Vaccine 31 (2013) 6225–6231

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

DNA immunization with eukaryotic initiation factor-2α of *Toxoplasma gondii* induces protective immunity against acute and chronic toxoplasmosis in mice

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ARTICLE INFO

Article history:
Received 6 July 2013
Received in revised form
20 September 2013
Accepted 8 October 2013
Available online 31 October 2013

Keywords:
Toxoplasma gondii
Toxoplasmosis
Eukaryotic initiation factor-2α (TgIF2α)
DNA vaccine
Protective immunity
Mice

ABSTRACT

Toxoplasma gondii infection is a serious health problem of humans and animals worldwide. *T. gondii* eukaryotic initiation factor-2α (TgIF2α) plays a crucial role in parasite viability and is an important virulence factor of *T. gondii*. To evaluate the vaccine potential of TgIF2α, we constructed a novel eukaryotic plasmid pVAX-IF2α expressing TgIF2α from the RH strain and validated expression and immunogenicity *in vitro* in the Marc145 cell expression system by indirect immunofluorescence (IFA). Administration of pVAX-IF2α intramuscularly induced specific humoral immune responses including high levels of specific TgIF2α IgG antibody and a mixed IgG1/IgG2a response with a predominance of IgG2a production. The cellular immune response was elicited, showing significant production of IFN-γ and IL-2 associated with Th1 type response, and thus strong cell-mediated cytotoxic activity with increased frequencies of IFN-γ parameters analyzed in both $CD4^+$ and $CD8^+$ T cell compartments ($CD4^+$ IFN-γ^+ T cells and $CD8^+$ IFN-γ^+ T cells). Immunization resulted in partial protection against acute and chronic toxoplamosis in outbred Kunming mice, demonstrated by a significantly prolonged survival time (15.9 ± 4.6 days) after challenge with the virulent RH strain and significant reduction in brain cysts (44.1%) against chronic infection with PRU cyst in contrast to control mice. Our data suggested that pVAX-IF2α could be used as a DNA vaccine candidate against both acute and chronic *T. gondii* infection by the activation of effective humoral and cellular immune responses.

1. Introduction

Toxoplasma gondii infects all warm-blooded animals and humans, with a worldwide distribution [1–4]. Most *T. gondii* infections in humans are asymptomatic, but may cause congenital infection and posterior uveritis [5,6]. Infection in immunocompromised individuals are often severe due to encephalitis and disseminated infection [6–9]. The infection is a major cause of abortion in livestock, especially in sheep and goats, and consumption of infected meat is the main route of transmission to humans [10–12].

Tremendous advances have been made in the studies of anti-*Toxoplasma* DNA-delivered vaccines inducing Th1 type and $CD8^+$ cytotoxic T-lymphocyte (CTL) responses. Many *T. gondii* antigens have been identified as vaccine candidates, including dense granule antigen GRA7 [13], microneme proteins MIC13 [14], rhoptry proteins ROP16, ROP18 and ROP13 [15–17], PLP1 [18], IMP1 [19], NTPase II [20], RON4 [21] and eIF4A [22]. However, these single antigen vaccines only induced partial protection and new vaccine candidate antigens need to be identified.

T. gondii eukaryotic initiation factor-2α (TgIF2α) is identified to posses a regulatory serine residue (Ser-71) [23] and the phosphorylation of TgF2α is critical for parasite viability [24]. The TgIF2α probably is relevant to RH virulence due to the reduced virulence of TgIF2α-S71A mutant parasites *in vivo* and a significant delayed acute toxoplasmosis in mice model [24]. This antigen may have the ability to induce considerable protective efficacy against *T. gondii* infection, in spite of its characteristics of a non-secreted translation factor.

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http://dx.doi.org/10.1016/j.vaccine.2013.10.034

In the present study, we constructed a eukaryotic plasmid pVAX-IF2α expressing TgIF2α, analyzed immune responses and protective efficacy in Kunming mice induced by pVAX-IF2α against lethal challenge with *T. gondii* RH strain (Type I) or chronic infection with *T. gondii* PRU strain (Type II).

2. Materials and methods

2.1. Mice

Specific-pathogen-free (SPF) grade female outbred Kunming mice of 6–8 week old were purchased from Lanzhou University Laboratory Animal Center (Lanzhou, China). All mice used for the experiments were raised and treated in strict accordance with good animal practices under the Animal Ethics Procedures and Guidelines of the People's Republic of China, according to the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences.

2.2. Parasites, cell and antigens

Two *T. gondii* strains were used in this study, the virulent RH strain (Type I) and the cyst-forming PRU strain (Type II). Tachyzoites of the RH strain and cysts of the PRU strain were prepared according to the methods described previously [15,22,25]. The obtained tachyzoites of the RH strain were used for the production of the TgIF2α clones, the challenge of mice used tachyzoites of the RH strain and cysts of the PRU strain. Preparation of *Toxoplasma* lysate antigen (TLA) was performed as previously described by Chen et al. [22].

