 min for vaccination 5,000 cercariae / dose / animal (Dean *et al.,* 1983 and Shi *et al.,* 1993). The animals were washed and cleaned with warm water. Then, animals were injected with thiopental and *S. mansoni* irradiated carcariae were applied to the lateral shaved abdominal skin. The vaccination process was carried out at 0, 25 and 51 days. Then, the animals were killed, the blood was collected in

clean sterile tubes, and the serum was isolated for further investigation.

2.4. Isolation and purification of IgG fractions from whole serum

The IgG proteins of normal and vaccinated rabbit sera (NRS and VRS) were purified by protein G agarose affinity chromatography. Readymade protein G agarose minicolumn (GiBCO BRL) was used in this study. The affinity chromatography was done as recommended by the manufacture manual for the purification of IgG fraction. A partial purification step has been carried out by precipitation of immunoglobulins using supersaturated ammonium sulphate solution. The concentration of the IgG was determined using the method of Lowery *et al.,* (1951). The purity of IgG fractions was measured using SDS-PAGE and western blot was used to detect the antigencity. The sera were kept at 4°C until used (Harlow and Lane, 1988).

2.5. Preparation of *S. mansoni* cercarial antigen

UV irradiated *S. mansoni* cercariae (~ half million cercariae), were homogenized with 400µl Tris-buffered saline (TBS) in glass-Teflon homogenizer, placed on ice. Then was transferred into a microcentrifuge tube and centrifuged at 12,000g for 10 minutes at 4°C. Supernatant was transferred to a new tube and protein concentration was estimated by using the method of Lowery *et al*., (1951).

2.6. Western blot analysis

The UV-irradiated cercarial proteins or IgG proteins were separated electrophoretically throughout 10% polyacrylamide gel under reducing conditions according to (Laemmli *et al.,* (*1970*). Then the I gG proteins were stained with Coomassie Blue brilliant stain or transferred onto nitrocellulose membrane (0.45pm pore size) in a Mini Bio-Rad protein transfer unit (Burneete, 1981), using transfer buffer (25 mM Tris, 192 mM glycine, 20% v/v methanol, pH 8.3) for 2 hr at 100 volts.

The nitrocellulose membranes were blocked in 10X TBST (Tris-buffered saline Tween-20) containing 3% BSA, for 1 hr. The membranes were rinsed in TBST, and incubated with antiserum (VRS and NRS) for 1 hr with gentle shaking. Then the blots were washed four times in TBST and incubated with alkaline phosphatase conjugated anti-serum for 1 hr. After incubation, the blots were washed in TBST and soaked in Nitro-blue tetrazolium (NBT) and 5-bromo 4-chloro3-indolyl phosphate (BCIP) **(Sambrook *et al.,* 1989).**

2.7. Construction of cDNA expression library

Total RNA was isolated from the irradiated *S. mansoni* cercariarae by using acid guanidium thiocyanate-phenol-chloroform extraction method (Chomcznski and Sacchi, 1987). The quality of the preparation was evaluated by spectrophotometric reading and agarose gel electrophoresis stained with ethidium bromide. Undegraded total RNA was subsequently to isolate poly A^+ RNA (mRNA) using Oligo-dT cellulose column as described by (Smbrook *et al.,* (1989). A cDNA expression library was constructed using the (λZAPII cDNA cloning system of Stratagene (Short *et al*., 1988), as described by the manufacturer's instruction (Stratagene, La Jolla, CA).

2.8. Immunoscreening of the cDNA Library

The constructed λZAPII cDNA expression library was immunoscreened as described by Huynh *et al.,* (1986)**.** In Brief, plaques were plated onto *E. coli* host strain XL1-Blue at a density of 10^3 plaques per plate and incubated at 42°C for 3 hr. Nitrocellulose filters were presoaked in 10 mM isopropyl-D-thiogalactoside (IPTG) placed onto the plates and incubated at 37°C overnight. The filters were washed in TBST (20 mM Tris-HCl pH 7.5, 150 mM NaCL with 0.05% Tween 20), and blocked in TBS containing 2% bovine serum albumin (BSA). The nitrocellulose filters were probed with the primary antisera VRS and normal rabbit serum (NRS) as a negative control. Then, the filters were washed, incubated in a secondary antibody (BRL, Gaithersburg, MD), diluted in TBS. Bound second antibody was detected using (NBT) and (BCIP).

