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Vaccination with recombinant paramyosin in Montanide ISA206 protects against *Schistosoma japonicum* infection in water buffalo

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ABSTRACT

Background: Schistosomiasis japonica is a zoonosis and presents significant public health problems in China and the Philippines. Vaccines targeting domestic animals constitute attractive control measures.
Methods: We conducted three vaccine trials to evaluate the protective efficacy of recombinant full-length paramyosin (rSj97) in water buffalo. Animals were immunized with 3 doses of rSj97 adjuvanted with ISA206 at 250 μg/dose or 500 μg/dose at 4 wk intervals before challenge with 1000 *Schistosoma japonicum* cercariae. The primary outcome was worm burden assessed by portal perfusion 8–10 weeks post challenge. Safety measures included weight, temperature, body condition score, hemogram and routine assays for hepatic and renal function.
Results: The three-dose regimen was well tolerated in all three trials. In the first trial, vaccinated buffalo had 51.5% lower worm burden post challenge compared to controls. In the second trial, buffalo immunized with 500 μg/dose of rSj97 had 57.8% lower worm burden compared to controls ($p = 0.026$). A similar but not significant reduction (60.9%) was observed with animals administered with 250ug rSj97/dose. In the third trial, buffalo immunized with a 500 μg/dose of rSj97 had 57.8% lower worm burden compared to controls ($p = 0.014$).
Conclusions: These findings indicated that rSj97 is a safe and promising vaccine candidate for schistosomiasis japonica in water buffalo.

1. Introduction

Schistosomiasis is a neglected tropical disease (NTD) caused by infection with any of the five *Schistosoma* species: *S. mansoni, S. japonicum, S. heaematobium. S. mekongi, or S. intercalatum* [1]. It is endemic in 76 countries and territories and affects over 250 million people [2]. A quality-of-life assessment defines a significant 9.5–24% disability with the most aggressive schistosome species, *S. japonicum* [3,4].

Schistosomiasis japonica is a major disease risk for more than 40 million people in China [5], and 7 million more in the Philippines [6]. It has more than 40 mammalian animals as reservoir hosts that play a role in maintaining the parasite and increasing the chances for human infection [7]. In China, there are several hundred thousand livestock, including over 200,000 buffalo currently infected [8]. Considering the zoonotic nature of schistosomiasis japonica and the important role buffalo and cattle play in environmental contamination and transmission to humans [9–15], a transmission blocking veterinary vaccine for domesticated bovines would provide an additional and unique approach to schistosomiasis japonica control.

Paramyosin is a 97-kDa myofibrillar protein with a coiled-coil structure found only in invertebrates. In addition to the muscles of all 3 developmental stages of *Schistosoma japonicum*, paramyosin is located on the tegumental surface of lung stage schistosomula and in the secretory glands of cercariae [16]. Immunization with paramyosin confers resistance to infection by *S. mansoni* in

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mice [17,18], and an anti-paramyosin monoclonal antibody confers resistance to infection with *S. japonicum* in mice [19].

We have demonstrated the vaccine potential of *S. japonicum* paramyosin (Sj97) in mice immunized with protein biochemically purified from *S. japonicum* adult worms [20]. This work has also been extended to domestic sheep, pigs and water buffalo immunized with recombinant fragments of Sj97 [21–24]. In immunoepidemiology studies conducted in an *S. japonicum* endemic region of the Philippines, we have demonstrated that individuals with a Th2 biased cellular response [25] or IgE biased humoral responses to Sj97 [26] are relatively resistant to reinfection following treatment compared to individuals without these responses.

Together, these data support paramyosin as a leading vaccine candidate for schistosomiasis, but the low yields of full-length recombinant protein have hampered its further development [27]. Our group overcame this scale-up challenge lately [28], which has enabled larger scale testing of both the safety and efficacy of recombinant full length Sj97. Here, we report the results of 3 vaccine-challenge experiments in buffalo conducted in 2008, 2013 and 2016 using full-length rSj97 produced at pilot scale.