Monkey kidney cells (Marc-145; preserved in our laboratory) were grown and maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (FCS), 100 mg/ml streptomycin, and 100 IU/ml penicillin at 37 °C with 5% CO_2.

2.3. Construction of DNA vaccine plasmid

The eukaryotic expression vector pVAX I was used as a DNA vaccine vector. To clone the TgIF2α gene, the total RNA of tachyzoites of RH strain was prepared using TRIzol reagent (Invitrogen) according to the manufacturer's instructions, and the coding sequence of TgIF2α (1044 bp, GenBank accession no. AY518935.1) was obtained by RT-PCR amplification, with designed specific primers (forward primer: 5′-CCGGAATTCATGGAGGCGAGAGACGGCAC-3′, reverse primer: 5′-GCTCTAGATCACGCATTTCCGTCATCGTTA-3′), in which *Eco*R I and *Xba* I restriction sites were introduced and underlined. The amplified RT-PCR products were inserted into pMD18-T vector (TaKaRa, China) and sequenced in both directions to ensure fidelity, formed recombinant plasmid pMD18-IF2α. The TgIF2α fragment cleaved from pMD-IF2α by *Eco*R I/*Xba* I was sub-cloned into the corresponding sites of pVAX I (Invitrogen) using T_4 DNA ligase, thus generated plasmid pVAX-IF2α. The recombinant plasmid was propagated in *Escherichia coli* DH5α and confirmed by specific PCR, restriction analysis and DNA sequencing. The positive recombinant plasmid was processed as described previously by Chen et al. [22]. The concentration of pVAX-IF2α was determined by spectrophotometer at OD_{260} and OD_{280}.

2.4. Expression of pVAX-IF2α plasmid in vitro

The recombinant plasmid pVAX-IF2α was transfected into Marc-145 cells, and the expression was examined as described previously [22]. In brief, 48 h post-transfection, cells were fixed with 100% acetone for 30 min and washed with PBS-0.1% Triton-X-100 (PBST) for three times, and then were processed for indirect immunofluorescence assay (IFA) followed by incubation with goat anti-*T. gondii* tachyzoites polyclonal antiserum and a FITC-labeled donkey-anti-goat IgG antibody (Proteintech Group Inc., Chicago, USA). The specific fluorescence was examined through a Zeiss Axioplan fluorescence microscope (Carl Zeiss, Germany). Marc-145 cells transfected with empty pVAX I served as the negative control.

2.5. DNA immunization and challenge infection

Mice were divided into four groups (35 mice in each group), three groups of mice were injected intramuscularly with 100 μg pVAX-IF2α DNA in 100 μl sterile PBS, 100 μg empty vector pVAX, PBS (100 μl/each), respectively, and one group of mice were not inoculated to constitute blank control. All experimental groups were vaccinated three times at weeks 0, 2 and 4. Blood was collected from the tail vein prior to each immunization and challenge infection, and sera were separated and stored at −20 °C until analyzed for specific antibodies.

Two weeks after the last immunization, 15 mice of each group were challenged intraperitoneally (IP) with 1×10^3 tachyzoites of the RH strain. The survival time for each mice and the percentages of mice survived were recorded daily until a fatal outcome for all animals.

Eight mice of each group were inoculated orally with 20 cysts of the PRU strain at day 14th after the third immunization. Mice were observed daily for mortality. Four weeks after infection, surviving mice were sacrificed and the mean number of cysts per brain was calculated as described previously [20,25]. In brief, the whole brain from each mouse was isolated, homogenized and diluted in 3 ml PBS, and 1 μl of the homogenized brain was examined to calculate the number of *T. gondii* tissue cysts under an optical microscope. This procedure was carried out in triplicate, and the mean of three counts was obtained and was then used to calculate the total number of *T. gondii* tissue cysts in each brain sample. Thereafter, the remaining mice in all groups were sacrificed and splenocytes were aseptically harvested for lymphocyte proliferation assay, cytokine measurements, and flow cytometric analysis. This analysis was performed in three independent experiments. Pre-immune serum samples were used as negative controls.

2.6. Antibody analysis

The levels of IgG, IgG1 and IgG2a antibodies in serum were measured by ELISA 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; provided by the commercial Kit) in 100 μl of phosphate buffered saline (pH7.4) overnight at 4 °C. The plates were washed with PBS plus 0.05% Tween-20 (PBS-T) and blocked with PBS containing 1% BSA for 1 h. Serum samples diluted in PBS were added to the wells and incubated for 1 h at 37 °C. After washing with PBS-T, the wells were incubated with 100 μl of horseradish-peroxidase (HRP) conjugated anti-mouse IgG diluted in 1:250 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, 0.03% 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.

2.7. Lymphocyte proliferation assays

The *in vitro* spleen cell proliferative response was measured as described previously [14,22], and the proliferative