2.9. Identification and characterization of a positive clones

The clones that remained positive through three rounds of purification were *in vivo* excised, using R408 helper phage (Stratagene, La Jolla, CA). The phagemids were transferred to *XL1-Blue* cells and plated on LB/ampicillin plates. The DNA of the positive clones were extracted and digested with *XhoI* and *EcoRI* to estimate their molecular size. The isolated clones having the most positive signal and the highest molecular size were chosen for further studies. Both strands of the DNA insert of the isolated clones were sequenced by the dideoxy-nucleotide termination method (Sanger *et al.,* 1977) using Sequnase Version 2.0 (Unistated State Biochemical). PC/GENE software (Intelligentsias, Inc.) and Basic Local Alignment Search Tool (BLAST) programs were used to perform sequence analysis and homology comparisons based on the deduced amino acid sequence (Altschul *et al.,* 1990).

3. Results

3.1. Isolation of IgG fractions of vaccinated and normal rabbit sera

The IgG fractions of normal rabbit serum (NRS) and vaccinated rabbit serum (VRS) were partially purified by precipitation of immunoglobulin using ammonium sulphate followed by affinity chromatography using protein G-agarose. To determine the purity of the isolated IgG fraction, the bound fractions (IgG) and unbound were subjected to 7.5% SDS- PAGE. The prepared IgG fractions have shown to be free of other serum proteins (Figure 1).

3.2. Western blot analysis

Western blot analysis was carried out; first to assess the ability of the VRS-IgG to detect the cercarial antigen bound to nitrocellulose membrane to determine the appropriate primary antibody dilution and secondly, to detect the presence of cross reactivity with phage lysate. Finally to check the avidity of the secondary antibody conjugate for the primary antibody for sera before and after treatment using different concentrations of *E. coli* phage lysate (25ng and 100ng) with one dilution of primary antibodies VRS-IgG and NRS-IgG (1:1000). The results indicated a highly background with high positive reaction as an indicator from cross reaction as showed in figure (2) lanes 3 and 4 (100ng) and lanes 5 and 6 (25ng). Therefore the serum was reabsorbed by immobilized on nitrocellulose filter before immunoscreening of the library. The VRS and NRS IgG protein were tested after the treatment with *E. coli* phage lysate by using the same concentration like before treatment and the same dilution of primary antibody (1:1000) as showed in figure (2) lanes 7, 8 (100ng), and 9 and 10 (25ng) of *E. coli* phage lysate. The low concentration of *E. coli* phage lysate antigen did not give visible colour with VRS-IgG protein lane 9 or with NRS- IgG protein lane 10 (Figure 2).

To assess the antibody dilution for immunoscreening and to ensure the efficiency of treatment in removing anti *E. coli* antigen, Western blot was carried out after treatment using 3 different dilutions of primary antibody (1:600; 1:1200 and 1:1800) with 2 different concentration of UV irradiated cercarial antigen 100 µg (figure 3 lanes 4, 6 and 8) and 25 µg (lanes 5, 7 and 9) compared with NRS-IgG (lanes 10 and 11) with the same dilution and concentration.

The results showed that irradiated cercarial proteins were recognized by VRS only. The treatment was efficient in removing anti. *E. coli* antigen, so, there is no need for additional treatment.

3.4. Identification and Characterization of UVIRSmC2

3.4.1. Restriction Enzymes

The immunoscreening of UV irradiated *S. mansoni* cercariae cDNA expression library using of VRS-IgG fraction identified eight positive clones that survived through three rounds of plaque purification. The pattern of DNA digestion of one of the identified positive clones, designated UVIRSmC2, with *EcoR*I and *Xho*I revealed that the DNA of the insert has two internal sites for *EcoRl* and the estimated molecular size around1 kb (Figure 4).

3.4.2. DNA sequences and computer software analysis

The sequence of UVISmC2 insert revealed that the insert has 1088 bp Long, which corresponds to a translation product of 184 amino acids corresponding to a protein of 21.7 kDa, and it revealed an open reading frame of 552 nucleotides starting by the first methionine codon, ATG, at the base pair 376 and ending by the codon TAG at the base 928; two polyadenylation signals were identified (Figure 5).

