Optimized codon usage enhances the expression and immunogenicity of DNA vaccine encoding *Taenia solium* oncosphere *TSOL*18 gene

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Received March 20, 2014; Accepted January 21, 2015

DOI: 10.3892/mmr.2015.3387

Abstract. Cysticercosis due to larval cysts of *Taenia solium*, is a serious public health problem affecting humans in numerous regions worldwide. The oncospheral stage-specific *TSOL*18 antigen is a promising candidate for an anti-cysticercosis vaccine. It has been reported that the immunogenicity of the DNA vaccine may be enhanced through codon optimization of candidate genes. The aim of the present study was to further increase the efficacy of the cysticercosis DNA vaccine; therefore, a codon optimized recombinant expression plasmid pVAX1/*TSOL*18 was developed in order to enhance expression and immunogenicity of *TSOL*18. The gene encoding *TSOL*18 of *Taenia solium* was optimized, and the resulting opt-*TSOL*18 gene was amplified and expressed. The results of the present study showed that the codon-optimized *TSOL*18 gene was successfully expressed in CHO-K1 cells, and immunized mice vaccinated with opt-*TSOL*18 recombinant expression plasmids demonstrated opt-*TSOL*18 expression in muscle fibers, as determined by immunohistochemistry. In addition, the codon-optimized *TSOL*18 gene produced a significantly greater effect compared with that of *TSOL*18 and active spleen cells were markedly stimulated in vaccinated mice. ^{3}H-thymidine incorporation was significantly greater in the opt-*TSOL*18 group compared with that of the *TSOL*18, pVAX and blank control groups ($P<0.01$). In conclusion, the eukaryotic expression vector containing the codon-optimized *TSOL*18 gene was successfully constructed and was confirmed to be expressed *in vivo* and *in vitro*. The expression and immunogenicity of the codon-optimized *TSOL*18 gene were markedly greater compared with that of the un-optimized gene. Therefore, these results may provide the basis for an optimized *TSOL*18 gene vaccine against cysticercosis.

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Key words: cysticercosis, *TSOL*18 gene, codon optimization, expression, immunogenicity

Introduction

Cysticercosis, which is caused by the larva of *Taenia solium*, has affected humans in numerous developing regions worldwide (1,2), and even in certain developed countries (3,4). Cysticercosis is a serious public health problem as well as a great threat to the animal industry, which may result in tremendous economic damages. This disease has been identified by the World Health Organization as one to be eliminated worldwide (5). In the life-cycle of *Taenia* (*T.*) *solium*, swine serve as the intermediate host, while humans are intermediate as well as definitive hosts. Humans contract cysticercosis through ingesting raw or poorly cooked meat contaminated with *T. solium* larvae. These larvae mature to become tapeworms, which are parasites of the small intestine. The gravid proglottids of tapeworms, which are filled with eggs, are discharged with the feces of the hosts; under circumstances of poor hygiene, swine and humans may intake food and water polluted by eggs, which may lead to cysticercosis. Efforts, including drug therapy, environmental sanitation, enhanced management of feces and quarantine measures, have achieved only minimally positive results in disease control (6). Vaccination has been confirmed as a potentially valuable novel method for the prevention of *T. solium* transmission (7,8). The *TSOL*18 gene, which encodes the *T. solium*-specific *TSOL*18 antigen, is a homologue of *T. ovis To*18 (9) and *T. saginata TSA*18 (10), and along with other oncosphere antigens, it was shown to contain a fibronectin type III domain (11). A previous study has shown that the *TSOL*18 antigen may be the most immunogenic and protective protein ever reported against cysticercosis (12-15). A number of different expression systems have previously been used to create proteins; however,

species-specific variations in codon usage often serve as one of the primary influences of the results (16,17). A study has demonstrated that highly expressed genes 'prefer' optimal codons (18) and the expression levels of optimized codons were markedly higher compared with those of non-optimal codons (16,19,20). In order to increase immunogenicity of *TSOL*18 through higher recombinant protein production, it is necessary to optimize the codon in the expression host due to codon bias.

