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Evaluation of protective immunity induced by recombinant calcium-dependent protein kinase 1 (TgCDPK1) protein against acute toxoplasmosis in mice

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ABSTRACT

Toxoplasma gondii is an intracellular zoonotic parasite that causes toxoplasmosis, which can cause economic losses and serious public health problems worldwide. A member of the *T. gondii* calcium-dependent protein kinases family, TgCDPK1 was recently identified as an essential regulator of exocytosis in *T. gondii,* and participated in direct parasite motility, host-cell invasion and egress. In the present study, the protective immunity of recombinant TgCDPK1 protein (rTgCDPK1) was evaluated against acute toxoplasmosis in mice. rTgCDPK1 were expressed and purified, BABL/c mice were intraperitoneally immunized with rTgCDPK1 and challenged with the highly virulent RH strain of *T. gondii*. The specific immune responses were analyzed by measuring the cytokine and serum antibody, and lymphocyte proliferation assays, flow cytometry of lymphocytes and the survival curve were employed to evaluate the protective efficacy. From the results we found that special humoral and cellular responses could be elicited in vaccine mice, and higher level of IgG antibody, and the significant increased levels of Th1-type cytokines IFN-γ, IL-12 (p70), IL10 and $CD3^+CD4^+CD8^-$ and $CD3^+CD8^+CD4^-$ T cells could also be detected comparing to control mice ($P < 0.05$). All vaccinated mice prolonged survival time (14.90 ± 2.89 days) challenge with 1000 tachyzoites of RH, while the control mice died within 8 days. These results indicated that TgCDPK1 protein was a potential vaccine candidate against acute toxoplasmosis.

1. Introduction

Toxoplasma gondii, an obligate intracellular apicomplexan parasite, is deemed to be one of the most widespread parasite of warm-blooded hosts [1]. It is such an extraordinarily successful parasite that about 1/3 of the world population were chronically infected. Humans are exposed to *T. gondii* through the ingestion of uncooked food contaminated with cyst or water and vegetables polluted by sporulated oocysts [2,3]. Human infection by *T. gondii* is usually asymptomatic and induces only a self-limiting disease in health people. However, infection on immunocompromised individuals was more likely to happen and even more severe. Abortion, stillbirth and significant congenital defects were the serious symptoms of toxoplasmosis when *T. gondii* was acquired in utero [4]. Additionally, toxoplasmosis in animals, especially livestock, is of great economic impact.

The control of toxoplasmosis has a great significance for not only livestock production but also public health [5]. Vaccination is the most effective way to take control of the infection of *T. gondii* due to the fact that no effective drugs have been developed for chronic infection. So far, only one (ToxoVax®, Intervet B.V.), based upon attenuated tachyzoites, has been applied in a handful of countries [6]. Considering the possibility for attenuated vaccines to regain virulence and further cause iatrogenic infection, this commercial vaccine is still deficient [7]. In addition, though many types of candidate vaccine such as engineering vaccines and subunit vaccines have been developed, they were all not satisfied with the complex epidemic status. Therefore, the exploration of an alternative and safe vaccine against *T. gondii* would have a great value for controlling the parasite.

In recent years, a group of plant-like calcium-dependent protein kinases (CDPKs) has become increasingly attention-getting. The CDPKs, belonging to a superfamily of kinases prominent in the calcium signaling cascades have been identified in plants, ciliates and

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apicomplexans but are absent from fungi and animals and human [8–10]. *T. gondii* CDPKs family (TgCDPKs) which contains 14 members plays a vital role during the whole lifecycle of *T. gondii* [9,11]. TgCDPK1 protein, reported as an essential regulator of exocytosis in *T. gondii,* participates in direct parasite motility, host-cell invasion and egress [12]. It has been shownTgCDPK1 protein was of protective efficacy against experimental toxoplasmosis in mice when injected as the form of DNA vaccine plasmid [13]. However, the efficacy of recombinant form of TgCDPK1 protein directly inoculated as a vaccine has not been evaluated.

Here, the objectives of this study were to express rTgCDPK1 and to evaluate the protective efficacy of this protein against acute toxoplasmosis caused by a challenge with the high virulence *T. gondii* RH strain in a BALB/c mouse model.

