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Immune responses to a recombinant attenuated *Salmonella typhimurium* strain expressing a *Taenia solium* oncosphere antigen TSOL18

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ABSTRACT

A tapeworm, *Taenia solium*, remains a great threat to human health, particularly in developing countries. The life cycle of *T. solium* is thought to be terminated *via* vaccination of intermediate hosts. In this study, we constructed a recombinant attenuated *Salmonella typhimurium* live vaccine strain χ4558 expressing a TSOL18 antigen. SDS-PAGE and Western blot confirmed the expression of the interest protein and its antigenic property. The recombinant strain stably propagated *in vitro*, of which the growth was not reversely influenced by TSOL18 protein expressed. It was also shown that mice survived 10^{12} CFU of *S. typhimurium* χ4558, while all mice infected with 10^7 CFU of the wild-type died within five days. The mouse experiment indicated that vaccine strain χ4558 induced a high titer of specific antibody for a long time. In contrast to the controls, the vaccinated mice had an obvious augment of $CD4^+$ and $CD8^+$ T lymphocytes and the percentage of helper $CD4^+/CD8^+$ T lymphocytes was significantly increased ($p < 0.01$). After oral administration, *S. typhimurium* χ4558 was first colonized mainly in the Peyer's patches and then predominantly in the mesenteric lymph nodes and spleens in the vaccinated mice. In addition, the high levels of specific anti-TSOL18 antibodies were also observed in pigs administrated with *S. typhimurium* χ4558. Collectively, these results demonstrate the possibility of use of an attenuated *S. typhimurium* strain as a vector to deliver protective antigens of *T. solium*.

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1. Introduction

Cysticercosis caused by *Taenia solium* is prevalent in humans and pigs in many developing countries or areas in Latin America, Asia, and Africa, and it has been recently reported in some developed countries [1,2]. This disease causes serious health and economic consequences [3]. The life cycle of *T. solium* includes a larval phase, at which pigs and occasionally humans serve as intermediate hosts after the ingestion of *T. solium* eggs. This parasite can cause neurocysticercosis when metacestodes encyst in the human central nervous system and can develop into tapeworms in the intestine when humans consume improperly cooked infected pork. In endemic regions, transmission of this disease is clearly related to low standards of hygiene and environmental sanitation control in the areas where rustic rearing of pigs, which can roam about freely in search of

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edibles and/or deliberately fed with human feces, is practiced by rural population [4,5].

Since pigs are a main natural intermediate host, transmission is eliminated by controlling the prevalence of cysticercosis in pigs. To this end, it has been focused on development of efficacious vaccines. Previous animal vaccination experiments using defined proteins, synthetic peptides, recombinant phages, and plasmid DNA have resulted in different levels of protection against experimental *T. solium* infection [6–11]. A gene encoding *T. solium* oncosphere antigen TSOL18, a homologue to *Taenia ovis* To18 [12] and *T. saginata* TSA18 [13] that induced protective responses in immunized sheep and cattle, respectively, was screened out from a cDNA expression library of *T. solium* oncospheres [14]. The TSOL18 antigen had been confirmed the existence of a secretory signal at the N terminal and at least one structural domain of Fibronectin III (Fn III) at the C terminal, which is likely to be involved in interactions between oncospheres and intermediate hosts and seems important in inducing host immune protection [15]. A number of studies have demonstrated that TSOL18 induces high immune protection in pigs against infection with *T. solium* oncospheres [16–18]. These vaccination studies prompt us to explore the efficient delivery of a TSOL18 antigen to pigs, paving a way towards development of more cheap practical vaccines without compromising efficacy.

Salmonella spp. has been well studied as a vaccine carrier. *Salmonella*-vectored live vaccines are being developed against a number of parasitic pathogens such as *Leishmania* species, *Schistosoma japonicum*, *Echinococcus multilocularis*, *Trypanosoma cruzi* and *Toxoplasma gondii* [19–23]. These vectored vaccines possess several advantages: cheap large-scale manufacture; room-temperature transportation and storage; and oral needle-free administration. These properties render *Salmonella* spp. to be a good delivery system to present antigens in the development of live vaccines.

