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Vaccination with DNA encoding ES 43-kDa /45-kDa antigens significantly reduces *Trichinella spiralis* infection in mice

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ABSTRACT

Trichinella spiralis is an intestinal nematode parasite that can cause trichinellosis in humans and animals worldwide. The most important known hosts of *T. spiralis* are pigs, horses, dogs and cats. Pork and its products are the main sources of infection in human trichinellosis. Vaccines against these infections are urgently needed. In this study, the genes encoding the 43-kDa or 45-kDa glycoprotein present in the excretory-secretory (ES) products from *T. spiralis* muscle larvae (ML) were cloned into the eukaryotic expression vector pVAX1, resulting plasmids pVAX1-Ts43 and pVAX1-Ts45, respectively. Then BALB/c mice were intramuscularly immunized with the DNA vaccine pVAX1-Ts43, pVAX1-Ts45, or both to evaluate their immunogenicity and host protective potential. After the third immunization, mice of each group were challenged with 300 *T. spiralis* ML. The results showed that the mice immunized with the DNA vaccine pVAX1-Ts43 or pVAX1-Ts45 developed significant numbers of FAS^+PNA^+ $B220^+$ B cells indicating the formation of the germinal centers (GCs), IFN-γ-secreting (mesenteric lymph nodes, MLN) cells, and IL-4-, and IL-10-secreting splenocytes. Mice immunized with the pVAX1-Ts43 or pVAX1-Ts45 vaccine elicited partial protective immunity against challenge infections with *T. spiralis* as shown by significant reduction in ML. Most notably, the combined immunity of pVAX1-Ts43 and pVAX1-Ts45 induced better immune responses than either of the DNA vaccines given alone and provided as high as 75.9% reductions in muscle larval burden. These results suggest that the plasmid DNA encoding the 43-kDa or 45-kDa glycoprotein could be considered as a potential vaccine candidate against *T. Spiraling* infection.

1. Introduction

Trichinellosis is a serious parasitic zoonosis and a globally endemic food-borne disease caused by *T. spiralis* (Murrell, 2016; Rainova et al., 2016) Parasitic nematodes of the *Trichinella* genus are widespread in nature and can infect most species of omnivorous and carnivorous animals, incidental hosts, and humans. > 100 species of mammals, birds and reptiles are susceptible to *T. spiralis*, according to epidemiological investigations. The most important known hosts of *T. spiralis* are pigs, horses, dogs and cats (Pozio and Zarlenga, 2013).

Trichinellosis is still a serious public health threat and regarded as an emerging or a re-emerging disease (Bai et al., 2017; Flis et al., 2017). Between 1964 and 2011, > 600 outbreaks of human trichinellosis infection were documented in China, affecting 38,797 people and causing 336 deaths (Cui and Wang, 2011; Cui et al., 2011). In recent years, trichinellosis outbreaks have occurred in Yunnan Province (Bai et al., 2017). The seroprevalence of *T. spiralis* in humans ranged from 0.26% to 8.43% in 10 out of 34 provinces/autonomous regions/municipalities (P/A/M) in China from 2004 to 2009 (Cui et al., 2011), while the seroprevalence of swine trichinellosis from seven P/A/M in China ranged from 0.01% to 29.95% from 2005 to 2009 (Cui and Wang, 2011). A recent survey revealed that the prevalence of swine *Trichinella* infection in small suburb pig farms in central China was from 0.61% to 3.79% (Jiang et al., 2016; Cui et al., 2013). And Pork and its products may be main sources of human acquired *T. spiralis* infection (Pozio, 2014).

Since the first study to specifically look at DNA as an immunogen was performed in the early 1990s, DNA vaccines have been proposed for a diverse range of diseases, including bacteria, viruses and parasites (Tregoning and Kinnear, 2014). Even it was identified as an ideal method against parasitic infections that require specifically tailored immune responses in order to confer protection. The vaccines are easy to construct, prepare and administer. Even cloning target genes directly from the pathogen makes it more attractive to deal with infectious

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diseases, especially for pandemic outbreaks (Tregoning and Kinnear, 2014). Application of this newly developing vaccination technology with regard to parasitic infection provides new hope for significant advances in anti-parasitic vaccine research.

T.spiralis muscle larva (ML) antigens were classified in eight groups (TSL-1 to TSL-8). The glycoproteins from ML of TSL-1 antigens with high immunogenicity play a major role in inducing of protection against the parasite as they represent complex stimuli that induce specific humoral and cellular immune responses in the host by reducing adult and ML burdens (Yépezmulia et al., 2010). The 43-kDa and 45-kDa glycoproteins are two major antigenic proteins in the ES products from *T.spiralis* ML. The 43-kDa glycoprotein, was firstly isolated from ES products of *T. spiralis* ML (Gold et al., 1990), and found to be expressed by precapsule and postcapsule muscle larvae, which suggested the importance of this protein for capsule formation (Sofronicmilosavljevic et al., 2015). The 40-mer synthetic peptides derived from 43-kDa glycoprotein administered orally, intranasally or subcutaneously with adjuvants and the 30-mer peptide derived from the 43-kDa TSL-1 antigen expressed in *Salmonella enteric* serovar Typhimurium were all able to induce significant protection against *T. spiralis* infection in mice, including a significant reduction in ML and adult worms as well as an increase in specific IgG levels in immunized animals (Bi et al., 2015; Castillo Alvarez et al., 2013; Pompa-Mera et al., 2014). The 45-kDa glycoprotein as well as the 43-kDa glycoproteins belong to a family of trypsin-like-serine proteases that comprise glycosylated and nonglycosylated isoforms encoded by separated genes (Robinson et al., 2007). These glycoproteins can be promising vaccine candidates because of their biologic characteristics, immunogenicity and protective efficacy.