2. Materials and methods

2.1. Experimental mice

Eight-week-old SPF female BALB/c mice were purchased from Center of Laboratory Animals, Lanzhou Institute of Biological Products, Lanzhou, China. All animals were handled in strict accordance with good animal practice according to the Animal Ethics Procedures and Guidelines of the People's Republic of China, and the study was approved by The Animal Administration and Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Permit No. LVRIAEC-2009-006).

2.2. Parasites and preparation of soluble tachyzoite antigens

The *T. gondii* RH strain (type I) were maintained in our laboratory and prepared from human foreskin fibroblast (HFF) cells, and HFF cells were cultured with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA).

The soluble tachyzoite antigens (STAg) was prepared as describe previously [14], tachyzoites of *T. gondii* RH strain were washed with phosphate buffered saline (PBS), and then purified by 3-μm polycarbonate filters. The purified tachyzoites were suspended in ice-cold PBS at a density of 10^8 tachyzoites/mL, and subjected to sonication on ice until the cells were completely lysed. The supernatants were cleared by centrifuged at 12,000 × *g* for 15 min at 4 °C. Finally, the STAg concentration was determined by the Bradford method using bovine serum albumin (BSA) as the standard and stored at −70 °C for further use.

2.3. Gene amplification and plasmid construction

Total RNA of *T. gondii* tachyzoites was extracted using Trizol reagent (Invitrogen, Carlsbad, USA). The first strand cDNA was synthesized by AMV reverse transcriptase using oligo(dT) primer. The 1524 bp fragment of the TgCDPK1 gene (GenBank accession No. AF333958) of *T. gondii* was amplified by PCR, using the following primers: forward: 5′-CATATGGGGCAGCAGGAAAGCAC-TCT; and reverse:3′-CTCGAGGT TTCCGCAGAGCTTCAAGAGC. The amplified products were purified from agarose gel using a DNA gel extraction kit (Tiangen, China), and was then inserted to pET-30a (Novagen). The recombinant plasmid was confirmed by sequencing and restriction enzyme analysis, and the positive plasmid designated as pET30a-TgCDPK1.

2.4. Expression and purification of the recombinant proteins

The protocol of transfection and induction of competent cells was done as described [15] with minor modifications. The BL21 (DE3) competent cell were transformed with pET30a-TgCDPK1 and selected on Luria-Bertani (LB) agar plate containing 50 μg/ml of kanamycin. The recombinant proteins were expressed with a His tag and purified as described previously [16]. Briefly, the positive BL21 (DE3) colony were grown in LB at 37 °C until the optical density at 600 nm (OD600) reached 0.6–0.8, and recombinant protein was induced with 0.1 mM IPTG for 4 h. Cells were harvested and resuspended in PBS and lysed by sonication. The cell lysates were centrifuged at 10,000 × *g* for 30 min at 4 °C, and the supernatant was loaded onto a 10 mL Ni Sepharose™ fast flow resin column (GE Healthcare), and washed with wash buffer (PBS containing 15 mM imidazole, pH 7.8), finally the purified fusion protein was eluted with elution buffer (PBS containing 250 mM imidazole, pH 7.8). Before inoculation, rTgCDKP1 were dialyzed against PBS and endotoxins were removed with Detoxi-Gel endotoxin removal gel (Pierce, Rockford, IL), filtered throughout a 0.22 μM-pore membrane and stored at −80 °C.

2.5. Western bolt analysis

The purified rTgCDPK1 protein was subjected for SDS-PAGE. After electrophoresis, proteins were transferred onto a nitrocellulose membrane, which were immuno-blotted with serum obtained from a *T. gondii*-infected mouse and diluted 1:200 in 5% nonfat dried milk–TNT (15 mM Tris-HCl, 140 mM NaCl, 0.05% Tween 20) as previously described [17]. Bound antibodies were detected with horseradish peroxidase- (HRP-) conjugated goat anti-mouse IgG (Sigma-Aldrich, USA), diluted 1:1000 in 5% nonfat dried milk–TNT. Finally, the immunoreaction was detected with freshly prepared diaminobenzidine (TIANGEN, China) as a chromogenic substrate for 5 min.

2.6. Immunization and challenge

Mice were randomly divided into three groups (15 mice in each group), and were intraperitoneally injected 3 times at a 14-day interval: group 1 with 100 μl (100 μg/ml) rTgCDPK1 protein, group 2 with 100 μl PBS, and group 3 without any treatment. The serum samples were collected from the tail vein from 3 mice in each group at the day before every immunization and stored at −20 °C for further analysis.