The use of the BLASTN (for DNA) program to search the similarity between the DNA sequence of the insert of the isolated clone, UVISmC2 and other DNA sequences, using non-redundant Gene Bank + EMBL + DDBJ + PDB sequences database has revealed a high degree with *S. mansoni* Sm21.7 (Francis and Bickle, 1992) and S. *mansoni* antigen (Ahmed *et al.,* 2001). Also, a significant similarity was detected with a score of 420 with S. *japonicum* 22.6 kDa tegumental associated antigen (Jeffs *et al.,* 1991 Waine *et al.,* 1994: Yang *et al.,* 1997) and a score of 360 with *S. mansoni Sm* 22.6 antigen (Stein and David, 1986; Jeffs *et al.,* 1991).

The search with BLASTP (for protein sequence) program to investigate similarity between the predicted amino acid sequence and other protein sequences, using non-redundant Gene Bank CDS translations +PDR+ Swiss port+ PIR database revealed a striking identity with score of 964 with S. *mansoni* 21.7 kDa antigens (Francis and Bickle, 1992: Ahmed *et al.,* 2001). Also, a similarity with a score of 276 was detected with S. *mansoni* 22.6 kDa antigen (Stein and David, 1996; Jeffs *et al.*, 1991) and with a score of 270 with *S. japonicum* 22.6 kDa antigens (Jeffs *et al.,* 1991; Waine *et al*., 1994; Yang *et al*., 1997). Figure (6) represents the alignment between the predicted protein encoded by UVISmC2 insert and 22.6 kDa antigens of S. *mansoni and S. japonicum.* Amino acid composition analysis of the DNA sequence revealed the presence of a high percent of amino acids lysine (9.78%) glutamic acid (9.23%) and serine (7.6%). The isoelectric point was found to be at pH 7.26 and the greatest point of hydrophilicity was located from amino acid 33 to 38 that is always found to be correlated with a known antigenic determinant (Hopp and Woods, 1981). PC/GENE Computer analysis revealed the presence of sites and signatures in the predicted protein sequence as follows: 4 protein kinase C phosphorylation sites, 5 casein kinase II phosphoylation sites, one tyrosine kinase phosphorylation site, one N-myriostylation site, and one EF-hand calcium- binding motif.

The identified EF-hand Ca^{2+} binding motif in 21.7 kDa antigen encoded by UVISmC2 was compared with previously reported EF-hand Ca^{2+} binding motif in 22.6 kDa antigen of S. *mansoni* and S. *japonicum.* In the 21.7 kDa antigen, the domain was located between amino acids 42-69; while for the 22.6 kDa antigen of S. *mansoni* and S. *japonicum,* the domain was located between amino acids 47-74 and 48-75 respectively (Figue 7).

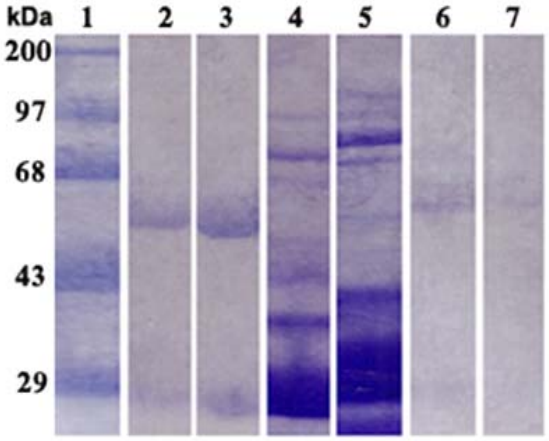

Figure (1): SDS-gel electrophoresis of fractions eluted from agarose affinity column. Lane 1: Low molecular weight protein marker. Lane 2: Unbound fraction of NRS. Lane 3: Unbound fraction of VRS. Lane 4: Bound fraction of VRS. Lane 5: Bound fraction of NRS. Lane 6: Normal rabbit sera (polyvalent). Lane 7: Vaccinated rabbit sera (polyvalent).