Therefore, the aim of the present study was to use codon optimization for the gene encoding *TSOL*18 of *T. solium*, construct an optimized plasmid vector (optimized pVAX1/*TSOL*18) for transfection and then evaluate the expression of the optimized gene *in vivo* and *in vitro* in order to determine its immunogenicity.

Materials and methods

Codon optimization, design and synthesis of the TSOL18 gene. According to the codon usage in mouse cells, 79 out of 130 amino acids of *TSOL*18 were modified into the most preferred ones, based on codon preference in host species, for mammalian using GeneOptimizer software (Geneart AG, Regensburg, Germany), without changing the amino acid sequence; then, the optimized full-length *TSOL*18 gene was synthesized by Jinsite Biotechnology Co. (Nanjing, China). The target fragments were amplified using polymerase chain reaction (PCR; S100 Thermal Cycler, Bio-Rad Laboratories, Inc., Hercules, CA, USA) and the following pair of 19 bp primers: P1, 5'-ATGGTGTGCAGGTTCGCCC-3' and P2, 5'-GGATCCTCAGCTTCTCCTC-3', were constructed. Restriction endonucleases *Hin*dIII and *Bam*HI (Fermentas, Vilnius, Lithuania) were added at the N-terminus and PCR was performed using the primers self-complemented through 30 cycles of pre-denaturizing (95˚C for 5 min), denaturizing (94˚C for 30 sec), annealing (55˚C for 30 sec) and extension (72˚C for 2 min). Reaction products were purified and preserved, then the optimized *TSOL*18 gene and expression vector (pVAX1) were double digested with *Hin*dIII and *Bam*HI, respectively, and ligated with T4 DNA Ligase (Fermentas) to construct the optimized expression vector pVAX1/*TSOL*18. Following transfection into *E. coli* DH5α, the positive transfectants were selected, cultured on 5 ml lysogeny broth medium (Oxoid Limited, Basingstoke, UK) (kanamycin+10 μg/ml) and confirmed through Hind*III*/Bam*HI* double digestion. The DNA sequence of the clones was confirmed by Shanghai Sheng Gong Biological Engineering Co., Ltd (Shanghai, China).

Chinese hamster ovary (CHO)-K1 cell culture and transfection. CHO-K1 cells (cat. no. CCL-61; American Type Culture Collection, Rockville, MD, USA) were cultured at 37˚C in a humidified atmosphere with 5% CO_2 in F12K medium (Gibco-BRL, Carlsbad, CA, USA) with 10% fetal calf serum (Gibco-BRL), 2 mM l-glutamine and 1.5 g/l $NaHCO_3$. For the experiment $5x10^5$ cells/ml were cultured per plate and medium was replaced every 24 h prior to transfection. CHO-K1 cells floating in F12K medium containing 10% fetal calf serum were seeded ($3.5x10^5$ cells/well) onto six-well cell culture plates and anchorage-dependent cells reached 50-70% confluence. Subsequently, 100 μl Sofast™ lipofection transfection reagent (Sunma Biotechnology Co., Ltd., Xiamen, China) diluent was added to 100 μl (1 μg/ml) optimized plasmid pVAX1/*TSOL*18 and 100 μl (1 μg/ml) plasmid pVAX1 (Invitrogen Life Technologies, Carlsbad, CA, USA). The mixtures were then added to the wells of a CHO-K1 cell culture plate with mixing. Following transfection, plasmid-transfected CHO-K1 cells were cultivated at 37˚C in a humidified atmosphere with 5% CO_2 in F12K medium with 10% fetal calf serum for 48 h, with replacement of the medium every 24 h. Transfected cells were harvested and preserved at -80˚C.