Two weeks after the last immunization, 10 mice in each group were challenged intraperitoneally with 1,000 tachyzoites of *T. gondii* RH strain. The survival number of mice was recorded every day.

2.7. Antibody analysis

The levels of antigen-specific antibodies (IgG, IgG1 and IgG2a) were measured using enzyme-linked immunosorbent assay (ELISA) kits (SBA Clonotyping System-HRP Kit, Southern Biotech Co., Ltd, Birmingham, USA) according to previous description [14]. In brief, 100 μl CDPK1 (10 μg/ml) were used to coat 96-well microtiter plates at 4 °C overnight. The plate was washed three times with 0.05% Tween 20 in PBS (PBST) and blocked with PBS containing 1% BSA at room temperature for 1 h. After washing 3 time with PBS containing 0.05% Tween-20 (PBST), the plates were added with mouse serum samples diluted with PBS (1:50), and incubated at room temperature for 1 h. After washing five times with PBST, the bound antibodies were detected by horseradish peroxidase-conjugated anti-mouse IgG (diluted 1:250), anti-mouse IgG1 or IgG2a (1:500) and incubated for 1 h at 37 °C. Adding 100 μl substrate solution (pH4.0) (1.05% citrate substrate buffer; 1.5% ABTS; 0.03% H_2O_2), and then incubated in dark for 20 min. The absorbance values were measured at 450 nm (Bio-TekEL x 800, USA). All samples were carried out in triplicate. The negative control was included in each experiment, and the cut-off value was calculated using 2 × the average OD value of negative controls.

2.8. Lymphocyte proliferation assays

Two weeks after the last immunization, spleens were aseptically isolated from 3 mice from each group. The splenocytes were obtained aseptically via mechanical filtration of splenic organ and erythrocyte

were lysed with using erythrocyte lysis buffer (0.15 M NH4Cl, 1.0 M KHCO3, 0.1 mM EDTA, pH 7.2). Dulbecco's Modified Eagle Medium (DMEM) supplemented with 2% fetal calf serum (FCS) was used to resuspended splenocytes. These obtained lymphocytes were then cultured in triplicate at a density of 2×10^5 cells per well in 96-well microtiter plate. Lymphocytes were stimulated with STAg (10 μg/ml), concanavalin A (Con A; 5 μg/mL; Sigma) or medium alone served as positive and negative controls, respectively. After incubation at 37 °C in a 5% CO_2 for 44 h, each well was added 10 μl CCK-8 reagent (provided by Enhanced Cell Couning Kit-8 Beyotime, China) and incubation continued for 4 h. The proliferative activity was evaluated by measuring absorbance at 570 nm. The stimulation index (SI) for each group was calculated as follows: ($OD_{570STAg}/OD_{570\ Control}$): ($OD_{570ConA}/OD_{570Control}$).

2.9. Cytokine assays

The splenocytes were co-cultured with STAg or medium alone (negative control) in flat-bottom 96-well microtiter plates as described in the lymphocyte proliferation assay. The supernatant of each well was collected after 24 h to measure the level of IL-4; 72 h for IL-10; 96 h for IFN-γ and IL-12 (p70) and then detected with commercial ELISA kits (Biolegend, USA) according to the manufacturer's instructions and precious description [18]. Each experiment was performed in triplicate.

2.10. Flow cytometry analysis

The splenic lymphocytes were collected as described above [18], The viability was determined with 0.04% trypan blue (viability > 90%) and alive cell concentration was adjusted to 1×10^6 cells/ml in PBS containing 2% FBS, and the lymphocytes were incubated with 3 different surface markers at 4 °C for 30 min in the dark [19,20], including phycoerythrin (PE)-labeled anti-mouse CD3, allophycocyanin (APC)-labeled antimouse CD4 and fluorescein isothiocyanate (FITC)-labeled anti-mouse CD8 (eBioscience). Then the cells were fixed with FACScan buffer (PBS containing 1% FCS and 0.1% Sodium azide) and 2% paraformaldehyde. The specific cells were analyzed according to the surface markers (CD3, CD4 and CD8) through a FACScan flow cytometer (BD Biosciences, USA).