Herein, we aimed to use an attenuated *Salmonella typhimurium* vaccine strain as a vector for development of an anti-cysticercosis vaccine. In administrated mice, tissue colonization, safety, and proliferative and immunological properties of a *S. typhimurium* live vaccine expressing TSOL18 were assessed. Moreover, the levels of specific anti-TSOL18 antibodies in pigs with oral administration of this vaccine were also evaluated.

2. Materials and methods

2.1. Animals

6- to 8-week female BALB/c mice were obtained from Lanzhou Institute of Biological Products, China. Mice were kept for at least one week before being administrated. 35–40 days aged healthy pigs were purchased from a local area without the occurrence of cysticercosis.

All animals were housed in an animal facility in Lanzhou Veterinary Research Institute, China. The experimental protocol was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China.

2.2. Bacterial strains and plasmids

Escherichia coli, attenuated *S. typhimurium*, and plasmids used in this study were listed in Table 1. *E. coli* χ6212 was used as an intermediate host strain for cloning and *S. typhimurium* χ3730 used as a modification strain. *E. coli* and *S. typhimurium* were grown in LB broth or LB agar at 37 °C [24]. When required, antibiotics were added into the media at the following concentration: 100 μg/ml ampicillin (TaKaRa), nalidixic acid (20 μg/ml, NA, Sigma) and diaminopimelic acid (50 μg/ml, DAP, Sigma), the last two of which were added for the proliferation of *Asd*$^-$ strains.

2.3. Construction of recombinant plasmids

TSOL18 DNA fragments were generated by PCR using the forward primer 5′-CCGGAATTC-CGAGCGGTGACCGTACATTCGG-3′ and the reverse primer 5′-ACGCGTCGACCTACGAACG***GCGG***ACCTTCTTGT-3′ (the underlined bases represented *EcoR* I and *Sal* I restriction enzyme sites, respectively, and the 4 italic bold bases in the reverse primer were site-directed mutants according to Graphical Codon Usage Analyser (http://gcua.schoedl.de/) and the signal peptide sequence at the N-terminal was removed [25]) and cloned into pYA3341. Then the recombinant plasmid pYA3346 was transformed into competent χ6212 cells prepared as described above. A single colony of the recombinant χ6212 was inoculated into 5 ml LB broth containing 20 μg/ml NA and cultured for 14 h at 37 °C, 200 rpm. Plasmid DNA was extracted using Plasmid Extract Kit (TaKaRa) and the insert was confirmed by PCR and sequencing.

2.4. Construction of *S. typhimurium* vaccine strains χ4557 and χ4558

S. typhimurium χ3730 and χ4550 competent cells were prepared as previously described [19]. Recombinant plasmid pYA3346 was electroporated into χ3730 competent cells under the following conditions: voltage 2500 V, capacitance 25 μF, electric resistance 200 Ω and discharge time 3–5 ms using Gene Pulser (Bio-Rad). A single colony of the transformants was inoculated into 5 ml LB broth containing 20 μg/ml NA followed by incubation for 14 h at 37 °C, 200 rpm. Recombinant plasmid χ3730 DNA extracted was electroporated into χ4550 competent cells under the same parameters as used for χ3730 competent cells, plated onto LB agar plates with 20 μg NA/ml NA, and incubated for 18 h at 37 °C. Positive recombinant strain harboring TSOL18 was designated as χ4558. The blank control strain χ4557 containing pYA3341 was prepared as the method used for preparation of the χ4558 strain.

2.5. SDS-PAGE and Western blot

A single colony of χ4557 or χ4558 was inoculated into 3 ml LB broth with 20 μg/ml NA and agitated for 14 h at 37 °C, 200 rpm. After centrifugation, the pellets were resuspended in SDS-PAGE loading buffer, boiled for 5 min, and separated on 12% SDS-PAGE gel. Protein bands were visualized by Coomassie brilliant blue R250 (Sigma). For Western

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