Reverse transcription (RT)-PCR analysis of optimized pVAX1/TSOL*18 mRNAs*. Total RNA was extracted from CHO-K1 cells using TRIzol (Invitrogen Life Technologies) 48 h following transfection, according to the manufacturer's instructions. The RNA precipitate was pelleted through centrifugation at 11,500 x g for 10 min and washed with 70% ethanol (Sigma-Aldrich, St. Louis, MO, USA). Following an additional centrifugation under identical conditions, the precipitate was dissolved in 20-30 μl 0.1% diethylpyrocarbonate (Sigma-Aldrich)-treated water and preserved at -80˚C. cDNA was synthesized using Revert Aid™ M-Mulv Reverse Transcriptase (Fermentas) with oligo (dT) primer (1 μl) from the total RNA (4 mg) in a final volume of 20 μl, according to the manufacturer's instructions under the following conditions: 25˚C for 5 min, 42˚C for 60 min, 70˚C for 5 min and then maintained at 4˚C. PCR was performed using 0.2 μl cDNA obtained from the RT-reaction, described above, as a template. The primer used for the optimized *TSOL*18 gene was the same as above. The reaction mixture (25 μl) contained 0.2 μg cDNA template, 10 mM deoxyribonucleotide, 0.5 μl forward primer, 0.5 μl reverse primer and 0.5 μl Taq polymerase (PrimeSTAR HS PCR kit; Takara Bio, Inc., Tokyo, Japan). PCR was performed under the following conditions: Preheating at 95˚C for 3 min, denaturing at 94˚C for 45 sec, annealing at 52˚C for 45 sec and extension at 72˚C for 1 min. Following 30 cycles of amplification, the PCR products were analyzed using agarose gel electrophoresis.

Western blot analysis of optimized pVAX1/TSOL*18 protein*. Transfected cells ($1x10^7$) were added to 100 μl 1X lysis buffer (1% NP-40, 0.5% deoxycholate and 0.1% SDS; Sigma-Aldrich). Following boiling for 5 min, centrifugation at 8,944 x g at 4˚C for 5 min was performed. The whole suspension was then applied to 12% SDS-PAGE at 100 V for 120 min, and the proteins were transferred to a nitrocellulose membrane (Millipore Corp., Bedford, MA, USA). Following blocking with 5% skimmed milk (Oxoid Limited) in phosphate-buffered saline (PBS) at 4˚C for 12 h, the membranes were probed with 1:2,000 dilutions of the human anti-oncosphere polyclonal antibody at 37˚C for 2 h, followed by the addition of horseradish peroxidase (HRP)-conjugated goat anti-human polyclonal immunoglobulin G (1:1,000; Wuhan Boster Biological Technology, Ltd., Wuhan, China) at 37˚C for 2 h. Reactive bands were visualized using diaminobenzidine (Wuhan Boster Biological Technology, Ltd.). Meanwhile, standard protein markers were cut off from the nitrocellulose membrane and dyed with Ponceaux (Amresco LLC, Solon, OH, USA) for 10-15 min.

Immunohistochemical analysis. A total of 12 eight-week-old wild-type female BALB/c mice were purchased from Anhui Medical University (Hefei, China), and the experiment was approved by the Institutional Ethical Review Committee of Bengbu Medical College (Bengbu, China). The mice were fed with food and water *ad libitum* in a specific pathogen-free environment maintained at 23±1˚C, in a 12 h light/dark cycle. The mice were randomly assigned to four groups (n=3/group) and intramuscularly injected in the hind legs with 50 μl Sofast™ lipofection transfection reagent diluent. In order to improve the efficiency of gene transfer, bupivacaine (80 μl, 6.7 mg/ml; Sigma-Aldrich) was injected at the same sites to regenerate muscle 24 h prior to the injection of the plasmids. The groups were vaccinated as follows: Group A, empty control, 10 μl 5% glucose (Sigma-Aldrich); group B, empty vector, 100 μg pVAX1; group C, 100 μg pVAX1/*TSOL*18; and group D, 100 μg optimized pVAX1/*TSOL*18.