2.11. Statistical analysis

The data analysis was performed by SPSS18.0 Data Editor (SPSS Inc., Chicago, IL, USA). The statistical differences of antibody responses, lymphoproliferation assays, cytokine production, and percentages of $CD3^+CD4^+CD8^-$ and $CD3^+CD8^+CD4^-$ T cells, between all the groups were calculated according to one-way ANOVA, and the difference were considered statistically significant when $P < 0.05$.

3. Results

3.1. The expression and purification of TgCDPK1

After expression and purification, rTgCDPK1 protein was subjected to SDS-PAGE and separated on a 12% polyacrylamide gel, a band of 65 kDa was visualized through coomassie-blue staining (Fig. 1). This results indicated that rTgCDPK1 was expressed efficiently.

3.2. Western blotting analysis

The antigenicity of rTgCDPK1 was confirmed by Western blotting. The results showed that rTgCDPK1 reacted with serum from mice infected with *T. gondii* tachyzoites, no reaction was detected with negative mice sera (Fig. 2).

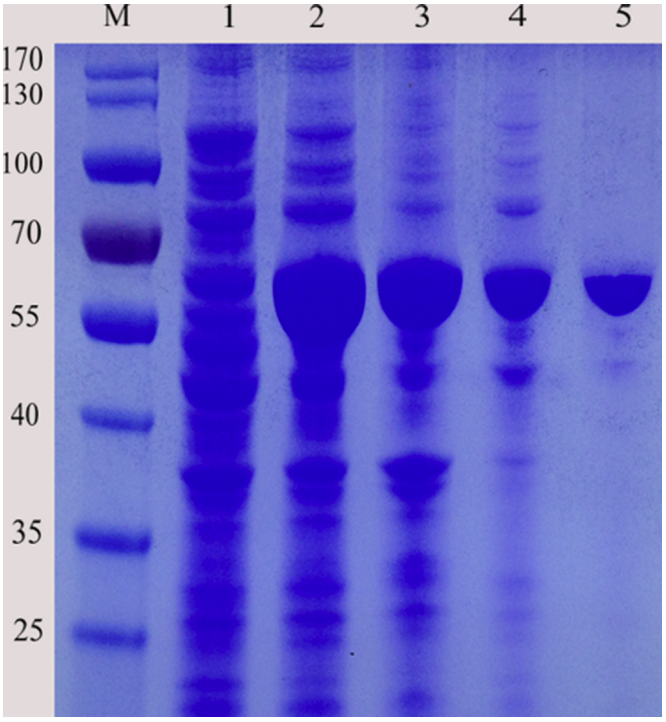

Fig. 1. SDS-PAGE analysis of TgCDPK1 expressed in *E. coli*. M: maker 25–170 kDa; (1) the negative control; (2) the lysate of *E. coli* transformed with pHis-TgCDPK1; (3) the centrifuged deposit of lytic *E. coli* transformed with pHis-TgCDPK1; (4) the centrifuged liquid supernatant of lytic *E. coli* transformed with pHis-TgCDPK1; (5) the purified TgCDPK1 of *T. gondii* expressed in *E. coli*.

3.3. Humoral response induced by recombinant antigen

To evaluate the level of anti-*T. gondii* antibodies, the serum samples from immunized and control groups were detected. From the results we could find that rTgCDPK1 induced a significant IgG response, the difference is significant between the immunized group and the control groups (PBS and blank) ($P < 0.05$). While, for the blank control and PBS groups, the levels of specific antibodies did not statistically increase with continuous immunization ($P > 0.05$) (Fig. 3).

In order to investigate the type of Th-response induced in the immunized mice, the expression level of IgG isotypes (IgG1 and IgG2a) were detected separately two weeks after last immunization. The results showed that the expression levels of IgG1 and IgG2a were significantly increased in immunized comparing with the control groups ($P < 0.05$), suggesting specific humoral response with a mixed Th1/Th2 profile was elicited by rTgCDPK1, with a predominant Th1 type immune response. For the control groups, no difference was detected in the expression levels of IgG1 and IgG2a ($P > 0.05$).

3.4. Splenocyte proliferation

The splenocyte proliferative levels simulated by STAg, ConA or blank were analyzed by Enhanced Cell Counting Kit-8. The proliferation stimulation index (SI)was shown in Table 1, proliferation values from mice immunized with rTgCDPK1 and stimulated with STAg indicated statistical difference compared with the control groups ($P < 0.05$), while the two control groups presented no significant differences ($P > 0.05$) (Table 1).

