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Protection against Asiatic *Taenia solium* Induced by a Recombinant 45W-4B Protein▽

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***Taenia solium* is a great threat not only to human health but also to the pig-raising industry. Oncospheral stage-specific 45W proteins are good candidates for the development of anticysticercosis vaccines. In this study, a recombinant 45W-4B protein was highly produced and used for vaccination. Two animal trials resulted in a significant reduction in parasite burden induced by the definite protein against Asiatic *T. solium*, up to 97.0% and 98.4%, respectively. These provide informative results for the development of effective 45W-4B vaccines against cysticercosis caused by both Chinese and Mexican *T. solium* isolates and even by other isolates.**

The disease cysticercosis is an important parasitic zoonosis, caused by infection of larval *Taenia solium*, and a serious public health problem in pigs and human beings in many developing areas or countries worldwide. Recently, serological investigation showed that the infectious rate of taeniasis on the United States-Mexico border was up to 3% and the serological positivity rate of cysticercosis in some areas of California reached 1.8% (1, 4), indicating that the disease has reemerged in the United States. The larvae in uncooked or poorly cooked meat products may encyst in the brain or other nervous system tissues of humans, leading to the disease called neurocysticercosis or even to death. Pigs infected by metacestodes not only impede international trade but also greatly threaten human safety.

The immunization of pigs with efficient and cheap vaccines is a useful and practical approach for the control of cysticercosis (10). The 45W gene family was first described in *Taenia ovis*, and the recombinant protein was highly efficient for protection against infection of *T. ovis* oncospheres in sheep (9) or of *T. solium* oncospheres in pigs (14). These promising results have promoted the development of genetically engineering anticysticercosis vaccines based on *T. solium* 45W antigens. The *T. solium* 45W gene family has been proven to comprise at least five members. They can be transcribed into different types of mRNAs (A, B, and C) by means of alternative splicing, forming many protein isoforms (7). The 45W-4B and 45W-1C antigens are absolutely conserved, whereas other 45W proteins are rather variant between Chinese and Mexican *T. solium* isolates (16). In this study, we investigated the protection of the recombinant 45W-4B antigen against Asiatic *T. solium* in growing pigs in order to evaluate the priority of the 45W-4B antigen for vaccine development.

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MATERIALS AND METHODS

Pigs and eggs. Fifteen 50-day-old healthy pigs were purchased from a local area without occurrence of cysticercosis, in which anticysticercosis antibodies were not detected by enzyme-linked immunosorbent assay (ELISA). The experimental protocol was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences.

An adult worm recovered from a patient with taeniasis was completely scissored to release *T. solium* eggs. After being washed three times in saline, the released eggs were recovered by centrifugation and counted with a McMaster egg counter under a microscope. Viable and mature eggs were judged mainly by their morphology, such as structural integrity and visible hooks.

High expression and purification of the recombinant 45W-4B protein. The recombinant plasmid with removal of encoding signal peptide bp 51 at the 5′ terminus and encoding hydrophobic amino acid bp 57 at the 3′ terminus, designated as pGEX-45W-4B, was constructed previously in our lab. The cloning and expression of the recombinant 45W-4B protein were conducted as previously described (11). Briefly, the positive recombinant *Escherichia coli* BL21 cells were inoculated into 2× YT medium and induced with a final concentration of 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside) at 37°C for 5 h. Afterwards, the cells were collected by centrifugation, washed in phosphate-buffered saline (PBS; pH 7.2) several times, resuspended in cell lysis buffer containing 0.1 mg/ml lysozyme, and lysed using five freeze-thaw cycles. The fusion protein was purified using glutathione Sepharose 4B (Amersham) according to the protocols described before (17). The purity level and concentration of the purified protein were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and a biophotometer (Eppendorf).

Preparation of crude antigens from fresh metacestodes. Crude extracts were prepared from *T. solium* cysticerci strictly according to the procedures as Cai et al. reported previously (2).