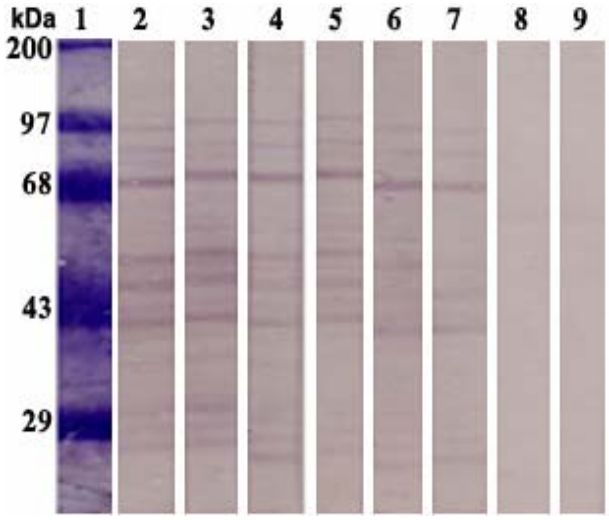

Figure (3): Western blot analysis of VRS and NRS-IgG treated against UV irradiated cercarial antigens. **Lane 1**: Low molecular weight protein marker. **Lane 2** :(150 µg /ml) UV. Irradiated cercarial protein with (1:500) treated VRS IgG. **Lane 3**: (75 µg /ml) UV irradiated cercarial protein with (1: 500) treated VRS IgG. **Lane 4**: (75 µg /ml) UV irradiated cercarial protein with (1:1000) treated VRS IgG. **Lane 5** :(25 µg / ml) UV irradiated cercarial protein with (1:1000) treated VRS IgG. **Lane 6** :(100 µg /ml) UV irradiated cercarial protein with (1:1500) treated VRS IgG. **Lane 7** :(25 µg /ml) UV irradiated cercarial protein with (1: 1500) treated VRS IgG. **Lane 8** :(100 µg /gm) UV irradiated cercarial protein with (1:500) treated NRS IgG. **Lane 9** :(25 µg /ml) UV irradiated cercarial protein with (1:500) treated NRS IgG.

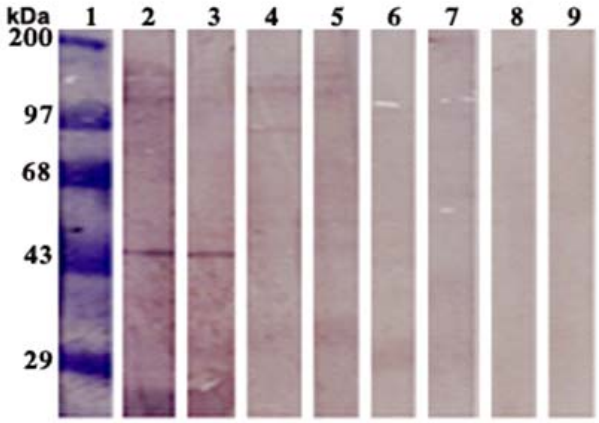

Figure (2): Western blot analysis of VRS and NRS-IgG treated and untreated against *E. coli XL-1-Blue* phage lysate. Lane 1: Low molecular weight standard; Lane 2:Untreated VRS (1:1000) with 150 ng of *E. coli* lysate; Lane 3:Untreated NRS (1: 1000) with 150 ng of *E. coli* lysate; Lane 4:Untreated VRS (1:1000) with 25 ng of *E. coli* lysate; Lane 5:Untreated NRS (l: 1000) with 25 ng of *E .coli* lysate; Lane 6:Treated VRS (1: 1000) with 150 ng of *E. coli* lysate; Lane 7:Treated NRS (l: 1000) with 150 ng of *E. coli* lysate; Lane 8: Treated VRS (1:1000) with 25 ng of *E. coli* lysate; Lane 9:Treated NRS (1:1000) with 25 ng of *E .coli* lysate.