3.5. Flow cytometric analysis

The percentages of $CD3^+$ $CD8^+CD4^-$ and $CD3^+$ $CD4^+CD8^-$ T cells from splenocyte in each group were analyzed by flow cytometry and

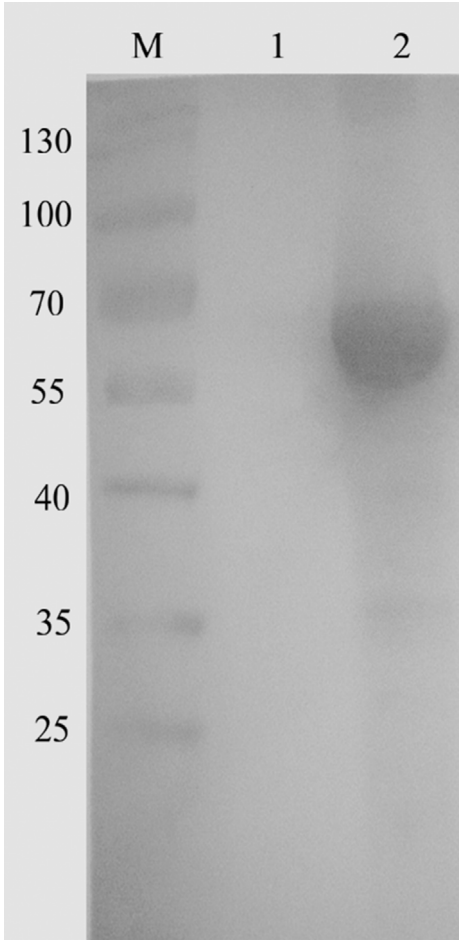

Fig. 2. Western blotting analysis of antigenicity of purified TgCDPK1. M: maker 25–130 kDa; (1) Western blot analysis using normalmice serum as the primary antibody; (2) Western blot analysis using serum obtained from a *T. gondii*-infected mouse.

summarized in Table 1. For the immunized group, the percentages of $CD3^+CD4^+CD8^-$ was 17.61% and $CD3^+CD8^+CD4^-$ was 6.49%, which were significantly increased in rTgCDPK1 immunized group comparing to the two control groups ($P < 0.05$). In contrast, the two T-cell subtypes were no significant increase in the two control groups ($P > 0.05$).

3.6. Cytokine production

The cytokines, including IL-4, IL-10, IFN-γ and IL-12 (p70), were analyzed by ELISA, and the results were summarized in Table 2. The ELISA results indicated that the levels of IFN-γ, IL-12 (p70), and IL-10 in rTgCDPK1 immunized group were increased significantly comparing to the two control groups ($P < 0.05$). While, the level of IL-4 from rTgCDPK1 immunized group showed no statistical difference between the two control groups ($P > 0.05$).

3.7. Protective efficacy of immunized mice

To investigate the protective efficacy against *T. gondii* infection induced by rTgCDPK1, ten mice from each group were challenged with 10^3 tachyzoites of RH strain at 2 weeks after last immunization. The survival time was recorded and the survival curve for each group of mice was shown in Fig. 4. The average survival times of mice in two control groups were no different, almost 7 days, the same with the previous studies [14,18], all mice were died within 8 days after challenge (blank control, 6.1 ± 0.3 days; PBS, 6.2 ± 0.4 days) ($P > 0.05$). The survival time of mice immunized with rTgCDPK1 was significantly prolonged (14.90 ± 2.89 days) compared to mice in the two control groups ($P < 0.01$). The immunization with rTgCDPK1 could prolong the survival time, but not totally protect the mice against the RH infection.

4. Discussion

The ideal vaccines against toxoplasmosis have been supposed to be a quite effective way to protect public health. In the last few years, many promising experiment vaccines were identified based on filtration of protective antigens, such as membrane-associated surface antigens [21–23], excreted-secreted dense granule proteins [24–27], rhoptry proteins [28–31] and microneme proteins [32,33]. However, these vaccines were limited to reduce partial protective immune responses. In the present study, the recombinant protein TgCDPK1 were evaluated as a subunit vaccine against experimental *T. gondii* infection in BALB/c mice model. The result showed immunization with recombinant TgCDPK1 protein is able to stimulate a significant proliferation of lymphocyte in vitro, which further elicited both humoral and cellular immune responses, resulting in prolonged survival time of mice challenged with virulent RH strain of *T. gondii*.

