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Partially protective immunity induced by the 14-3-3 protein from *Trichinella spiralis*

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ABSTRACT

Trichinellosis, a widespread zoonosis, is considered to be an emerging or re-emerging infectious parasitic disease. The development of vaccines to prevent *Trichinella* infection in domestic animals and humans is important for disease control. In a previous study, we identified *Ts*14-3-3 as an immunodominant protein from *Trichinella spiralis* (*T. spiralis*) adult worms recognized by early infection sera from pigs and mice. In this study, we further confirmed that *Ts*14-3-3 mRNA is expressed in both adult worms and in the larval stages of *T. spiralis*. Immunostaining with anti-*Ts*14-3-3 mouse sera further confirmed that native *Ts*14-3-3 is highly expressed on the surface of *T. spiralis* muscle larvae. The immune recognition by infected sera, its expression in both adult and larval stages and its exposure on the surface of the parasite led us to explore *Ts*14-3-3 as a vaccine antigen. Recombinant *Ts*14-3-3 formulated with an ISA50v2 adjuvant produced strong total IgG and balanced IgG1 and IgG2a responses in vaccinated mice and stimulated mouse splenocytes to produce high levels of Th1 (INF-γ, IL-2) and Th2 (IL4, IL5) cytokines. These results indicate that *Ts*14-3-3 is highly immunogenic and is able to induce balanced Th1/Th2 immune responses. These vaccine-induced immune responses resulted in a reduction in muscle larvae of up to 46.2% in vaccinated mice upon subsequent larval challenge relative to the number of larvae in mice received PBS control. The significant reduction in muscle larvae in vaccinated mice suggests that *Ts*14-3-3 is a promising vaccine target for potential use in domestic pigs to prevent trichinellosis transmission.

1. Introduction

Trichinellosis, caused by infection with *Trichinella* spp., is one of the most important food-borne infectious diseases, and its occurrence has been reported in 66 countries (Murrell and Pozio, 2011). Trichinellosis is considered as an emerging/re-emerging disease in various parts of the world (Dupouy-Camet, 2000; Dorny et al., 2009). Between 1986 and 2009, 65,818 cases and 42 deaths were reported from 41 countries (Devleesschauwer et al., 2015). In China, between 2005 and 200915 outbreaks of human trichinellosis were reported, with 1387 cases and four deaths (Cui et al., 2011). Most trichinellosis outbreaks are associated with consumption of raw or undercooked meat and meat products from domestic (dogs, horses, pigs) and wild (walruses, wild boars, bears) animals containing *Trichinella spiralis* (*T. spiralis*) larvae (Pozio, 2007; Ortega-Pierres et al., 2015). The primary source of human infection is domestic pork and pork-related products, which are consumed at the highest rate by humans when compared with the consumption of other meat products (an estimated 1 billion pigs were consumed in 2015) (Gottstein et al., 2009; Ortega-Pierres et al., 2015). In addition to its worldwide public health hazards in both developed and developing countries, trichinellosis poses an economic problem to the pig production industry and food safety (Medina-Lerena et al., 2009; Frey et al., 2009). Disease control should focus primarily on preventing pig-to-human transmission (Ortega-Pierres et al., 2015). Accordingly, the development of vaccines capable of preventing infection in domestic animals, which would therefore prevent transmission of the infection to humans, would make a substantial contribution to disease control.

The complete life cycle of *T. spiralis* occurs in a single host, including all stages of the adult worms (Ad), newborn larvae (NBL), and muscle larvae (ML). After ingestion by the host, the ML is released with the aid of digestive enzymes in the host's stomach

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and migrates to the small intestine, where they molt and develop into Ad. NBL is released from adult female worms over a period of 5–10 days after infection and then penetrate the intestine and migrate to muscle tissue, where they form encysted ML. During all developmental stages, *T. spiralis* express many immunodominant antigens that have the ability to elicit a protective immune response (Zocevic et al., 2011). In recent years, significant effort has been focused on immunizing hosts against *T. spiralis* infections using recombinant proteins or epitope immunogens (Bi et al., 2015; Li et al., 2013; Pompa-Mera et al., 2014; Gu et al., 2013). However, a vaccine that provides adequate protection against *Trichinella* infection has not yet been developed to the point of commercialization. More effort is needed to identify antigens that induce stronger protective immune responses for use as vaccine candidates. The immunorecognition of parasite antigens by antibodies from infected sera is a practical and feasible approach for identifying protective immunodominant antigens (Wang et al., 2014; Wu et al., 2009).

In a previous study, we used an immunoproteomic approach to identify sixty-four antigens that were recognized by early infection sera from pigs and mice infected with *T. spiralis*. The 14-3-3 protein of *T. spiralis* (*Ts*14-3-3) is one of these strongly recognized antigens (Yang et al., 2015). Here, we describe the screening and molecular characterization of this protein and its protective efficacy against *Trichinella* infection in a murine model.