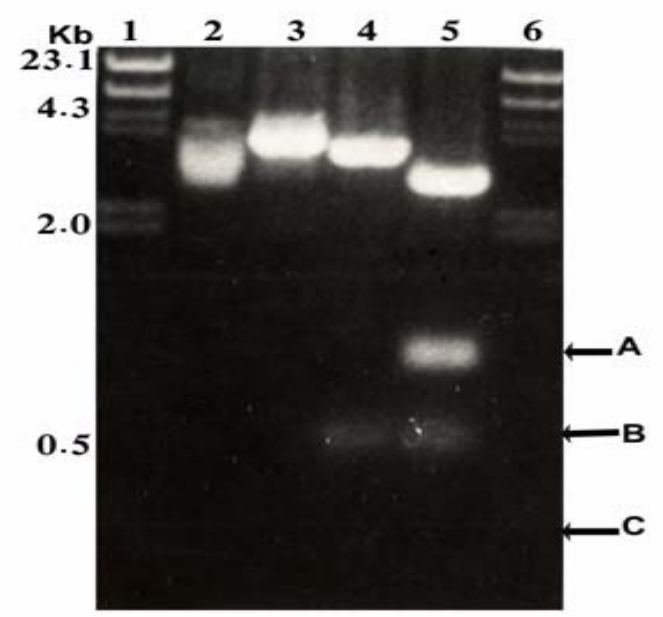

Figure (4): Agarose gel electrophoresis pattern of UVISmC2 phagemid. Lanes 1 and 6 represent Lambda DNA marker digested with *Hind*III (23.1-0.5kb). Lane 2 undigested DNA. Lane 3: *XhoI* digested DNA. Lane 4, *EcoRI* digested DNA. Lane 5 *XhoI* and *EcoRI* digested DNA of UVISmC2, respectively.

Figure (5): cDNA sequence of UVISmC2 clone with the predicted amino acid sequence immediately beneath. Nucleotides are numbered from the end of *Eco*RI adaptors and the amino acids from the initiator methionine; **M**. Two potential polyadenylation sequences are shown underlined.

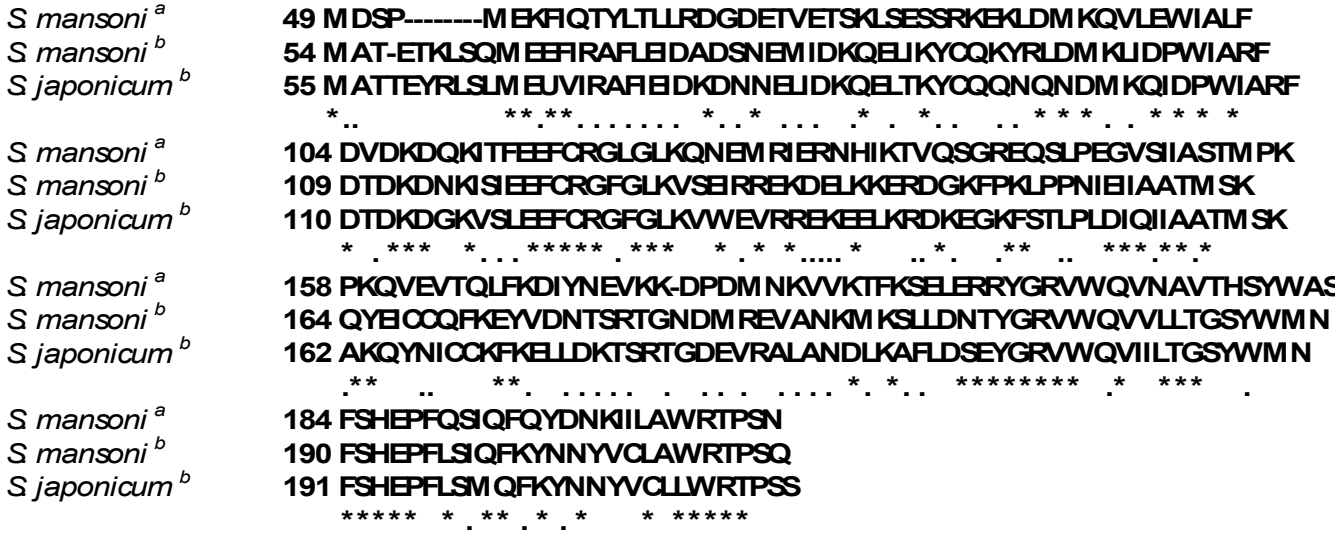

Figure (6): Alignment between the predicted proteins encoded by UVISmC2 insert, *S .mansoni* 21.7 kDa antigens and 22.6kDa antigen of *S. mansoni* and *S. japonicum.*

*a position in the alignment is perfectly conserved a position is well conserved.