When *T. gondii* invasion launches in vitro, B cell responses can be activated and produced large amount of anti-*T. gondii* antibodies which adhered to the surface of parasites and limited their spread by forbidding the attachment of tachyzoites to host cell receptors. Those antibody-coated parasites were then eliminated by macrophages [34]. At the present study, the levels of IgG, detected two weeks after each immunization, revealed that our recombinant TgCDPK1 protein was good immunogenic. Moreover, the antibody levels of experimental group increased with successive immunization compared to the control group, suggesting a successful and continuous B cell response were induced by the subunit vaccine.

Many previous studies confirmed Th1 response is necessary for

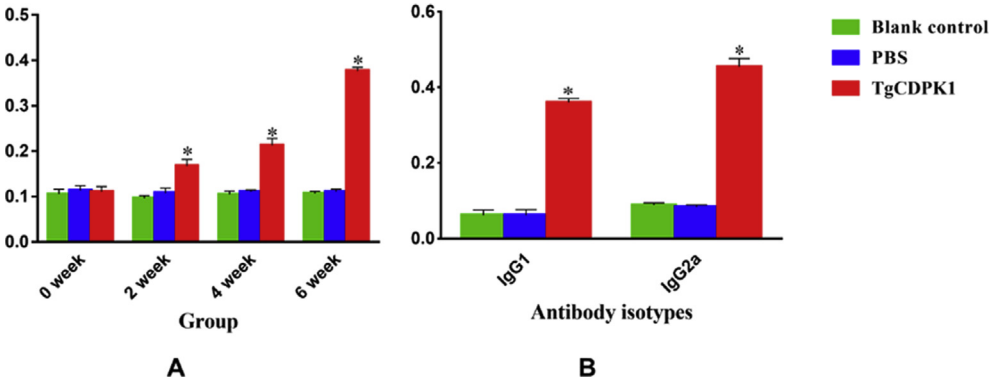

Fig. 3. The effect of subunit vaccine immunization on the antibody response. (A) Determination of total IgG antibodies in the sera of BALB/c mice immunized at weeks 0, 2, 4 and 6 post-primary vaccination as labeled. (B) Determination of the specific IgG isotype profile in the sera of BALB/c mice. Results are presented as means ± SD and statistically significant difference ($P < 0.05$) are indicated by an asterisk (*).

Table 1
Splenocyte proliferative responses and the percentages of $CD3^+CD4^+CD8^-$ and $CD3^+CD8^+CD4^-$ T cells in immunized mice 2 weeks after the last immunization.

Group	SI (Mean ± SD)	$CD3^+CD4^+CD8-$(%)	$CD3^+CD8^+CD4-$(%)
rTgCDPK1	1.97 ± 0.17*	17.61 ± 4.07*	6.49 ± 2.34*
PBS	1.08 ± 0.13	9.21 ± 2.23	3.23 ± 0.85
Blank control	1.15 ± 0.16	11.03 ± 2.32	3.34 ± 0.21

Data are presented as the mean ± SD (n = 3); (*) p value <0.05 is significant.

effective protection against *T. gondii* infections [34]. It is an imply that one of important conditions to be a good vaccine is able to induce a Th1 rather than Th2 type response in the T-helper cells. Therefore, the IgG isotype was detected by ELISA assay to determine the T-helper response in immunized mice. As result, high level of IgG1 and IgG2a were induced by rTgCDPK1 protein in the experimental group in contrast to the control group, which is consistent to previous findings [17,35–38]. The result indicated both the two humoral response were occurred simultaneously. By contrast, the level of IgG2a is higher than the level of IgG1, suggesting the response placed emphasis on the orientation of Th1- type response. In addition, these results also proofed by the aspect of different type of cytokines production.

Secretion of Th1-type cytokines like interferon-γ(IFN-γ) interleukin-12 (IL-12), and tumor necrosis factor-β (TNF-β) cooperating with cellular immunity is the key mechanism against *T. gondii* infection [39]. The production of IFN-γ by NK- or T-cells can restrict the growth of *T. gondii* in the acute or chronic phase of infection through the transcription factor STAT1 [40,41]. Meanwhile, the production of IL-12 by accessory cell can virtually promotes the ability of NK- and T-cells to make IFN-γ [42]. The immunogenicity and reactivity of rTgCDPK1 were detected by the specific IgG response, and then STAg of *T. gondii* were used to stimulated splenocytes mimicking the second infection by *T. gondii*, and different cytokines were measure to evaluate the response of cellular humoral immunity. In the present study, significantly high level of IFN-γ and IL-12 (p70) were detected in the mice of rTgCDPK1 group in contrast to the control groups while the levels of IL-4 and IL-10 were relatively low. That mean Th1-type response was mainly induced during the immunization protocol, which was in accordance with the previous IgG isotype results.