2. Materials and methods

2.1. Ethical statement

All animal procedures in this study were reviewed and approved by the Capital Medical University Animal Care and Use Committee (Permission No. AEEI-2015-140).

2.2. Animals

Female BALB/c mice, 6–8 weeks of age and free of specific pathogens, were obtained from the Laboratory Animal Services Center of Capital Medical University (Beijing, China). The mice were maintained under specific pathogen-free conditions at an appropriate humidity and temperature.

2.3. Parasites preparation

T. spiralis (strain ISS533) was maintained in female ICR mice. ML was isolated from infected mice via the standard pepsin digestion method as previously described (Gamble et al., 2000).

2.4. Expression of recombinant Ts*14-3-3 protein and the production of antisera*

*Ts*14-3-3 was expressed in *E. coli* (BL21) as a recombinant protein with a His-tag at the C-terminus under IPTG induction and purified using immobilized metal ion affinity chromatography (IMAC), as described previously (Yang et al., 2015). Antiserum against r*Ts*14-3-3 was produced in mice via subcutaneous immunizations with 25 μg of r*Ts*14-3-3 emulsified in an equal volume of ISA50v2 adjuvant (Seppic, France).

2.5. Reverse transcription polymerase chain reaction (RT-PCR) to detect Ts*14-3-3 mRNA expression*

Total RNA was extracted from *T. spiralis* Ad and ML using a Total RNA Kit (TIANGEN, China). The *Ts*14-3-3 mRNA from both developmental stages of the parasite was detected using RT-PCR with *Ts*14-3-3 specific primers as previously described (Bi et al., 2015). The housekeeping gene GAPDH from *Trichinella* was amplified as a positive control.

2.6. Immunofluorescent test (IFT)

Anti-r*Ts*14-3-3 sera was collected from the mice immunized with r*Ts*14-3-3 and used to detect the expression and localization of native *Ts*14-3-3 in *T. spiralis* ML. *T. spiralis* ML was collected from infected mice and fixed in 3% (v/v) paraformaldehyde. Sections of *T. spiralis* ML were blocked with normal goat serum for 1 h and then incubated with anti-r*Ts*14-3-3 mouse sera (1:100) for 2 h. Sections were incubated for 1 h with the Dylight 488-conjugated goat-anti-mouse IgG secondary antibody at a dilution of 1:100. Larval sections incubated with normal mouse serum under the same conditions served as the negative control. After being washed five times with PBS, these sections were examined under a fluorescence microscope (Leica, Germany) (Bi et al., 2015).

2.7. Immunization of mice

BALB/c mice were divided into three groups of 12 animals each. The first group of mice was immunized subcutaneously with r*Ts*14-3-3 (25 μg/mouse) emulsified with an equal volume of the water-in-oil adjuvant ISA50v2 (Seppic, France) and boosted twice at intervals of 2 weeks. The second and third groups, which served as controls, were injected with ISA50v2 emulsified with PBS or with PBS alone, following same immunization schedule.

2.8. Serological antibody detection

The levels of anti-*Ts*14-3-3-specific total IgG, IgG1 and IgG2a antibodies in the sera of immunized mice collected one week after each immunization were determined via ELISA as described previously. Briefly, plates were coated with r*Ts*14-3-3 (1 μg/ml) and the mouse sera diluted in PBS at 1:1000 was added. HRP-conjugated goat-anti-mouse IgG, IgG1, or IgG2a were used as secondary antibodies. 3,3′,5,5′-tetramethylbenzidine (TMB) (BD, USA) was used as a substrate. Absorbance at 450 nm was measured with a microplate reader.

2.9. Cytokine analysis

To examine the specific cellular immune responses against r*Ts*14-3-3, the cytokine profiles in splenocytes from immunized mice were analyzed upon *in vitro* stimulation with the r*Ts*14-3-3 antigen using a modified ELISPOT assay (Yang et al., 2013). One week after the final immunization, six mice from each group were sacrificed and their spleens were processed under aseptic conditions. A total of 2×10^6 splenocytes were added to each well of ELISPOT plates pre-coated with anti-mouse IFN-γ, IL-2, IL-4, and IL-5 antibodies. After stimulation with r*Ts*14-3-3 (2 μg/ml) for 48 h at 37 °C, the cells were incubated with biotinylated secondary antibodies (BD, USA) for 2 h at room temperature. The cells were incubated with ConA as a positive control and RPMI 1640 medium alone as a negative control.

2.10. Challenge experiments

Ten days after the last immunization, three groups of mice (6 mice per group) were challenged orally with 500 ML per mouse. The ML was collected from each group 45 days post infection (dpi). The protective immunity was calculated based on the worm reduction rate of recovered larvae per gram (LPG) musclein r*Ts*14-3-3-immunized mice compared with that in the control groups.

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