The 12 mice were sacrificed by CO_2 inhalation 48 h following immunization to examine histological changes within the injection site. Injected muscle tissues were removed and fixed in 10% formalin (Sigma-Aldrich), embedded in paraffin (Polysciences, Inc., Warrington, PA, USA) and sectioned for microscopic examination. Muscle tissue sections were incubated at 4˚C for 12 h with human anti-oncosphere polyclonal antibody at dilutions of 1:200, 1:300, 1:400 and 1:500, followed by the addition of alkaline phosphatase (ALP)-conjugated rabbit immunoglobulin G secondary antibody for immunohistochemistry. Binding of antibodies to the muscle sections was evaluated using light microscopy (BX41; Olympus Corporation, Tokyo, Japan) at magnifications of x100 and x400. Slides were reviewed to evaluate the staining result of the protein by two pathologists who were blinded to the experiment at the First Affiliated Hospital of Bengbu Medical College (Anhui, China). Scores were determined by combining the proportion of positively stained muscle cells and the intensity of staining. Mice muscle cell proportions were scored as follows: 0, no positive cells; 1, <10% positive cells; 2, 10-35% positive cells; 3, 35-75% positive cells; and 4, >75% positive cells. Staining intensity was graded on a four-point scale: 1, no staining; 2, light yellow; 3, yellow brown; and 4, brown. Total scores of ≤1 were regarded as negative (-), 2 as low expression (+), 3 as moderate expression (++) and ≥4 were taken as high expression (+++).

Proliferation measurement by ^{3}H-thymidine. Eight-week-old wild-type female BALB/c mice were randomly assigned to four groups (n=10/group): Optimized pVAX1/*TSOL*18 (150 μg/injection), pVAX1/*TSOL*18 (150 μg/injection), pVAX1 empty vector (150 μg/injection) and PBS (150 μg/injection). The four groups were immunized intramuscularly in the hind legs for a total of three immunizations administered at two-week intervals. In order to improve the efficiency of gene transfer, 6.7 mg/ml bupivacaine was injected at the same sites to regenerate muscle 1 h prior to plasmid injection. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Institute for Bengbu Medical College (permit no. LAEC-2009-027). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Two weeks following the final immunization, mice were sacrificed, spleens were excised and single viable cell suspensions were isolated. Erythrocytes were lysed by ammonium chloride (155 mM; Sigma-Aldrich) and splenocyte cultures from individual animals were prepared using a syringe plunger, pressing spleen tissue through a cell strainer. Following washing in RPMI 1640 media (Gibco-BRL), supplemented with 10% fetal bovine serum, 2 mmol/l glutamine, 1 mmol/l sodium pyruvate, 100 U/ml penicillin (Sigma-Aldrich), 100 μg/ml streptomycin (Sigma-Aldrich) and 12.5 U/ml nystatin (Sigma-Aldrich), lymphocytes were counted using trypan blue to identify viable cells and were resuspended to a concentration of $1x10^6$ cells/ml. Cells ($2x10^5$ in 200 μl) were plated in 96-well plates and stimulated in triplicate using the corresponding *TSOL*18 protein (1 μg/μl , 20 μl per well; as previously prepared in our lab). The positive control was conA. Following 48 h of incubation at 37˚C with 5% CO_2, cells were pulsed with 1 μCi ^{3}H-thymidine for 24 h to measure proliferation. Cells were then harvested and thymidine incorporation was determined using a liquid scintillation counter (FJ-2107P; Xi'an Nuclear Instrument Factory, Xi'an, China) at 72 h.

Statistical analysis. Values are presented as the mean ± standard error of the mean. All data were analyzed using SPSS 17.0 software (International Business Machines, Armonk, NY, USA). Comparisons between the means were made using a one-way analysis of variance. $P<0.05$ was considered to indicate a statistically significant difference between values.

Results

Codon optimization, design and synthesis of the TSOL18 gene. A comparison of the *TSOL*18 gene and codon-optimized *TSOL*18 gene is shown in Fig. 1. A total of 79 out of 130 amino acids of *TSOL*18 were modified, and identical residues are marked in yellow.