[a] The *S .mansoni* 21.7 kDa antigen encoded by UVISmC2 insert is indicated by *S. mansoni*

[b] The *S. mansoni* and *S. japonicum* 22.6 kDa antigen sequences are indicated by *S. mansoni* and *S. japonicum* respectively.

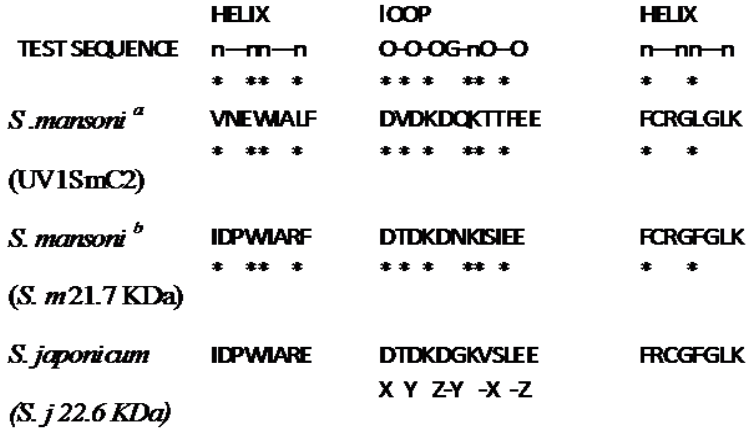

Figure (7): Comparison of the EF hand of test sequence and the deduced protein of UVISmC2 insert *S .mansoni* 21.7 kDa antigens (a) and 22.6 kDa antigens of *S .mansoni* and *S .japonicum.* In the test sequence **(Strynadka and James, 1989)** O denotes residue with side chain oxygen (D, E, N, Q, S, T); n denotes a non-polar residue; G denotes glycine; - denotes any amino acid may appear in this position; * denotes residue matching the test sequence, X, Y, Z, -X,-Y, -Z refer to the six Ca^{2+} ion ligating positions in the loop.

[a] The *S. mansoni* 21.7 kDa antigen encoded by UVISmC2 insert is indicated by *S. mansoni*

[b] The *S. mansoni* and *S .japonicum* 22.6 kDa antigen sequences are indicated by *S. mansoni* and *S. japonicum,* respectively.

5. Discussion

The radiation-attenuated (RA) schistosome vaccine is highly effective under laboratory conditions but, for ethical and practical reasons, cannot be used in humans. Nevertheless, it serves as a compelling model for the development of a recombinant vaccine **(**Coulson, 1997; Wilson and Ivens, 2006). The protective capabilities of the RA vaccine were originally established in rodents (Dean, 1983) and subsequently extended to primates (Eberl *et al.,* 2001, Soisson *et al.,* 1993 and Yole *et al.,* 1996a,b), with up to 86% protection obtained after five vaccinations of the olive baboon (*Papio anubis*) (Kariuki *et al.,* 2004). The baboon has the capacity to harbor a substantial schistosome infection long term, unlike mice, which succumb to egg-induced pathology even with low worm burdens (Warren *et al.,* 1972). This makes it an ideal host to investigate the interaction of infection and vaccination. Another advantage of baboons is that they are closer phylogenetically and in body scale to humans, so that experimental results are likely to have a greater relevance (Nyindo and Farah, 1999).

Irradiated cercariae have been shown to stimulate the host immune system and confer a high level of resistance without causing the pathological symptoms of schistosorniasis (Richter *et al.,* 1995). Thus, in the present study, we tried to clone some genes encoding proteins (antigens) isolated from UV-irradiated *S. mansoni* expression library recognized by protective antibodies (IgG fraction) isolated from rabbits vaccinated with irradiated cercariae. Identified cDNA clone, UVISmC2 encoding an antigen showed 100% identity at the amino acid level with previously identified *S. mansoni* clones, encoding 21.7 kDa antigens, isolated from *S. mansoni* sporocyst and 25 days schistosomula cDNA expression libraries (Francis and Bickle, 1992; Ahmed *et al.,* 2001). However, a lower degree of identity with other previously identified *S. mansoni* and *S. japonicum* clones, encoding 22.6 kDa antigens, isolated from *S. mansoni* and *S. japonicum* adult worm expression library was detected (Stein and David, 1986; Jeffs *et al.,* 1991; Waine *et al.,* 1994: Yang *et al.,* 1997). At the nucleic acid level, the DNA sequence homology search revealed a striking degree of identity with *S. mansoni* antigen, encoding 21 .7 kDa antigen, in the open reading frame (ORF) sequence (Francis and Bickle, 1992; Ahmed et *al.,* 2001).

