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DNA vaccination using recombinant *Schistosoma mansoni* fatty acid binding protein (smFABP) gene

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ABSTRACT

Schistosomiasis is a fatal disease that has a negative impact on health and economics. Praziquantel (PZQ) is the drug of choice for schistosomiasis treatment, but it has no prophylactic effect; therefore, vaccination is an essential requirement in schistosomiasis control. This work was carried out to investigate the possible effect of DNA vaccination against *Schistosoma mansoni* infection using recombinant *S. mansoni* fatty acid binding protein (rsmFABP). The *smFABP* gene was cloned into the eukaryotic expression vector pcDNAI/Amp in order to obtain an smFABP-pcDNAI recombinant plasmid (DNA vaccine) and was used for the intramuscular DNA vaccination of out-bread Swiss albino mice prior to infection with *S. mansoni* cercariae. Infected groups, either DNA vaccinated or unvaccinated, were treated with PZQ at week 6 post-infection. After 8 weeks post-infection, all mouse groups were sacrificed and parasitological, immunological and histopathological parameters were studied. DNA vaccinated mice showed a high titer of anti-smFABP-IgG antibodies and acquired significant protection (74.2%, $p < 0.01$) against *S. mansoni* infection, with a reduction in ova and granuloma counts. DNA vaccinated and PZQ treated animals had higher titers of anti-smFABP-IgG antibodies and decreased (87%, $P < 0.001$) parenchymal granulomas compared to the DNA vaccinated PZQ untreated group. Infected mice, either non DNA vaccinated or vaccinated, had very high collagen content and fibrous granulomas (74%) compared to the PZQ treated group (10.3% fibrous granuloma) and PZQ treated + DNA vaccinated group (0% fibrous granuloma). In conclusion, DNA vaccination had protective and anti-pathological effects in naive mice and greatly improved the pathological status in PZQ-treated animals, suggesting an immunological and pathological modulating effect of PZQ treatment.

1. Introduction

Schistosomiasis, an acute and persistent parasitic disease, is the result of infection with blood trematode worms belonging to the genus Schistosoma. Reports estimated that at least 218 million people needed preventive treatment in 2015. To prevent or reduce morbidity, a preventive remedy must be repeated many times for many years. Seventy-eight countries reported endemic schistosomiasis transmission, but preventive pharmacotherapy against schistosomiasis on a large-scale was carried out in only 52 nations (WHO, 2017). In Egypt, schistosomiasis is one of the major public health problems (El-Khoby et al., 2000; Tendler et al., 2015; Dawaki et al., 2016). About 5–6 million schistosomiasis cases were estimated to have occurred in 1990 (Barakat, 2013). The number of cases increased to 7 million in 2006 (Steinmann et al., 2006) and reached 7.2 million by 2012 (Hotez et al., 2012). *Schistosoma mansoni* and *haematobium* are present in Saudi Arabia. A study at the genotype level using random amplified polymorphic DNA (RAPD) for isolates of *S. mansoni* from Egypt, Saudi Arabia and Puerto

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Rico showed that Egyptian strains are strongly correlated to Saudi strains, but Puerto Rican strains are not (Jamjoom, 2006). In Saudi Arabia, infection with *S. haematobium* was found mainly in Tihama Aseer and the lowland coastal plains in the southern areas, whereas infection with *S. mansoni* was shown to occur mainly in the highlands of the western areas and some parts of the Central and Northern Regions (Lotfy and Alsaqabi, 2010).

Praziquantel (PZQ) is an available and effective medicine for schistosomiasis treatment, but the re-infection rates increase in populations that are exposed to contaminated water (Mossallam et al., 2015; Rollinson et al., 2013).

The development of a human schistosomiasis vaccine is recognized as a priority and would complement existing control measures (Bergquist et al., 2002; El Ridi et al., 2014). There is no effective vaccine against schistosomiasis at present, but research to develop a vaccine against schistosomiasis is ongoing (McManus and Loukas, 2008; Mossallam et al., 2015). The majority of research has examined a single possible schistosomiasis vaccine antigen rather than investigating combined approaches. It seems likely that the use of a single antigen would not achieve the desired protective immune response because Schistosoma has a complicated life cycle (Mossallam et al., 2015).

More than 100 schistosome antigens have been identified as vaccine candidates against schistosomiasis. However, only four of them are being examined for human use: one from *S. haematobium* [the glutathione S-transferase (Sh28GST)] and three from *S. mansoni* [the tetraspanin (Sm-TSP-2), *S. mansoni* fatty acid binding protein (Sm14) and Smp80 (calpain)] (Tebeje et al., 2016). *Schistosoma mansoni* fatty acid binding protein (smFABP) or Sm14, a 14-kDa protein, is one antigen that induces partial protection in mice following vaccination in cercarial models (Tendler et al., 2015). smFABP is found in the tegument and muscles of adult worms and has putative calcium binding and actin-bundling activities (Tendler et al., 1996, 2015) and the ability to bind to linolenic and palmitic acids *in vitro* (Moser et al., 1991).

smFABP is immunogenic in irradiated cercariae immunization models. smFABP can be recognized by serum IgG in mice that are vaccinated with irradiated cercariae. It was reported that fatty acids are involved in down regulation of granuloma production *in vitro* (Oliveira et al., 1999; Alebie et al., 2014). Therefore, smFABP could be a candidate for protective immune responses induced against *S. mansoni*.