Fig. 2 shows the results of the electrophoresis of optimized pVAX1/*TSOL*18 and recombinant plasmid-optimized pVAX1/*TSOL*18 following digestion with *Hin*dIII and *Bam*HI. Following electrophoresis, two different sets of DNA fragments were detected. The band at ~3,000 bp indicated the full-length recombinant plasmid vector DNA and the band at ~414 bp indicated the optimized pVAX1/*TSOL*18 DNA. These results were consistent with the theoretical base-pair calculation and the fragments corresponding to the predicted DNA length. In addition, the inserted fragment (414 bp) was confirmed to be recombinant plasmid-optimized pVAX1/*TSOL*18, as determined using directed sequencing.

Expression of optimized TSOL18 in eukaryotic cells. The functions of optimized pVAX1/*TSOL*18 gene vaccine rely on its expression of a certain amount of protein. To verify whether the optimized pVAX1/*TSOL*18 gene could be cultured in the eukaryotic cell system, CHO-K1 cells were transfected and the mRNA expression of optimized *TSOL*18 was analyzed using RT-PCR. The results showed that the size of product was in accordance with the expected size (~414 bp) (Fig. 3); the internal control β-actin was detected at similar

Figure 1. Alignment of *TSOL*18 wild-type nucleotide sequence with the optimized *TSOL*18 sequence. Identical residues are marked in grey. First line, *TSOL*18 nucleotide sequence; second line, codon optimized *TSOL*18 nucleotide sequence; and third line, amino acid sequence.

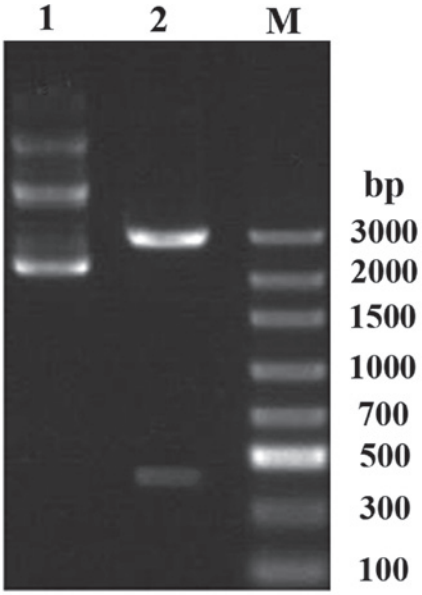

Figure 2. Protein expression of optimized pVAX1/*TSOL*18 and recombinant plasmid optimized pVAX1/*TSOL*18 following digestion with *Hin*dIII and *Bam*HI. Lane 1, optimized pVAX1/*TSOL*18, two sets of DNA fragments were detected; lane 2, recombinant plasmid optimized pVAX1/*TSOL*18, the first band (~3,000 bp) indicated the full-length of recombinant plasmid vector DNA and the band (~414 bp) indicated the optimized pVAX1/*TSOL*18 DNA; and lane 3, DNA molecular weight markers.

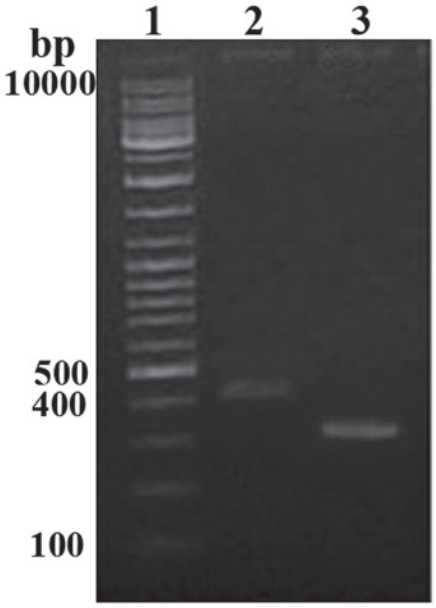

Figure 3. Expression of optimized pVAX1/*TSOL*18 messenger RNAs in eukaryotic cells detected using reverse transcription PCR. Agarose gel electrophoresis analysis showed: Lane 1, DNA marker; lane 2, PCR products of optimized pVAX1/*TSOL*18 gene (~414 bp); and lane 3, PCR products of β-actin used as an internal control (300 bp). PCR, polymerase chain reaction.