In addition, a slightly higher proliferative response of splenocytes induced by immunization with recombinant TgCDPK1 protein were presented by the proliferation stimulation index (SI), suggesting an activated cellular immune response. The percentages of both $CD3^+CD4^+CD8^-$ and $CD3^+CD8^+CD4^-$ cells increased, which were synergistic to cytotoxic activity against *Toxoplasma* infection and also responsible for the secretion of the increased levels of IFN-γ. These findings were similar to the previous results reported by other researchers [36,43,44].

At last, the survival rate of vaccinated mice in the face of a lethal *T. gondii* should be the most direct index to evaluate a candidate vaccine. As result, the survival time of all the mice in the TgCDPK1 group were significantly prolonged. Twenty percentage mice immunized with TgCDPK1 even survived for 17 days while all the mice in the two control groups died within 8 days.

In the present study, we found that intraperitoneal immunization

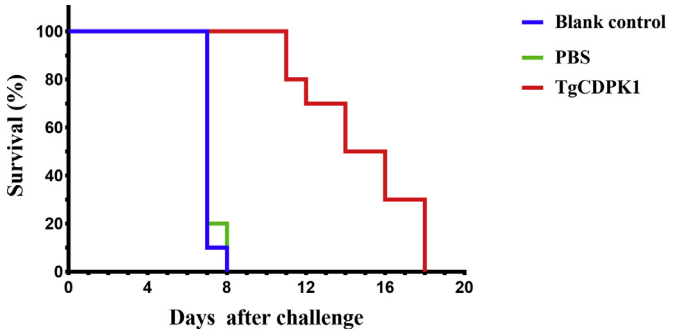

Fig. 4. Survival curves of mice immunized with PBS, rTgCDPK1 or blank control and challenged with 1,000 tachyzoites of *T. gondii* RH strain 2 weeks after the last immunization. Each group was composed of 10 mice and survival times were monitored daily after the challenge. The mice in control groups (PBS, blank control) were all dead within 8 days after challenge.

with recombinant TgCDPK1 protein can significantly prolonged the mice survival time, but cannot prevent them from death. The efficiency of this recombinant subunit was inferior to DNA vaccine of TgCDPK1, which may due to different mice model and variant mechanism of these two type of candidate vaccine [13]. The adjuvant is also very important for the efficiency of protein vaccine, there are so many different adjuvants, so the best adjuvant need to be screened in the future research. In addition, considering that TgCDPK1 gene is conserved among main types of *T. gondii* (Type I, Type II and Type III), which is expressed in the bradyzoite as well as the tachyzoite stage (unpublished data), we believed that the protection induced by recombinant TgCDPK1 protein purified from type I *T. gondii* could also be effective against type II and type III*T. gondii*. The details, especially the reduction of brain cyst of type II *T. gondii* in the immunized mice, need to be explored in the further investigation.

5. Conclusion

In summary, this study demonstrated recombinant TgCDPK1 protein is a novel that can induce strong humoral and cellular immunity response and provide a partial protection to experimental *T. gondii* infection, suggesting it is a promising vaccine candidate against acute toxoplasmosis. Nevertheless, further studies are needed to induce stronger immune protection by combining protein and DNA vaccine.

Table 2
Cytokine productions of splenocytes induced by soluble tachyzoite antigens of *T. gondii* (STAg).

Group	Cytokine production (pg/ml)			
	IFN-γ	IL-12 (p70)	IL-10	IL-4
rTgCDPK1	608.57 ± 36.13*	340.62 ± 7.42*	351.26 ± 15.47*	9.69 ± 4.16
PBS	88.54 ± 19.87	< 15	< 15	13.24 ± 0.88
Blank control	63.04 ± 25.76	< 15	< 15	10.97 ± 3.23

Data are presented as the mean ± SD (n = 3); (*) p value <0.05 is significant.

