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Immunogenicity and anti-fecundity effect of nanoparticle coated glutathione S-transferase (*Sj*GST) DNA vaccine against murine *Schistosoma japonicum* infection

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ABSTRACT

There is still urgent need for a vaccine against schistosomiasis, especially in *Schistosoma japonicum* endemic areas where even a vaccine that will interrupt zoonotic transmission will be potentially effective as an intervention tool. We had developed a novel nanoparticle gene delivery system, which has proven efficacious in gene transfection to target immune cells with complementary adjuvant effect and high protective efficacy in several diseases. Here, we applied this nanoparticle system in combination with *S. japonicum* glutathione S-transferase (*Sj*GST) DNA vaccine to show the immunogenicity and anti-fecundity effect of the nanoparticle coated vaccine formulation against murine schistosomiasis. The nanoparticle-coated DNA vaccine formulation induced desired immune responses. In comparison with the nanoparticle coated empty vector, it produced significantly increased antigen-specific humoral response, T-helper 1 polarized cytokine environment, higher proportion of IFN-γ producing $CD4^+$ T-cells and the concomitant decrease in IL-4 producing $CD4^+$ T-cells. Although there was no effect on worm burden, we recorded a marked reduction in tissue egg burden. There was up to 71.3% decrease in tissue egg burden and 55% reduction in the fecundity of female adult worms. Our data showed that *Sj*GST DNA vaccine, delivered using the nanoparticle gene delivery system, produced anti-fecundity effect on female adult schistosomes as previously described by using conventional subunit vaccine with adjuvant, proving this DNA vaccine formulation as a promising candidate for anti-pathology and transmission blocking application.

1. Introduction

Schistosomiasis is still an important helminthic infection in terms of severe morbidity that can result as a consequence of infection. It disproportionately affects people with limited access to potable water and sanitation in the tropics and subtropics, with over 240 million people infected and more than 700 million people at risk of getting infected [1]. Although the disease can be effectively treated with the drug of choice Praziquantel (PZQ), reinfection occurs rapidly after mass drug administration (MDA). An effective vaccine used singly or in combination with chemotherapy is the optimal approach, especially in *Schistosoma japonicum* endemic areas where zoonotic transmission from non-human mammalian reservoirs complicates control efforts [2]. The potential for the emergence of PZQ-resistant parasite progenies underscores the need for a vaccine [3]. While an anti-schistosomiasis vaccine that elicit reduction in worm burden is desirable, anti-fecundity vaccines will be equally effective since the parasite eggs account for both the severe pathogenesis [4–6] and continued transmission [2]. In fact, Bilhvax (*Sh*GST), the most advanced vaccine against urogenital schistosomiasis in phase III clinical trial, induces anti-fecundity targeted immune response against *Schistosoma haematobium* [7,8]. Such anti-fecundity vaccine is urgently needed in *S. japonicum* endemic areas where they could initially be deployed to interrupt zoonotic transmission [9–14].

One of the major challenges in schistosomiasis vaccine development is the identification of antigens that can induce the desired immune responses leading to host resistance to reinfection. Several

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human studies in endemic areas have provided insight into the potential resistance inducing immune response for schistosomiasis [2,15–19]. Although this subject is still strongly debated, there is a consensus on the involvement of Th1 type of immune response for induction of protective immunity against human schistosomiasis [16,18], and a correlation between levels of IL-10 and susceptibility to schistosomiasis [16,20]. Indeed, protective immunity elicited by vaccination with radiation-attenuated cercariae and other promising vaccine candidates have shown to be Th1 mediated [21–30]. Co-administration of several vaccine antigens with adjuvants with capacity to potentiate Th1 responses and reduce immunoregulatory responses have also shown improved protective efficacy [28,31–38]. *S. japonicum* 26 kDa GST which has consistently proved to be one of the most promising candidates against Asian schistosomiasis has been shown to induce mainly Th1 response, leading to high antifecundity effect with very significant reduction in liver and intestinal egg burden, albeit with minimal but significant reduction in worm burden [13,14,28,39,40].

DNA vaccination has distinct advantage over other traditional systems, and is considered safe and amenable for application in disease interventions [41]. However, one of the major challenges of this novel approach is the gene delivery system for the optimum delivery of the vaccines to target cells, and adjuvant for the induction of desired type of immune response [41,42]. To address this, we had developed a number of novel nanoparticle (NP) formulations for optimum gene delivery and induction of desired immune response [43–47]. One of the most promising novel nanoparticles is a ternary complex of plasmid DNA, polyethylenimine (PEI) and gamma polyglutamic acid (γ-PGA) [43]. It is a very efficient gene delivery system which is taken up by the target cells through γ-PGA specific receptor mediated energy-dependent process, with consistently high transfection efficiency and low toxicity to mammalian cells [43]. This nanoparticle gene delivery system has been tested for DNA vaccination against many diseases, with results showing consistently high transfection efficiency and significantly improved vaccine effects [47–50]. Here, we have applied this system for DNA vaccination against murine *S. japonicum* infection using the promising 26 kDa *Sj*GST vaccine candidate. Our results showed induction of mainly Th1 immune response, which significantly reduced the fecundity of the female adult worms, even with limited reduction in worm burden.