levels (~300 bp). These results demonstrated that the recombinant plasmid DNA vaccine expressed *TSOL*18 in eukaryotic cells. The cell lysate was then analyzed by western blotting, which revealed an obvious strong protein band at 18 kDa in the optimized pVAX1/*TSOL*18 group, whereas the empty vector

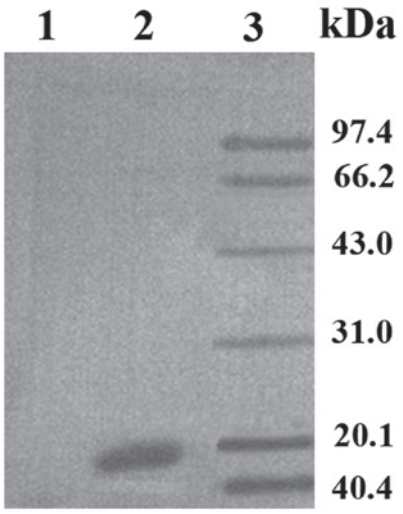

Figure 4. Expression products of the optimized pVAX1/*TSOL*18 protein analyzed by western blotting. Lane 1, pVAX1 group (no bands); lane 2, optimized pVAX1/*TSOL*18 group (an obvious strong protein band at 18 kDa); and lane 3, protein molecular weight markers.

pVAX1 group had no band (Fig. 4). These results demonstrated that the recombinant plasmid was able to express protein with immunocompetence in CHO-K1 cells.

Expression of optimized TSOL18 in vivo. In order to determine whether optimized codon usage enhances *TSOL*18 gene expression *in vivo*, immunohistochemical analysis was performed to observe its expression in the hind leg muscles of mice (Fig. 5). The results revealed that immunostaining in the muscle of the optimized pVAX1/*TSOL*18 group was positive (+++), with brown-yellow color pellets in the muscle fibers and more pellets were present in the optimized pVAX1/*TSOL*18 group compared with that of the pVAX1/*TSOL*18 group (++). In addition, the pVAX1 group muscle was negative for *TSOL*18 expression (-~+).

Cell proliferation effect induced by the TSOL*18 protein*. In order to further verify whether the optimized pVAX1/*TSOL*18 induced a good immunity response, the cell proliferation rate was determined using ^{3}H-thymidine. As shown in Fig. 6, ^{3}H-thymidine incorporation was significantly greater in the optimized pVAX1/*TSOL*18 group compared with that in the pVAX1/*TSOL*18, pVAX1 and blank control groups ($P<0.01$). This suggested that the number of spleen cells in the optimized pVAX1/*TSOL*18 group stimulated by the *TSOL*18 protein was significantly increased compared with that in the pVAX1/*TSOL*18 group, indicating that the optimized pVAX1/*TSOL*18 demonstrated enhanced immunogenicity compared with that of pVAX1/*TSOL*18.

Discussion

Numerous factors may impact the expression of a protein, including the suitability of the promoter, codon bias, the position of the Shine-Dalgarno sequence and the stability of mRNA (18,21). If a gene experiences strong selection pressure