The UVISmC2 clone, isolated from UV irradiated *S. mansoni* cercariae cDNA library, was characterized by the differences at the level of the nucleotide sequence restricted to the 5' and 3' non-coding sequences. The first 295 nucleotides 5' non-coding sequence was completely different from the previously sequenced 5' non-coding sequence of Sm21.7, and particuar1y SMS01 that nearly encodes the full length sequence of 21.7 kDa antigen and the 3' non-coding sequence that has a truncated non-coding sequence of 107 bp preceding the poly A tail. Also, an ambiguous unmatched T preceding the poly A tail was detected.

The identified EF-hand Ca^{2+}-binding motif in 21.7 kDa antigen encoded by UVISmC2 has been compared with previously reported EF-hand Ca^{2+}-binding motif in 22.6 kDa antigen of *S. mansoni* and S. *japonicum* (Strynadka and James, 1989), which was based on the earlier work of Tufty and Krestinger, (1975). The comparison study revealed divergence in one of the helices flanking the binding loop of 21.7 kDa antigen of UVISmC2 and 22.6 kDa antigens of *S. mansoni* and S. *japonicum.* Previous studies (Marsden *et al.,* 1988; MacManus *et al.,* 1990) indicated that a complete helix-Loop-helix unit is not required for the Ca^{2+}- binding. Hence, it is possible that the loop can bind Ca^{2+} even if the flanking helices do not match the test pattern exactly. This may explain why the Ca^{2+}-binding motif in *S. japonicum* antigen is functional.

Neither S. *mansoni* 21.7 kDa nor 22.6 kDa antigens loops bind calcium. The crucial difference in the Sm22.6 upon which its inability to bind Ca^{2+} was explained is that it lacks the glycine in the Ca $^{2+}$-binding loop (Stein and David, 1986). The same may also hold true for the 21.7 kDa protein encoded by

UVISmC2 in which glycine at position 6 is replaced by glutamic acid. From the previous findings, it is clear that the 21.7 kDa antigen (encoded by UVISmC2) is highly related to 22.6 kDa antigen which was identified in different schistosome species. Francis and Bickle (1992) suggested that the 21.7 kDa and 22.6 kDa antigens may be derived from an ancestral protein that had a functional calcium binding domain.

Sites annotated analysis of the predicted protein encoded by UVISmC2 has shown that it has many kinase phosphorylation sites along the protein. Protein kinases represent critical nodes for the amplification and distribution of signals (Watson *et al.,* 1992). N-myristoylation site also was detected in the predicted protein; it is post-translational modification of the protein sequence. An appreciable number of eukaryotic proteins are acylated by the covalent addition of myristate (a C14-saturated fatty acid) to their N-terminal residue via an amide linkage (Towler *et al.,* 1988).

The highest point of hydrophilicity (Hopp and Woods, 1981) was detected in the predicted protein encoded by UVISmC2 insert from amino acid 33 to amino acid 38. Hydrophilicity is associated with antigenic determinants because hydrophilic regions are on the outside of a protein. Also, the most Polar Regions may provide the greatest interactions for bonding between the antigen and antibody. This finding may be in agreement with the assumption of Francis and Bickle (1992) of the presence of immuno-dominant epitope(s) at the 5' end of the gene encoding 21.7kDa antigen, based on the observation that all of the separately derived clones encoding 21.7 kDa produced a similarly sized product corresponding to the whole gene.

In this study, the encoded by UVISmC2 clone (21.7 kDa) was isolated from cercariae cDNA expression library. Northern blot analysis demonstrated that mRNA for Sm21.7 is present in the sporocyst, 3 hours schistosomula and adult stages (Francis and Bickle, 1992). In addition, the SMS01 was isolated from schistosomula 25 days (Ahmed *et al.,* 2001). Hence, the 21.7 kDa antigen is expressed in all post snail stages of the S. *mansoni* life cycle.

Immunocytochemical localization on cryosections of adult worms using specific antibodies produced by the immunization of rabbits with purified SMS01 protein (21.7 kDa) showed that this antigen is located in the tegumental region and dispersed among the parenchymal cells of the adult schistosome parasite (Ahmed *et al.,* 2001).