Immunization and experimental infection of pigs. In trial 1, 15 pigs were separated into three groups of five animals each. Vaccination of pigs with recombinant 45W-4B or crude antigens was performed prior to an experimental challenge infection with *T. solium* eggs. Immunizations were given intramuscularly in the neck. Pigs in group A were immunized with PBS used as the control. Group B was immunized with 1,200 μg of crude antigens extracted from *T. solium* metacestodes, and group C was immunized with 200 μg of purified 45W-4B. Twenty-one days later, a booster was conducted at the same site. Each pig was experimentally infected with 25,000 mature viable *T. solium* eggs 7 days after the booster. Control and immunized pigs were slaughtered 93 days after challenge. Naked-eye inspection was swiftly performed as soon as the experimental pigs were humanely slaughtered. Pig organs and tissues, including muscles, brains, and tongues, were sliced into small pieces with knives and scissors. Afterwards, dissected cysticerci were collected and counted.

In order to further evaluate the efficiency of the recombinant 45W-4B protein as an anticysticercosis vaccine, the vaccination trial in pigs was repeated as described for trial 1. As mentioned above, pigs in group A were immunized with PBS used as the control, and those in group B were immunized with 200 μg of purified 45W-4B. In the groups with immunization of recombinant 45W-4B or crude antigen in the two trials, an adjuvant, ISA 206 (Seppic, France), a kind of

TABLE 1. Detection of specific antibodies in control and 45W-4B-immunized pigs in trial 1

Group	OD_{492} value in ELISA[a]						
	BI	15 days PI	40 days PI	55 days PI	75 days PI	105 days PI	117 days PI
A	0.19 ± 0.03	0.15 ± 0.01	0.16 ± 0.02	0.17 ± 0.03	0.15 ± 0.03	0.16 ± 0.01	0.16 ± 0.02
C	0.17 ± 0.03	0.74 ± 0.19[b]	1.37 ± 0.08[b]	2.21 ± 0.08[b]	2.12 ± 0.07[b]	1.65 ± 0.27[b]	1.18 ± 0.29[b]

[a] BI, before immunization; OD_{492}, optical density at 492 nm.
[b] $P < 0.01$, compared to PBS group.

water-in-oil-in-water adjuvant, was utilized as an immune enhancer. The significance of the reduction induced by vaccination was determined using the Mann-Whitney test.

Detection of specific antibody in growing pigs by ELISA. Serum samples were collected and separated from each pig in the first vaccine trial 15, 40, 55, 75, 105, and 117 days postimmunization (PI). Specific antibody levels were detected by ELISA by the protocols described previously (17). In ELISA, the purified recombinant 45W-4B was used as a coating antigen. The antibody levels were compared using an unpaired *t* test.

RESULTS

Identification of the recombinant 45W-4B. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis results showed that the recombinant 45W-4B protein with a molecular mass of 40 kDa was mainly in the supernatant of the lysate. Using affinity chromatography, the highly purified target protein was obtained and its final concentration reached 3.2 mg/ml.

Anti-45W-4B specific antibody in the experimental pigs in trial 1. Compared with pigs in group A, pigs immunized with the recombinant 45W-4B vaccine obviously showed increases in the level of anti-45W-4B specific antibody 15 days PI (Table 1). Moreover, the antibody level of all the immunized pigs in group C reached a summit 30 days after the booster and lasted for long time, up to at least 3 months.

Protection against *T. solium* egg challenge. In trial 1, vaccination with the recombinant 45W-4B antigen significantly reduced the number of viable cysticerci recovered ($P < 0.01$), giving a 97.0% reduction, 1.1% higher than the 95.9% reduction in the pigs with immunization of crude extracts of metacestodes (Table 2). No significant differences were observed between the group immunized with 45W-4B and that immunized with crude antigens. There was only one pig completely protected against experimental infection with *T. solium* eggs, each, in groups B and C.