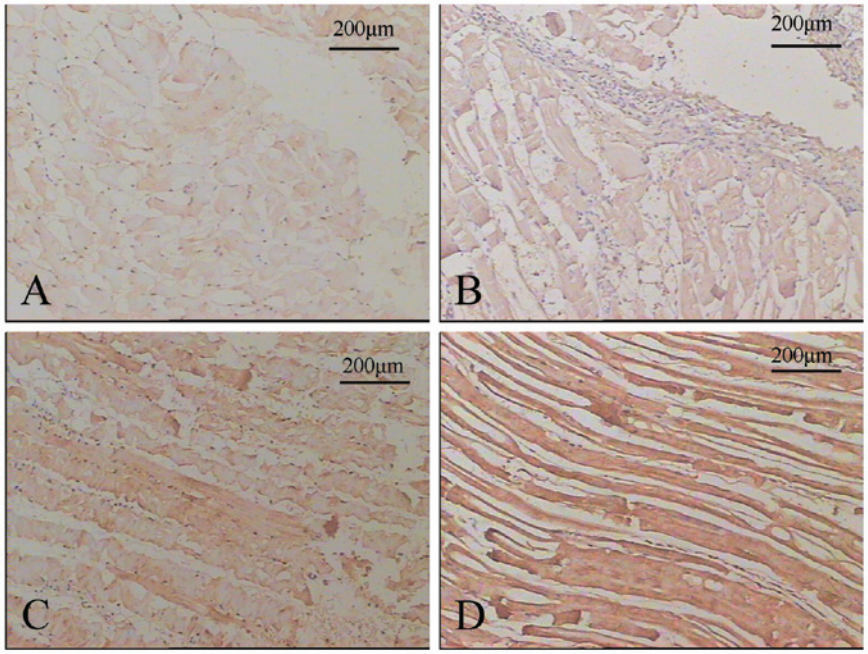

Figure 5. Immunohistochemical analysis of the expression of optimized *TSOL*18 in hind leg muscles of mice. (A) Negative control group injected with 10 μl 5% glucose. No brown-yellow color pellets were observed in the muscle fibers (-~+). (B) Control plasmid group injected with 100 μg empty vector pVAX1 No brown-yellow color pellets were observed in the muscle fibers (-~+). (C) pVAX1/*TSOL*18 group were injected with 100 μg pVAX1/TSOL18. Moderate positive staining indicated by brown-yellow color pellets were observed in the muscle fibers (++). (D) Optimized pVAX1/*TSOL*18 group injected with 100 μg optimized pVAX1/TSOL18. Strongly positive staining indicated by brown-yellow color pellets was observed in the muscle fibers (+++). Magnification, x100.

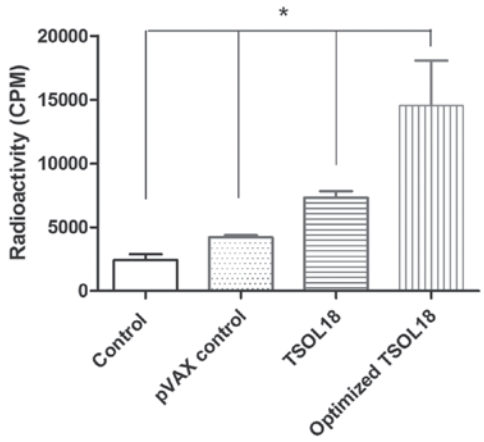

Figure 6. Cell proliferation was induced by the *TSOL*18 protein, as detected using ^{3}H-thymidine. Values are presented as the mean ± standard error of the mean. *P<0.01: *TSOL*18 group, P<0.01 vs. blank control and PVAX groups; optimized *TSOL*18 group, P<0.01 vs. blank control, PVAX and *TSOL*18 groups.

(such as high expression), it may have more usage codon bias and thus, a higher translation efficiency (22). Using codon modification, Zheng *et al* (23) successfully enhanced the expression of the glycoprotein gene in *E. coli*. In addition, Mani *et al* (24) reported that the codon adaptation index and guanine-cytosine content of the genes in optimized DNA was significantly enhanced.

In the present study, in order to improve *TSOL*18 expression, the codon optimization method was used to match codon frequencies in animal (mouse) organisms, modify ribosome binding sites and mRNA degradation sites, as well as adjust translational rates to allow various domains of the protein to fold properly (25). The codon usage for *TSOL*18 was mapped. RT-PCR amplification and gel electrophoresis revealed an obvious band at ~414 bp in the recombinant plasmid optimized pVAX1/TSOL18 transfected cells, indicating that the constructed plasmid had been successfully transfected into the CHO-K1 cells. It has been reported that the induction of a specific immune response only occurs when the gene accessing the host body expresses a certain amount of protein (26,27). Western blot analysis of the cell lysates revealed an obvious strong protein band at 18 kDa in the optimized pVAX1/*TSOL*18 group, whereas the empty vector pVAX1 group had no band. These results demonstrated that the recombinant plasmid was able to express protein with immunocompetence in eukaryotic cells.

