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Protective immunity induced by a DNA vaccine expressing eIF4A of *Toxoplasma gondii* against acute toxoplasmosis in mice

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ABSTRACT

Toxoplasma gondii is an obligate intracellular protozoan parasite infecting humans, mammals and birds. Eukaryotic translation initiation factor (eIF4A) is a newly identified protein associated with tachyzoite virulence. To evaluate the protective efficacy of *T. gondii* eIF4A, a DNA vaccine (pVAX-eIF4A) encoding *T. gondii* eIF4A (Tg-eIF4A) gene was constructed. The expression ability of this recombinant DNA plasmid was examined in Marc145 cells by IFA. Then, Kunming mice were intramuscularly immunized with pVAX-eIF4A and followed by challenge infection with the highly virulent *T. gondii* RH strain. The results showed that vaccination with pVAX-eIF4A elicited specific humoral responses, with high IgG antibody titers and specific lymphocyte proliferative responses. The cellular immune response was associated with significant production of IFN-γ, IL-2 in Kunming mice, and a mixed IgG1/IgG2a response with predominance of IgG2a production, indicating that a Th1 type response was elicited after immunization with pVAX-eIF4A. In addition, the increase of the percentage of CD8+ T cells in lymphoid in mice suggested the activation of MHC class I restricted antigen presentation pathways. After lethal challenge, the mice vaccinated with the pVAX-eIF4A showed a significantly prolonged survival time (23.0 ± 5.5 days) compared with control mice which died within 7 days of challenge ($P < 0.05$). These results demonstrate that pVAX-eIF4A could elicit strong humoral, Th1-type cellular immune responses and increase survival time of immunized mice, suggesting that eIF4A is a promising vaccine candidate against acute *T. gondii* infection in mice.

1. Introduction

Toxoplasma gondii is a ubiquitous apicomplexan parasite of many warm-blooded animals including humans, capable of causing toxoplasmosis [1–4]. Naturally, the vast majority of infections in adults are asymptomatic due to the hosts' cellular immune responses [1,5], however, *T. gondii* can cause severe diseases in immune-deficient individuals such as AIDS patients, those with tumors or transplantation operations, as well as congenitally infected persons [6]. In addition, infection with *T. gondii* may lead to abortion or congenital toxoplasmosis in animals, especially in sheep and goats [7], and thus cause considerable economic losses to the livestock industry [8,9]. Therefore, the human and animal significance of toxoplasmosis makes it urgent to search for alternative ways for the prevention and control of *T. gondii* infections.

The ideal measures of controlling and preventing the consequences of acute or chronic toxoplasmosis would be the use of an effective vaccine against *T. gondii*, because there are no available drugs that can effectively eliminate the parasite from the infected hosts. Under the present scenario, several types of vaccines have been developed such as subunit vaccines, genetically engineered vaccines, especially, a live and attenuated vaccine of *T. gondii* S48 strain has been licensed and used in farm animals, but it has limitations of safety concern and inadequate efficacy [10,11]. Immunization with DNA vaccine can elicit effective humoral and cellular immune responses that produce specific antibodies and both CD4+ T helper cells and CD8+ cytotoxic T cells against cellular pathogen invasions in animal models [12–14], which are critical for control of *T. gondii* infection during both acute and chronic phases of the infection [15–18].

The eukaryotic translation initiation factor eIF4A is an RNA helicase that facilitates translation initiation by unwinding otherwise

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inhibitory mRNA structure [19], which is required for a number of mammalian internal ribosome entry sites (IRESes) [20]. Also, eIF4 factors including eIF4A are capable of controlling cell proliferation and morphogenesis [19,21], and considered to be critical for parasite growth and survival [22,23]. The *T. gondii* eIF4A protein is expressed in the tachyzoite but massively down-regulated in the bradyzoite, and considered to be associated with tachyzoite virulence [21]. Regardless of the possible roles of eIF4A protein mentioned above, no previous studies have evaluated the vaccine potential of eIF4A against *T. gondii* infection.

The objectives of this study were to determine the immunogenicity of *T. gondii* eIF4A in mice by construction of the eukaryotic plasmid pVAX-eIF4A, expressing eIF4A of *T. gondii* in vitro, to analyze various immune responses to pVAX-eIF4A vaccination, and to estimate protective effect of the DNA vaccine based on pVAX-eIF4A in mice against lethal challenge with *T. gondii* RH strain.

2. Materials and methods

2.1. Animals and parasites

Specific-pathogen-free (SPF) grade female Kunming mice of six to eight weeks old were purchased from Lanzhou University Laboratory Animal Center (Lanzhou, China). All mice were handled in strict accordance with good animal practices according to the Animal Ethics Procedures and Guidelines of the People's Republic of China, and the present study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Permit No. LVRIAEC-2011-10). Tachyzoites of the highly virulent *T. gondii* RH strain (Type I) were used to challenge mice. The RH strain was preserved in our laboratory (Laboratory of Parasitology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences), maintained by serial intraperitoneal passage in Kunming mice and purified according to the method described previously [24].

