Vaccine 28 (2010) 2735–2742

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Oral vaccination with Ts87 DNA vaccine delivered by attenuated *Salmonella typhimurium* elicits a protective immune response against *Trichinella spiralis* larval challenge

Yaping Yang, Zhifei Zhang, Jing Yang, Xiaoqin Chen, Shijuan Cui, Xinping Zhu*

Department of Parasitology, School of Basic Medical Sciences, Capital Medical University, 10 Xitoutiao, You An Men, Beijing 100069, China

ARTICLE INFO

Article history:
Received 18 November 2009
Received in revised form
30 December 2009
Accepted 13 January 2010
Available online 25 January 2010

Keywords:
Trichinella spiralis
Ts87 antigen
DNA vaccine
Salmonella typhimurium

ABSTRACT

We have previously reported that Ts87 is an immunodominant antigen that induces protective immunity against *Trichinella spiralis* larval challenge. In this study, the Ts87 gene was cloned into an expression plasmid, pVAX1, and the recombinant Ts87 DNA was transformed into attenuated *Salmonella typhimurium* strain SL7207. Oral immunization of mice with Ts87 DNA delivered in *S. typhimurium* elicited a significant local mucosal IgA response and a systemic Th1/Th2 immune response. Cytokine profiling also showed a significant increase in the Th1 (IFN-γ) and Th2 (IL-5, 6, 10) responses in splenocytes of immunized mice upon stimulation with Ts87 antigen. An immunofluorescence assay performed with antisera revealed that the recombinant Ts87 protein was expressed in mesenteric lymph nodes of immunized mice. The mice immunized with *Salmonella*-delivered Ts87 DNA displayed a statistically significant 29.8% reduction in adult worm burden and a 34.2% reduction in muscle larvae following challenge with *T. spiralis* larvae, compared with mice immunized with empty *Salmonella* or a PBS control. Our results demonstrate that Ts87 DNA delivered by attenuated live *S. typhimurium* elicits a local IgA response and a balanced Th1/Th2 immune response and produces partial protection against *T. spiralis* infection in mice.

1. Introduction

Trichinellosis is a widespread zoonosis acquired by the ingestion of raw or inadequately cooked meat products containing encapsulated larvae of the *Trichinella* parasite. Outbreaks of trichinellosis have been regularly reported over the past two centuries; in addition, there are evidences suggesting that this parasitic disease has been emerging or re-emerging in some countries in recent years [1,2]. Human trichinellosis outbreaks occur in many parts of the world, and it has been estimated that as many as 11 million people are infected with this parasite [2]. In China alone, 17 outbreaks of meat-borne human trichinellosis, with 828 cases and 11 deaths, were recorded from 2000 to 2003 [3]. The wide distribution of domestic and wild animal reservoirs and sources of human infection makes the prevention and control of trichinellosis exceptionally difficult [4]. Thus, the development of vaccines that prevent infection in domestic animals and humans is a promising approach for controlling the disease.

In our previous study, a novel gene named Ts87 was cloned by immunoscreening a *Trichinella spiralis* cDNA library with immune sera [5], and the recombinant Ts87 protein (rTs87) was expressed in prokaryotic and eukaryotic expression systems [6,7]. The Ts87 protein was found to be an excreted–secreted (ES) product of *T. spiralis* worms. Vaccination with rTs87 produced a partial reduction in the muscle larval burden following *T. spiralis* larval challenge in BALB/c mice [8]. These results indicate that the Ts87 protein is a promising vaccine candidate for *T. spiralis* infection.

T. spiralis infection is established by the ingestion of meat containing parasite larvae. Newborn larvae penetrate the intestine and migrate to muscle tissue, where they form cysts. Therefore, the intestinal mucosa is the first barrier to encounter parasites, and mucosal immunity is likely to be important in protecting the host against *Trichinella* infection. There are evidences that the intestinal mucosa of infected animals produces antibodies specifically against *T. spiralis* [9,10], and the production of mucosal IgA mediates the protective immunity [11].

Recent studies have shown that attenuated *Salmonella typhimurium* is an effective vector for oral delivery of heterologous antigens to the immune system, resulting in long-lasting mucosal and systemic responses and thus providing an efficient platform technology for the design of novel vaccination strategies [12–14]. In this study, DNA encoding the protective antigen Ts87 was cloned into a eukaryotic expression vector, and the recombinant DNA, delivered by a genetically attenuated strain of *S. typhimurium*, was used to orally immunize mice to induce protection in a murine model of *T. spiralis* infection.

* Corresponding author. Tel.: +86 10 83911436; fax: +86 10 83911436.
E-mail addresses: zhuxping@ccmu.edu.cn, yyptwo@163.com (X. Zhu).

0264-410X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2010.01.026

2. Materials and methods

2.1. Bacterial strains

The attenuated *S. typhimurium* SL7207 strain, which has a deletion in the aroA gene in the aromatic amino acid biosynthetic pathway, was kindly provided by Prof. J.S. He of Beijing Jiaotong University. This mutant strain of bacteria is not pathogenic to mice via oral delivery [15]. The bacteria were grown in LB broth.