In the present study, pVAX1 was selected as the carrier vaccine expression vector, the safety of which has been approved by the Food and Drug Administration; in addition, CHO-K1 cells were previously found to be suitable for the expression of the pVAX1 plasmid (28). In the present study, the cationic polymer Sofast™ was applied in order to insert the reconstruction plasmid pVAX1/*TSOL*18 into CHO-K1 cells. Due to the high transfection efficiency and low cytotoxicity of Sofast™, the procedure is simple and quick (29).

DNA vaccines combine pathogen genes, encoding effective immunogens, and plasmids in order to get into the host cells through direct immunization and express the protective antigens (30). Thus, plasmid DNA vaccines must be injected into animal models in order to verify their effectiveness. In the present study, mice were selected instead of pigs as an animal model due to their small size, clear genetic background, easy use and exhibition of similar immune

effects. At present, gene vaccine immunization is primarily performed using intramuscular injections, the gene gun method, mucosal immunization or intravenous and celiac injections (31). The route and delivery method used for genetic immunization studies have numerous implications affecting the outcome of the immune response. In the present study, intramuscular injection was selected as the method of immunization as experiments have shown that the DNA is readily absorbed by the host cells (Fig. 5D). Sheep were immunized intramuscularly with TO45w plasmid DNA in a study by Rothel *et al* (32), the results of which demonstrated high levels of immunoglobulin G1. In addition, BALB/c mice were immunized intramuscularly with plasmid DNA (pCDI-K45w, pcDI-K45 sec and pcDI-K45wTR) in a study by Drew *et al* (33), which reported that the cellular localization of the DNA vaccine antigen had a significant effect on the production of antibody.

The results of the present study revealed that immunostaining with anti-oncosphere antibodies in plasmid-injected muscle showed positive staining for *TSOL*18, whereas immunostaining in empty pVAX1-injected muscle showed negative results. In addition, an increased number of brown-yellow color pellets was observed in the optimized pVAX1/*TSOL*18 gene group compared with that of the pVAX1/*TSOL*18 group; this therefore indicated that the optimized pVAX1/*TSOL*18 gene expressed more protein compared with that of the un-optimized gene.

^{3}H-thymidine, as a DNA precursor, can be used to indicate the degree of cell proliferation (34-36). In the present study, the lymphocyte transformation degree was determined according to ^{3}H-thymidine radiation in the lymphocytes. This method is objective, sensitive and accurate (34-36). The results revealed that the number of spleen cells stimulated in the optimized pVAX1/*TSOL*18 gene group was significantly increased compared with that of the pVAX1/*TSOL*18 gene and empty contrast groups. These results indicated that following codon optimization, the quantity of protein expression increased and the active spleen cells were stimulated to rapidly proliferate, and therefore induced improved immunogenicity compared with that of the pVAX1/*TSOL*18 group.

In conclusion, the eukaryotic expression vector containing the codon-optimized *TSOL*18 gene was successfully constructed and expressed *in vivo* as well as *in vitro*. In addition, the expression and immunogenicity of the codon-optimized *TSOL*18 gene were markedly greater compared with those of the un-optimized gene in mice. These results provided the basis for developing an optimized *TSOL*18 gene vaccine against cysticercosis.

Acknowledgements

The authors would like thank Professor Junjie Sun, Department of Nuclear medicine, Bengbu Medical College and Professor Baiqing Li, MD, PhD, Department of Immunology, Bengbu Medical College, for their technical support. The present study was supported by grants from The Natural Science Foundation of China (no. 30600518), Anhui Provincial Natural Science Research Project (no. KJ2011B095) and Bengbu Medical College Fund (no. BY1001).

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