In the repeated vaccination, the reduction induced by the 45W-4B vaccine was up to 98.4%, slightly higher than that in group C in trial 1, and was obviously significant ($P < 0.01$) in comparison with the control. No viable cysticerci were found in three of five pigs immunized with the recombinant protein.

TABLE 2. Reduction in growing pigs immunized with the 45W-4B vaccine or crude antigens from metacestodes

Vaccine group	No. of cysticerci in individual pigs	Mean no. of cysticerci	Reduction (%)[a]
Trial 1			
A	78, 98, 17, 140, 306	127.80	
B[b]	0, 13, 7, 1	5.25	95.9
C	4, 0, 2, 7, 6	3.80	97.0
Repeated trial			
A	5, 97, 138, 78, 306	124.80	
B	4, 0, 6, 0, 0	2.00	98.4

[a] Reduction rate (%) was calculated by the following formula: [1 − (mean no. of viable metacestodes with vaccination/mean no. of viable metacestodes for PBS) × 100%]. $P < 0.01$, compared to PBS group.
[b] One pig (immunized with the crude antigens of *T. solium* larvae) died during the trial for unknown reasons.

DISCUSSION

At present, important progress in the development of effective and practical vaccines against cysticercosis has been made. At laboratory and/or field levels, many vaccines, including definite recombinant vaccines (2, 6), DNA vaccines (8), synthesized peptide vaccines (3), and phage vaccines (12), have been proven efficient for protection against *T. solium* infection in pigs or hamsters. Most recently, attenuated *Salmonella enterica* serovar Typhimurium was used as a vector for development of an anticysticercosis oral vaccine and induced a high level of specific antibody in mice. Vaccination with such a live vector vaccine in pigs is under investigation (5).

On a global scale, *T. solium* is divided into two geographical genotypes, Asian and African/Latin American, based on mitochondrial markers (13). The Chinese isolate belongs to the Asiatic genotype, and the Mexican isolate is grouped into the African/Latin American genotype. It is a good idea to select antigens which are absolutely conserved among different *T. solium* isolates as vaccine candidates. Previous studies showed that, with the exception of 45W-4B and 45W-1C genes, there existed noticeably genetic morphology of other 45W genes between Chinese and Mexican *T. solium* isolates (16). In this study, 45W-4B was therefore selected for development of a recombinant vaccine. The results of the two animal trials showed that a parasitic load in pigs with immunization of the recombinant 45W-4B antigen decreased greatly, and one and three immunized pigs were completely protected from challenge with *T. solium* oncospheres, respectively, suggesting that the 45W-4B antigen is a good candidate for the development of vaccines against infection with both Chinese and Mexican *T. solium* isolates and even other isolates. In a previous study, a similar reduction in a parasitic load was also reported by using a recombinant 45W-A1 protein (6), another splicing isoform of 45W antigens in *T. solium*. Although a high decrement in a parasitic load was obtained in this study, a few animals (1/5 and 3/5 in the two respective trials) immunized with the recombinant 45W-4B antigen were completely protected, and it is necessary to optimize the recombinant vaccine and to further assess its efficacy at a laboratory level before extensive field trials.

In immune responses to the 45W vaccine in sheep, immunoglobulin G1, which is able to effectively activate the pathway of complement, was a predominant antibody subtype, and its titer was up to $>10^4$ (15). The *T. ovis* 45W protein is believed to function in antibody-dependent, complement-mediated lysis of the oncospheres to kill parasites. Sequence alignment revealed that the 45W-1A protein from *T. solium* might play a role in signal transduction and regulation of cell proliferation and differentiation during early embryogenesis (7). The 45W-4B gene is obviously distinguished from other 45W genes of *T. solium* at the nucleotide and amino acid levels (7, 16). There are so far no reports related to the functions of 45W-4B. Further experiments will be focused on the exploration of biological processes in which the protein is involved, which will not only lead to a better understanding of the pathogenesis of *Taenia* species but also help to improve the efficacy of 45W-4B vaccines.

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