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The protective efficacy aganist *Schistosoma japonicum* infection by immunization with DNA vaccine and levamisole as adjuvant in mice

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Summary Levamisole (LMS) as an adjuvant enhances cell-mediated immunity in DNA vaccination; we investigated the efficacy and liver immunopathology alleviation of a DNA vaccine, VR1012-SjGST-32, in a LMS formulation in the murine challenge model. Compared to controls, the VR1012-SjGST-32 plus LMS can reduce worm and egg burdens, as well as, immunopathological complications associated chronic inflammation significantly in liver, which were apparently associated with Th1-type response. Together, these results suggest that the LMS as a potential Schistosome DNA vaccine adjuvant can enhance both worm killing and disease prevention, which is possibly mediated through the induction of a strong Th1-dominant environment in immunized mice.

Abbreviations: Sj, *Schistosoma japonicum*; SjGST-32, SjGST26 fused with 32 kDa asparaginyl proteinase; CFSE, carboxyfluorescein diacetate succinimidyl ester; TMB, tetramethyl benzidine.

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Introduction

Schistosomiasis is a chronic parasitic disease that affects more than 200 million people in 74 countries worldwide, mostly in developing countries, causing approximately 20,000 deaths per year [1]. Schistosomes are also important pathogens for several domestic animal species and cause economic losses in endemic areas. The disease is associated with daily production of eggs by adult worms. The eggs that fail to escape the body are deposited into the liver, intestine, and genitourinary tract, where they stimulate a strong inflammatory reaction and granuloma formation that eventually leads to death [2]. Currently, Schistosomiasis control strategy is mainly based on the treatment of infected individuals by chemotherapy. However, drug treatment does not prevent individuals from reinfection that is constantly observed in individuals living in endemic area. Moreover, the development of parasite resistance to the drugs being used in mass chemotherapy has already been reported [3—5]. Thus the development of a vaccine is apparently the only practical measure for disease control. The use of irradiated cercariae for vaccination is the best animal model described hitherto, leading up to 90% or more protection against challenge infection [6]. However, culturing of the parasitic pathogen in large amounts for the purpose of vaccine preparation is completely impractical. Hence, the identification of relevant immunogens and their preparation by synthetic or recombinant DNA technologies are imperative for the development of an anti-Schistosome vaccine [7]. Several studies are in progress testing different anti-Schistosomiasis vaccine candidates and different vaccination protocols [8—10]. Many researchers agree that a vaccine aimed at reducing worm burden and/or egg production would be the most effective and cost-efficient way to control Schistosomiasis. It has been determined that a vaccine resulting in at least 40% reduction in worm burden would significantly reduce morbidity and transmission rates [3,11]. However, DNA vaccination alone is limited in that it often generates only weak immune response, particularly the cellular response. Various approaches have been developed recently in order to improve its vaccine potency, particularly with the respect to choice of a better adjuvant. Adjuvants are believed to function as a depot for prolonged antigen release, as a non-specific stimulus for the immune system, or both. Recently, it has also been realized that different adjuvants as ligands can preferentially directly interact different type of toll-like receptors (TLR) on antigen presenting cells that monitor any pathogenic invasion and in turn to active the host's immune responses [11—13].

Levamisole (LMS), a synthetic and soluble phenylimidazolthiazole, has been widely used as a pesticide for domestic animals over 30 years and also used in several human clinical trials as an anti-cancer drug [14]. Our laboratory has recently reported that LMS injected with a DNA vaccine against the foot mouth disease virus (FMDV) stimulated cellular immune responses in conjunction with a strong production of IFN-γ [13]. Furthermore, we have also demonstrated that LMS has been used effectively for the killed viral vaccines [11]. Early studies with experimental animal models established that vaccination with SjGST-32 which is a fusion protein of SjGST and Sj asparaginyl endopeptidase induced partial protection against cercarial challenge [9,15].

Our results demonstrate that LMS has a potent adjuvant activity to enhance the protective immunity induced by VR1012-SjGST-32 and leads to the reduction of liver pathology as a result of the induction of a Th1 response.

Materials and methods

Mice and parasites

Female C57BL/6 mice (H-2^b), 6—8 weeks of age, were purchased from The Animal Institute of the Chinese Medical Academy (Beijing, China), housed under a 12-h light cycle, and fed with pathogen-free food and water. The life cycle of a Chinese strain of *Schistosoma japonicum* was maintained in a laboratory of the Hunan Institute of Schistosomiasis Control. The parasites were originally collected in *Oncomelania hupensis* snails from Guichi County, Hunan Province, China. Cercariae of *S. japonicum* was maintained routinely in *O. hupensis* snails in the same laboratory and prepared by exposing infected snails to light for 1 h to induce shedding. Cercarial numbers and viability were examined under a dissective microscope prior to infection.

Fluorescent dye and antibodies

Fluorescent-labeled anti-mouse monoclonal antibodies including, anti-IL4-PE, anti-IFN-γ-FITC, anti-CD4-FITC, anti-CD4-PE, anti-IL10-PE and isotype controls were purchased from eBiosciences (CA, USA).

Parasite antigens and DNA vaccines

The VR1012-SjGST-32 gene consists of fused SjGST and Sj-32 genes was described previously [15]. Briefly, the SjGST coding sequence was obtained by PCR amplification using plasmid pMD-18-T-SjGST as the template and the primers containing PstI and BamHI restriction sites. The PCR product harboring the SjGST gene was cloned into the PstI and BamHI sites of 4.9 kb VR1012 plasmid (Vical Inc., San Diego, CA) downstream from the human cytomegalovirus promoter and designated as the VR1012-SjGST. Using the BamHI and EcoRI digested Sj32 gene from the pET28a plasmid, was then subcloned into VR1012-SjGST at same sites to yield the VR1012-SjGST-32, where the SjGST and Sj32 genes formed one open reading frame. Constructs and its empty vector were extracted by alkaline lysis, purified by PEG8000 precipitation and diluted in saline solution. The open reading frame of the fused gene was subcloned into the pGEX-3X-1 (Invitrogen Inc., USA), expressed in *Escherichia coli* (BL21 (DE3) pLysS) and purified by glutathione—sepharose chromatography.

Immunization schedule

Mice were randomly divided into five groups (nine per group), and immunized intramuscularly on days 0, 14, and 28 with either VR1012-SjGST-32 alone or formulated in 1% LMS as an adjuvant, empty VR1012 vector was used as a