Many vaccination trials have been carried out in the last decade using different recombinant antigens (Riveau and Capron, 1996; Dupre et al., 1999). In the time that most of these antigens were characterized, vaccines against schistosomiasis were not yet developed. DNA vaccines are considered to be subunit vaccines, as they allow proteins to be expressed in mammalian cells when introducing plasmids or viral vectors carrying the selected protective antigen. The advantages of using DNA vaccines are that they produce nearly native antigenic proteins and induce powerful humoral and cellular immunities in many animal models against many diseases such as HIV, tuberculosis and cancer (Taracha et al., 2003; Fonseca et al., 2004; Lee et al., 2004).

This work aimed to evoke specific protective immune responses towards the antigen smFABP using DNA vaccination strategies. Also, due to the probable synergistic effects of PZQ in animals that are first treated and then vaccinated, we studied the effect of vaccination using this tegmental antigen on histopathological and parasitological status in mice infected or treated with PZQ. Because the DNA vaccine is recommended for humans with high precautions, we targeted animals with the vaccine in order to prevent the reservoir cycle of the worm.

2. Materials and methods

2.1. Parasites and animals

The Egyptian strain of *S. mansoni* maintained by laboratory passage in *Biomphalaria alexandrina* snails and golden hamsters was employed in the Theodore Bilharz Research Institute (TBRI), Giza, Egypt. Cercariae were obtained as described by Gregoire et al. (1987).

Female CD1 Swiss albino mice (weight 20 g ± 2 g), bred and maintained at the SBSC, TBRI, Egypt, were used for DNA vaccination and protection experiments. Animal experiments were carried out according to international guidelines and ethical conditions.

2.2. Preparation of recombinant smFABP-pcDNAI/Amp expression vector

The smFABP coding sequence was obtained from GenBank (accession No: M60895.1). The coding sequence from base 123 to base 524 was used. The sequence was subjected to internal restriction site and open reading frame analysis using the Gene Runner program (version 6.0.028.). Restriction sites *Bam*HI (GGATCC) and *Sph*I (GCATGC) were added to the 5′ end after removal of the start codon (ATG) and to the 3' end after removal of the stop codon (TAA). The indicated coding sequence was synthesized and cloned into pUC57 at GenScript HK Limited (Hong Kong) to obtain recombinant vector 1. Using Gene Runner program DNA sequences of smFABP, the gene was converted into amino acid sequences using mammalian codon preferences. Using the Protean program of Lasergene, the 3D configuration, epitopes and antigenic sites were analyzed.

Competent DH5α bacteria were prepared by the method described by Chung et al. (1989) and transformed with recombinant vector 1. Recombinant vector 1 was extracted from bacterial cells using the Qiaprep spin miniprep kit (Qiagen) according to the manufacturer's protocol. Approximately 4 μg of purified recombinant vector 1 was cut by the restriction enzymes *Bam*HI and *Sph*I (Invitrogen) simultaneously in the same reaction. The insert fragment (smFABP) was purified from the 1% gel using the Qiaquick gel extraction kit (Qiagen) according to the manufacturer's protocol. pCDNAI/Amp vector was digested and purified using the same protocol as for the insert. T4-DNA ligase (Promega) was used to ligate the insert to the linearized pCDNAI/Amp vector to prepare the expression construct (smFABP-pCDNAI/Amp). DH5α competent cells were transformed with 4 μl of ligation reaction, and recombinant clones harboring the insert were verified by restriction digestion and DNA sequence analysis. Large-scale plasmid preparations of smFABP-pcDNAI/Amp and pcDNAI/Amp vector were carried out (Green and Sambrook, 2012). Endotoxin content of the purified plasmid was done using Genscript ToxinSensorTM Chromogenic LAL Endotoxin Assay Kit.

2.3. DNA vaccination and protection experiments using smFABP-pcDNAI/Amp

Mice were divided into 7 groups (6 animals each). Group 1: non-infected and untreated throughout the whole experiment. Group 2: infected untreated control mice. Group 3: PZQ treated group in which mice were orally treated with PZQ (500 mg/kg on 2 successive days) at 6 weeks post infection. Group 4: infected and vaccinated in which mice were vaccinated with expression construct by injecting mice intramuscularly in the right and left tibialis anterior muscles (100 μg/mouse) 4, 3, 2 and 1 week before infection. Group 5: Mice were treated as in group 4 but injected with pcDNAI/Amp instead of expression construct. Group 6: infected, vaccinated and treated the same as in group 4 and treated with PZQ as in group 3. Group 7: infected, pcDNAI/Amp vector and treated, in which mice were injected with pcDNAI/Amp vector the same as in group 5 and treated with PZQ as in group 3.

Infection of mice was carried out by challenging with *S. mansoni* cercariae (100/animal) using the tail immersion method (Olivier and Stirewalt, 1952). Eight weeks post-infection, animals were perfused, sacrificed and subjected to various studies.

Two weeks after the 2nd booster with DNA expression construct, all groups were tail-bled and equal volumes of sera of the same group were pooled to test the titer of polyclonal antibodies against the smFABP protein by ELISA (Hillyer and Gomez de Rios, 1979). The antigen used to coat ELISA plates was recombinant smFABP expressed in a pET-3a

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