Since the tegument is the outer covering of the parasite and serves as an interface between the host immune system and the parasite, the antigens associated with the tegument would be the major focus for development of a vaccine and/or immunodiagnostic reagents for schistosomiasis (Bergquist, 1990). So, the localization of the 21.7 kDa protein in the tegumental and subtegumental layers and its expression in all post snail stages of the parasite draw the attention to the importance of this antigen.

In the vaccinated rabbit serum (VRS) model, this antigen has been isolated several times using different strategies which means that this antigen may be one of the target antigens for protection in this model. In addition, this antigen was designated "vaccine dominant" as the antigen was recognized preferentially by mouse vaccine sera compared with mouse infection sera (Francis and Bickle, 1992).

On the empirical vaccination level, the 21.7 kDa antigen encoded by UVISmC2 succeeded to induce a good degree of protection in experimental animals. Immunization experiments in mice have revealed 40-70% protection against challenge infection with S. *mansoni* cercariae. Also, the antigen may be considered an antipathology vaccine since it plays an important role in the reduction of granuloma formation (Ahmed *et al.,* 2001). In experimental schistosomiasis, because sterilizing immunity has not yet been attained and because the disease is largely caused by the host's response to eggs, vaccination to reduce

granulomatous inflammation and fibrosis is suggested as an alternative approach (Botros *et al.,* 1996). All of these findings support the importance of this antigen, 21.7 kDa, encoded by UVISmC2 in the irradiated cercariae as a vaccine candidate.

UV light was reported to induce mutations (Griffiths *et al.,* 1996). DNA was identified as the principle biological target for UV irradiation (Imlay and Linn, 1988; Wales and Kusel, 1992). Therefore, the changes detected in the sequence of the 5' and 3' noncoding regions raise many questions about their reason and significance. As there is little data dealing with the molecular basis of the UV irradiated vaccination model, it is not easy to ascertain that these changes are a direct response to the effect of UV irradiation. If these changes were related to the effect of UV irradiation, why were they restricted to the noncoding region sequences and what are their significance.

Also, the results of the present study have shown no direct effect of UV irradiation on the codon sequence of the ORF of encoded by UVISmC2 insert. Wales and Kusel (1992) postulated that irradiation distorts the structure of schistosmular proteins, converting normally weak immunogens into highly immunogenic conformations. Such abnormal, non-native antigens may be created by the absorption of high-energy radiation and by reaction with oxygen radicals or the defective proteins may be produced following synthesis on RNA and DNA templates damaged by the irradiation. Also, Wales *et al.,* (1993) reported that UV irradiation causes an immediate and striking alteration in the carbohydrate antigens expressed by schistosome larvae and postulated that the structure of glycocalyx antigens released by irradiated larvae is modified in a way that alters the pattern of processing by the proteolytic enzymes of the antigen presenting cells resulting in presentation of new antigenic determinants to T helper, stimulating their potent protective immunity.

Hence the results of the present study, in the light of these postulations, expose to view those defects or abnormalities in the post translational processing of the proteins should be a point of consideration in the UV vaccination model. Further studies, however, are required to substantiate this suggestion. On the other hand, as the reported changes in encoded by UVISmC2 sequence were restricted to the noncoding sequence, characterization of the other identified positive clones may shed some light in this respect, keeping in the mind that radiation has haphazard and unoriented effects.

6. Conclusion

In conclusion, the proven value of irradiation as attenuating agent for schistosomiasis deserves to be examined by various means. The importance of antigen 21.7 kDa encoded by UVISmC2 as a candidate vaccine is currently under investigation. Although it is well known that irradiated cercariae is an effective vaccine against schistosomes in the experimental model, the actual effect of irradiation on DNA, RNA and protein to produce this protection is unknown. In this work for the first time we have try to isolate post-irradiation gene transcripts encoding antigens with immunogenic potentiality. So far in the schistosomiasis research a post-irradiation expression library has been constructed that induce the UV affected transcriptions. This library was immunoscreened with protective sera from UV irradiated rabbits to isolate genes encoding immunogenic antigens which may acquire its immunogenicity after UV irradiation due to unknown mechanism.

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