2. Materials and methods

2.1. Recombinant protein production and characterization

Pilot scale production of recombinant full-length paramyosin (rSj97) has been described previously [28]. Briefly, kanamycin-resistant recombinant pET30 plasmids were transformed into BL21(DE3) and expressed at the pilot scale using our published fermentation and chromatographic purification process (see Fig. S1 of Ref. [28]). Lyophilized, vialed rSj97 was resuspended in 1 ml of LPS-free water. LPS (endotoxin) levels were determined using an FDA-cleared LAL-assay (Lonza, USA). The protein concentration was assessed with a BCA-based protein assay containing bovine serum albumin as the standard (Pierce, Rockford, IL). Protein purity was assessed with 4–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and colloidal Coomassie staining (Gelcode Blue; Pierce, Rockford, IL). Proteins on stained SDS-PAGE gels were excised and subjected to trypsin digestion and nano-liquid chromatography-tandem mass spectrometry-based peptide sequencing (ProtTech, Norristown, PA). Residual SDS was measured using a quantitative colorimetric assay as described elsewhere [29]. Secondary and tertiary protein structure analyses were performed on 0.6 nM solutions of rSj97 in the presence and absence of 0.05% SDS by circular dichroism on a spectropolarimeter (J-185; Jasco Inc., Easton, MD) with a 0.2-mm-path-length cuvette with temperature control at 25 °C. Raw data in millidegrees were converted to ellipticity and used to calculate helix fractions [30] and the presence of a coiled-coil tertiary structure [31]. The recombinant Sj97 was functionally characterized for IgG and collagen binding by enzyme-linked immunosorbent assay (ELISA)-based assays as described elsewhere [28].

2.2. Vaccination experiments

The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee at the Shanghai Veterinary Research Institute (ShVRI) in China, and the Animal Welfare Committee of the Office of Research Administration for the Lifespan IACUC.

Montanide™ ISA206 adjuvants were from Seppic, France. Adjuvant-protein formulation was prepared the day before vaccination following the manufacturer's recommendation. In brief, LPS-free water reconstituted rSj97, or lyophilization buffer (50 M sodium phosphate, 0.05% tween-20, 0.3% sucrose, pH7.4) as the control, was added to ISA206 at 50:50 (w:w) ratio. The mixtures were stirred at a low shear rate (300 rpm) for 10 min at 30 °C in a glass beaker to form the water-in-oil-in-water emulsion [32].

Male and female water buffalo aged 10 to 17 months were purchased from non-schistosomiasis endemic area in Jiangsu, China. Animals were pre-screened for intestinal helminthes using 50 g of stool with the modified saturated saline floatation method. An anti-soluble egg antigen (SEA) ELISA test was also carried out on all the buffalo using pre-immune serum in order to confirm their schistosomiasis-free status. All buffalo received 15 mg/kg albendazole orally 4 weeks before the 1st immunization for presumptive treatment of potential geohelminth infections.

Vaccination protocols were based on those of Xu et al. [33,34]. Water buffalo were vaccinated at 0, 4 and 8 weeks by injecting emulsions subcutaneously at the lower third (near the shoulders) of a buffalo neck. At 12 wk, all animals were challenged with 1000±3 cercariae shed from the infected snails percutaneously. *Schistosoma japonica* infected *Oncomelania hupensis hupensis* snails were kept in the lab of ShVRI for lifecycle maintenance.

2.2.1. The 2008 trial

Sixteen water buffalo were equally allocated into 2 groups based on their gender, age and weight. One group received 3 vaccinations of 250 μg rSj97-ISA206 at 4-week intervals, and the unvaccinated control group was injected with ISA206 emulsified with the lyophilization buffer with the same schedule.

2.2.2. The 2013 trial

Thirty-two water buffalo were allocated into 2 vaccination groups with 11 buffalo each and 1 control group with 10 buffalo based on their gender, age and weight. One group received 3 doses of 250ug rSj97-ISA206 at 4-week intervals; the second group received 500 μg/dose for 3 injections at 4-week intervals. The controls were injected with ISA206 emulsified with the lyophilization buffer.

2.2.3. The 2016 trial

Sixteen water buffalo were allocated into 2 groups with 8 buffalo each based on their gender, age and weight. One group received 3 vaccinations of 500 μg /dose rSj97-ISA206 at 4-week intervals; the other group received injection of ISA206 emulsified with the lyophilization buffer.

2.3. Sample collection

Serum samples were collected from the animals pre- and 4 weeks post each vaccination and challenge infection, as well as at the time for perfusion. In the 2016 trial, we collected serum 2, 4 and 8 weeks post-challenge corresponding to weeks 0, 4, 8, 12, 14, 16, 18 and 20 of the experiment. Whole blood (K_2EDTA-anticoagulated) was collected at 0, 4, 8 and 12 weeks for hemogram and chemistry analysis to monitor buffalo health status after vaccination.

2.4. Worm antigen preparation

SWAP (soluble worm antigen preparation) and SEA (soluble egg antigen) were prepared under endotoxin free conditions according to standard procedures [35]. In brief, 7–8 weeks after *S. japonicum* cercarial exposure, infected rabbits (~2500 cercariae/rabbit) were perfused and adult worms and rabbit livers were collected and rinsed with LPS-free PBS. The collected worms and purified eggs were re-suspended in PBS and sonicated for 4 times of 1 min on ice on full power (Fisher scientific model F60 sonic dismembrator, USA), followed by centrifuging at 30,000*g* for 30 min at 4 °C. The resulting supernatant was stored at −80 °C.