2.2. Preparation of Toxoplasma lysate antigen (TLA)

Purified tachyzoites of the *T. gondii* RH strain were disrupted by three cycles of freezing at −20 °C and thawing, and then sonicated on ice at 60 W/s. The prepared cellular lysate was centrifuged for 30 min at 10, 000 × *g* at 4 °C, and the supernatants were pooled, sterile filtered with 0.2 μm sterile nitrocellulose filters (Sartorius). Finally, the TLA concentration was determined via the Bradford method using bovine serum albumin (BSA) as the standard, aliquoted and stored at −70 °C until use.

2.3. Construction of the eukaryotic expression plasmid

The coding sequence of the *T. gondii* eIF4A gene (1,239 bp; sequence positions 271 to 1509; GenBank accession no. AJ320155.1) was obtained by PCR amplification from the *T. gondii* RH strain tachyzoite cDNA, with a pair of specific primers (forward primer: 5′-CCGGAATTCACCATGGAGAACAACGAAGATAAAC-3′, reverse primer: 5′-GCTCTAGACTAGAAGAACTCCGCGACTTCC-3′), in which *Eco*R I and *Xba* I restriction sites were introduced and underlined. The amplified PCR product was inserted into pMD18-T vector (TaKaRa, China) and sequenced in both directions to ensure fidelity, formed a recombinant plasmid pMD-eIF4A. The eIF4A fragment cleaved from pMD-eIF4A by *Eco*R I/*Xba* I was subcloned into the *Eco*R I/*Xba* I sites of pVAX I (Invitrogen). Following the screening by PCR amplification and enzymatic digestion, the positive colonies were sequenced in both directions to ensure fidelity, and generated plasmid pVAX-eIF4A

Plasmids were then purified from transformed *Escherichia coli* DH5α cells by anion exchange chromatography (EndoFree Plasmid Giga Kit, Qiagen Sciences, MD, USA) following the manufacturer's instructions, dissolved in sterile endotoxin-free TE buffer and stored at −20 °C until use. The concentration of pVAX-eIF4A was determined by spectrophotometer at OD_{260} and OD_{280}.

2.4. pVAX-EIF4A plasmid expression in vitro

Marc-145 cells grown in 6-well plates were transfected with recombinant plasmid pVAX-eIF4A using lipofectamine™ 2000 reagent (Invitrogen) as instructed by the manufacturer. Marc-145 cells transfected with empty pVAX1 was prepared as the negative control. Forty eight hours post-transfection, the cells were fixed with cool acetone for 15 min, and washed with PBS-0.1% Triton-X-100 (PBST) for three times. The plasmid pVAX-eIF4A expression in the cells was detected using the indirect immunofluorescence assay (IFA). Briefly, the cells were incubated with anti-*T. gondii* polyclonal antiserum (goat) diluted 1: 50 in PBST at 37 °C for 60 min and washed by PBST, followed by a FITC-labeled donkey-anti-goat IgG antibody diluted 1:100 in PBST at 37 °C for 45 min (Proteintech Group Inc., Chicago, USA). After extensive washings with PBST, the specific fluorescence was imaged through a Zeiss Axioplan fluorescence microscope (Carl Zeiss, Germany).

2.5. Immunization and challenge

Female Kunming mice (23 mice for the experimental group) were vaccinated by bilateral intramuscular injection into the quadriceps three times at two week intervals with 100 μg plasmid encoding pVAX-eIF4A dissolved in 100 μl sterile phosphate buffered saline (PBS). As controls, groups of mice (23 mice for each group) were injected with empty pVAX I vector or PBS respectively, and one group of 23 mice was not inoculated to constitute blank control. Blood was collected from the tail vein prior to immunization, and sera were separated and stored at −20 °C until analyzed for specific antibodies.

Two weeks after the last immunization, three mice per group were sacrificed and splenocytes were aseptically harvested for lymphocyte proliferation assay, cytokine measurements, and flow cytometric analysis, and thereafter the remaining mice in all groups were intraperitoneally (IP) challenged with 1×10^3 tachyzoites of the virulent *T. gondii* RH strain. Pre-immune serum samples were used as negative controls.

2.6. Antibody titers and isotype determination

Anti-Tg-eIF4A IgG, IgG1 and IgG2a antibodies in serum samples were determined using SBA Clonotyping System-HRP Kit according to the manufacture's instruction (Southern Biotech Co., LTD, Birmingham, USA). In brief, microtiter plates were coated with capture antibody (10 μg/ml) in 100 μl of phosphate buffered saline (pH 7.4) overnight at 4 °C. The plates were washed with PBS containing 0.05% Tween20 (PBST) and then blocked with PBS containing 1% BSA for 1 h. Mice serum samples diluted in PBS were added to the wells and incubated for 1 h at 37 °C. After washing with PBST, the wells were incubated with 100 μl of horseradish-peroxidase (HRP) conjugated anti-mouse IgG diluted in 1:500 for 60 min at 37 °C, or anti-mouse IgG1 or IgG2a in 1:500, which were used for determination of antibody levels and isotype analysis, respectively. Binding was visualized by incubating with 100 μl substrate solution (pH4.0) (1.05% citrate substrate buffer; 1.5% ABTS; 30% H_2O_2) for 20 min. The absorbance was measured at 405 nm using an ELISA reader (Bio-TekEL × 800, USA). All samples were run in triplicate.