2. Materials and methods

2.1. Ethics statement

This study adhered to the recommendations in the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, Japan (Notice No: 71). All animal experiments were performed according to Japan guidelines for use of experimental animals and approved by the Ethics Committees on Animal Care and Experimentation of Nagasaki University (Approval No: 1307291083-2) and Tokyo Medical and Dental University (Approval No: 0140275A).

2.2. Parasites and experimental animals

Six weeks old female BALB/c mice were purchased from SLC Inc. Labs, Japan. *S. japonicum* (Yamanashi strain) life cycle was maintained using female ICR mice (SLC, Japan) and the snail host *Oncomelania hupensis nosophora* at Tokyo Medical and Dental University, Japan. *S. japonicum* cercariae used for challenge infection were shed from crushed snails following the method we previously detailed [51].

2.3. Construction of S. japonicum glutathione S-transferase plasmid DNA vaccine

The coding sequence of *Sj*GST (GenBank: M14654) was amplified from adult worm cDNA library by PCR using the following pair of primers with *Sal*I and *Bam*HI restriction sites on the forward and reverse primers respectively: Forward-5′-GA***GTCGAC***GTCATGTCCCCT ATACTAGGT-3′ and Reverse-5′-CA***GGATCC***TTATTTTGGAGGATGGTC GCC-3′. The PCR product was directly cloned into the *Sal*I and *Bam*HI restriction sites of the pVR1020 vector (Vical, San Diego, CA) to obtain the pVR1020-*Sj*GST plasmid construct. The plasmid DNA was transformed into Top10′ chemically competent *Escherichia coli* cells (Invitrogen, USA). Ten colonies were picked and analyzed by restriction endonuclease digestion and automated DNA sequencing to identify clones with insert in the correct open reading frame. Large scale DNA vaccine was prepared by amplifying and purifying large amounts of a positive clone using QIAGEN Plasmid Mega kit (QIAGEN, USA), according to manufacturer's instructions. The purified plasmid DNA vaccine was re-suspended in 5% glucose and stored in aliquots at −80 °C until use.

2.4. Nanoparticle (NP)-coated SjGST DNA vaccine formulation

The NP-coated *Sj*GST was prepared by formulation of the ternary complex of plasmid DNA (pVR1020-*Sj*GST), polyethylenimine (PEI) and gamma polyglutamic acid (γ-PGA) as earlier detailed by [43]. Briefly, plasmid DNA (pVR1020-*Sj*GST) solution in 5% glucose (1 mg/mL) and PEI solution (pH 7.4) were mixed by pipetting thoroughly and incubated at room temperature for 15 min. pVR1020-*Sj*GST/PEI complex was then mixed with γ-PGA polyanion by pipetting and again incubated for 15 min at room temperature. The ternary complex (pVR1020-*Sj*GST/PEI/γ-PGA) was constructed at a theoretical charge ratio of 1:8:6 for phosphate of plasmid pDNA:nitrogen of PEI:carboxylate of γ-PGA poly-anion, with 73.4 ± 11.5 nm particle size and −22.8 ± 1.4 mV electric charge (ζ-potential). The size and ζ-potential of the complex were determined using Zetasizer Nano ZS (Malvern Instruments, UK).

2.5. Immunization of mice with nanoparticle-coated DNA vaccine and Challenge infection and assessment of protective efficacy

Six weeks old BALB/c mice (n = 13) were immunized three times biweekly with either 100 μg of the pVR1020-*Sj*GST/PEI/γ-PGA nanoparticle DNA vaccine or pVR1020/PEI/γ-PGA containing NP-coated empty vector, by intra-peritoneal route of administration. Two weeks after the last vaccination, mice were bled from the tail vein to prepare sera for antibody ELISA, and 3–5 mice per groups were sacrificed and spleen aseptically collected for FACS, ELISPOT assay and cytokine analyses.

Two weeks after the last immunization, mice (n = 8–10 per group) were challenged percutaneously with 40 *S. japonicum* (Yamanashi strain) cercariae by abdominal penetration using the cover glass method. Seven (7) weeks post infection; mice were perfused to estimate the worm burden. At the time of perfusion, mice liver and intestines were also collected, weighed and digested overnight in 4% KOH at room temperature with shaking. After washing 3 times with distilled water, the worm burden was determined by microscopy and the tissue egg burden was estimated as a combination of intestinal and liver egg burden. The fecundity was determined by calculating the mean number of egg recovered in tissues per female worm.

2.6. Measurement of antibodies levels by ELISA

IgG and IgG subtype profiles were determined by ELISA using sera collected 2 weeks after last immunization. Briefly, 96-well ELISA plates