In this study, pVAX1-Ts43 and pVAX1-Ts45 plasmids were constructed by cloning the 43-kDa or 45-kDa glycoprotein gene into the expression vector pVAX1. Then the immune response and protective effects of these experimental DNA vaccines were evaluated in BALB/c mice immunized with either the purified plasmid pVAX1-Ts43 or pVAX1-Ts45 or both.

2. Materials and methods

2.1. Mice and parasite

Six-week-old female BALB/c mice were purchased from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences. They were raised with food and water provided ad libitum and artificial light for 12 h per day. All experiments with animals were conducted in compliance with the regulations concerning the use of laboratory animals of Jilin Agricultural University and in accordance with the national animal protection guidelines approved by the Institutional Animal Care and Use Committee. ML of *T. spiralis* (strain ISS534) were obtained from rats 35 days post-infection by digestion of minced skeletal muscle in 1% pepsin, 1% HCl for 3 h at 37 °C with agitation as previously described (Despommier et al., 1977).

All animal research was conducted according to the experimental practices and standards approved by the Animal Care and Ethics Committees of Jilin Agriculture University (Approval ID: JLAU08201705).

2.2. Plasmid construction

The total RNA was extracted from the fresh ML using the RNeasy Mini Kit (Qiagen, Germany). The RNA was reverse transcribed to cDNA using the Prime Script 1st strand cDNA Synthesis Kit according to the manufacturer's instructions (Takara, Otsu, Japan). The full-length coding sequences of the 43-kDa and 45-kDa glycoproteins were amplified by RT-PCR using specific primers from the cDNA template, respectively. The primers were designed according to the published sequences in GenBank (accession number M95499 and U01847, respectively) (Table 1).

Table 1
Primers used for the construction of the plasmids.

Primers	Nucleotide sequence (5′-3′)	Enzymes	length of fragments
43 k-F	CGCGGTACCATGCGAATATACATTTTTCTTAG	Kpn I	1200 bp
43 k-R	CGAGGATCCTTAGCTGTATGGGCAA	BamH I	
45 k-F	CGCGGTACCATGAAACTCTTGCTTTTAACA	Kpn I	876 bp
45 k-R	GCGGATCCTTAGCCTTGCTTAGAGAG	BamH I	

Note: Underline sequences are cleavage sites of restriction enzyme.

The target gene was sub-cloned into the plasmid pMD18-T. After transforming *E. coli* strain DH5α, the recombinant clones were selected on LB plates containing 100 μg/mL ampicillin incubated overnight at 37 °C. The plasmid DNA was extracted and identified by PCR using gene specific primers and nucleotide sequencing. Then the gene fragments were inserted into the expression vector pVAX1 (Invitrogen, Carlsbad, California, USA), generating recombinant plasmids pVAX1-Ts43 and pVAX1-Ts45 expressing the 43-kD and 45-kD glycoproteins, respectively. The positive plasmids were confirmed by agarose gel electrophoresis after digestion with appropriate restriction enzymes and DNA sequencing.

2.3. Western blot analyses

To confirm that the various DNA constructs were functional and to confirm the expression of the Ts43 and Ts45 proteins, BHK-21 cells were transfected with 2 μg of pVAX1, pVAX1-Ts43 or pVAX1-Ts45, using Lipofectamine™ 2000 Reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's protocol. Seventy-two hours post-transfection, the cells were harvested and lysed. Then proteins from the lysates were subjected to SDS-PAGE and western blot analyses as described elsewhere (Wang et al., 2013). After blotting the membrane was stained with mouse serum against *T. spiralis* as the first antibody and horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG (BIOSS) as the second antibody. Immunostained proteins were visualized using a 3, 3′-diaminobenzidine (DAB) reagent.

2.4. Immunization of mice

Plasmid DNA pVAX1-Ts43 and pVAX1-Ts45 were purified using the Qiagen EndoFree Plasmid Kit and diluted to 1 μg/μL in phosphate buffered saline (PBS). Totally 100 six-week-old female BALB/c mice were divided randomly into five groups with 20 animals each group, subjected to either PBS, pVAX1, pVAX1-Ts43, pVAX1-Ts45, or a mixed plasmid in both sides of their hind legs (50 μL/site) at a dose of 100 μg in a volume of 100 μL, respectively. The mixed vaccine was composed of 50 μg pVAX1-Ts43 and 50 μg pVAX1-Ts45. The mice were boosted twice at an interval of 1 week.

2.5. Infection of immunized animals with ML

One week after the third injection, ten mice of each group were challenged by oral infection with 300 infectious *T. spiralis* larvae per animal. Six weeks after challenge, the muscle larvae were examined from each group using a routine digestion method previously described (Wang et al., 2009). The protective immunity was calculated based on the mean number of muscle larvae collected from the group immunized with pVAX1-Ts43, pVAX1-Ts45, or both compared with those from the PBS/pVAX1 control group using the following formula:

$$\%\ \text{Larva reduction} = 1 - \frac{\text{mean number of larvae per gram muscle in vaccinated mice}}{\text{mean number of larvae per gram muscle in control mice}}$$