2.2. Plasmid construction and transformation

The full-length Ts87 gene was obtained by PCR amplification using the following primers: 5′-GCAAGCTTACCATGGGCACGAGATTTTATGCTATAGCTA-3′ (forward), 5′-GCGGATCCTTAGGTGCATGCATCCAAGTTCTTC-3′ (reverse). The HindIII and BamHI sites are underlined. The amplified DNA fragment was cloned into the eukaryotic expression vector pVAX1 (Invitrogen, Carlsbad, USA). The recombinant plasmid was named pVAX1-Ts87. To make competent cells, attenuated *S. typhimurium* strain SL7207 was grown in LB broth at 37 °C overnight to an OD_{600} of 0.6–0.8. Following centrifugation, the bacterial pellet was washed three times in sterile ice-cold water, resuspended finally in water. The recombinant plasmid pVAX1-Ts87 and the negative control plasmid (pVAX1) were subsequently electroporated into the bacteria in a 2-mm cuvette under the following conditions: 2.5 kV, 25 μF and 200 Ω (Gene Pulser Xcell, Bio-rad, Hercules, CA). This produced the strains SL7207/pVAX1-Ts87 and SL7207/pVAX1. The positive transformants were selected on LB agar containing 50 μg/ml kanamycin and identified by PCR amplification and restriction enzyme digestion. The PCR products were sequenced (TaKaRa, Dalian, China) to further confirm the introduction of plasmids in SL7207 and verify the reading frame of the cloned gene.

2.3. Stability of plasmid in vitro

To determine the persistence of the transformed plasmid maintained in a population of attenuated *S. typhimurium in vitro*, the transformed bacteria were cultured for up to 7 days (168 h) without antibiotic selection. The stability of the plasmid was determined by comparing the number of bacteria containing the plasmid (measured by survival on plate containing kanamycin) to the viable count on media without kanamycin at each 12 h interval [16]. Ten colonies of bacteria were randomly picked from plate with kanaymin and the plasmid DNA was extracted for digestion with restriction enzymes.

2.4. Parasites

The *T. spiralis* (ISS 533) parasites used in this study were maintained in ICR mice in our laboratory by serial passage infections. Each mouse was orally infected with 400 *T. spiralis* larvae. Adult worms were collected from the intestines of infected mice [11], and muscle larvae were recovered from the muscle of infected mice by a standard pepsin–hydrochloric acid digestion method [17].

2.5. Expression and purification of recombinant Ts87 protein (rTs87)

The full-length Ts87 gene was cloned into the pET28a (+) expression vector (Novagen, Madison, USA), and rTs87 was expressed in *E. coli* under 1 mM IPTG induction as described previously [6]. The recombinant protein was purified using Ni-affinity chromatography (Qiagen, Valencia, USA) according to the manufacturer's instructions. The mouse antiserum was made by immunizing mice with rTs87 as described previously [8].

2.6. Immunization schedule and sample collection

Female BALB/c mice aged 6–8 weeks and free of specific pathogens were provided by the Laboratory Animal Center, Academy of Military Medical Sciences and divided into three groups with 80 animals each. On day 0, the first two groups of mice were inoculated orally with 1×10^8 cells of SL7207/pVAX1-Ts87 or SL7207/pVAX1 in 100 μl of PBS. The third group of mice was given 100 μl of PBS only. Thirty minutes prior to oral inoculation, mice were given 100 μl of 10% sodium bicarbonate to neutralize stomach acidity. Subsequently, mice were boosted with the same dose at weeks 2 and 6.

At weeks 0, 1, 3, 5, 7, 9, 11, and 13 post-immunization, 10 mice from each group were sacrificed to obtain blood samples, intestines, and spleens [11].

2.7. Evaluation of humoral immune responses

The levels of antigen-specific total IgG, IgG2a and IgG1 antibodies in serum samples of immunized mice were determined by standard ELISA using goat anti-mouse IgG, IgG2a and IgG1 as described previously [18]. Quantification of the reactions was determined by absorbance at 492 nm.

2.8. Measurement of total IgA and rTs87-specific IgA in intestinal washings

For each sacrificed mouse, 10 cm of small intestine beginning from the gastroduodenal junction was cut, and the interior of the small intestine was flushed twice with a total of 2 ml of cold PBS. After centrifugation at $800 \times g$ for 10 min, the supernatants of the intestinal washings were harvested and stored at −80 °C for IgA analysis. Intestinal total IgA was assessed with a sandwich-type ELISA by trapping intestinal mucosal IgA as described previously [19]. The specific anti-Ts87 IgA was measured by standard ELISA using a rTs87-coated plate [11].

2.9. Detection of Ts87 mRNA transcription and recombinant Ts87 expression in vivo

Seven weeks after the first immunization, transcription of the Ts87 mRNA was determined in mesenteric lymph nodes (MLN), spleen and liver tissues of mice by using reverse transcription polymerase chain reaction (RT-PCR) with the Ts87-specific primers listed above. Total RNA was isolated from MLN, spleen and liver tissues of mice using TRIzol (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Reverse transcription of total RNA to synthesize first strand cDNA was performed at 42 °C for 20 min in a 20 μl reaction. The cDNA was amplified for 35 cycles at 94 °C for 30 s, 57 °C for 40 s and 72 °C for 1 min using the Ts87 primers. The control mouse β-actin transcript was amplified using the following primers: β-actin-1 5′-GTGGGCCGCTCTAGGCACCAA-3′; β-actin-2 5′-CTCTTTGATGTCACGCACGATTTC-3′. PCR products were detected by electrophoresis on 1% agarose gels.

To determine the expression of recombinant Ts87 *in vivo*, MLN of mice immunized with SL7207/pVAX1-Ts87 were fixed, frozen and cryosectioned. Sections were washed three times with cold PBS and blocked with 5% normal goat serum (NGS, diluted with PBS, pH 7.6) at room temperature for 30 min. After incubation with rTs87-immunized mouse serum at a 1:2000 dilution in PBS plus 5% NGS at 4 °C overnight, the tissue section was incubated with DyLight™ 488-conjugated goat anti-mouse IgG at a 1:200 dilution. The section incubated with serum from an unimmunized mouse at the same