Ethics approval

This study was approved by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences. All animals were handled in accordance with good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

[1] L.M. Weiss, J.P. Dubey, Toxoplasmosis: A history of clinical observations, Int. J. Parasitol. 39 (2009) 895–901.
[2] A.M. Tenter, A.R. Heckeroth, L.M. Weiss, *Toxoplasma gondii*: from animals to humans, Int. J. Parasitol. 30 (2000) 1217–1258.
[3] D. Hill, J.P. Dubey, Toxoplasma gondii: transmission, diagnosis and prevention, Clin. Microbiol. Infect. 8 (2002) 634–640.
[4] Montoya, Toxoplasmosis, Lancet 363 (2004) 1965–1976.
[5] A. Kijlstra, E. Jongert, Control of the risk of human toxoplasmosis transmitted by meat, Int. J. Parasitol. 38 (2008) 1359–1370.
[6] G.M. Bhopale, Development of a vaccine for toxoplasmosis: current status, Microb. Infect. 5 (2003) 457–462.
[7] E.A. Innes, P.M. Bartley, S. Maley, F. Katzer, D. Buxton, Veterinary vaccines against Toxoplasma gondii, Mem. Inst. Oswaldo Cruz 104 (2009) 246–251.
[8] J.F. Harper, A. Harmon, Plants, symbiosis and parasites: a calcium signalling connection, Nat. Rev. Mol. Cell Biol. 6 (2005) 555–566.
[9] K. Nagamune, L.D. Sibley, Comparative genomic and phylogenetic analyses of calcium ATPases and calcium-regulated proteins in the apicomplexa, Mol. Biol. Evol. 23 (2006) 1613–1627.
[10] O. Billker, S. Lourido, L.D. Sibley, Calcium-dependent signaling and kinases in apicomplexan parasites, Cell Host Microbe 5 (2009) 612–622.
[11] J.L. Wang, S.Y. Huang, T.T. Li, K. Chen, H.R. Ning, X.Q. Zhu, Evaluation of the basic functions of six calcium-dependent protein kinases in Toxoplasma gondii using CRISPR-Cas9 system, Parasitol. Res. 115 (2016) 697–702.
[12] S. Lourido, J. Shuman, C. Zhang, K.M. Shokat, R. Hui, L.D. Sibley, Calcium-dependent protein kinase 1 is an essential regulator of exocytosis in toxoplasma, Nature 465 (2010) 359–362.
[13] J. Chen, Z.Y. Li, S.Y. Huang, E. Petersen, H.Q. Song, D.H. Zhou, et al., Protective efficacy of Toxoplasma gondii calcium-dependent protein kinase 1 (TgCDPK1) adjuvated with recombinant IL-15 and IL-21 against experimental toxoplasmosis in mice, BMC Infect. Dis. 14 (2014) 487.
[14] W.B. Yang, D.H. Zhou, Y. Zou, K. Chen, Q. Liu, J.L. Wang, et al., Vaccination with a DNA vaccine encoding Toxoplasma gondii ROP54 induces protective immunity against toxoplasmosis in mice, Acta Trop. 176 (2017) 427–432.
[15] H. Kothari, P. Kumar, N. Singh, Prokaryotic expression, purification, and polyclonal antibody production against a novel drug resistance gene of Leishmania donovani clinical isolate, Protein Expr. Purif. 45 (2006) 15–21.
[16] M.S. Picchio, V.R. Sanchez, N. Arcon, A.S. Soto, M. Perrone Sibilia, M.L.A. Aldirico, et al., Vaccine potential of antigen cocktails composed of recombinant Toxoplasma gondii TgPI-1, ROP2 and GRA4 proteins against chronic toxoplasmosis in C3H mice, Exp. Parasitol. 185 (2018) 62–70.
[17] H. Nabi, I. Rashid, N. Ahmad, A. Durrani, H. Akbar, S. Islam, et al., Induction of specific humoral immune response in mice immunized with ROP18 nanospheres from Toxoplasma gondii, Parasitol. Res. 116 (2017) 359–370.
[18] K. Chen, J.L. Wang, S.Y. Huang, W.B. Yang, W.N. Zhu, X.Q. Zhu, Immune responses and protection after DNA vaccination against Toxoplasma gondii calcium-dependent protein kinase 2 (TgCDPK2), Parasite 24 (2017) 41.
[19] N.Z. Zhang, S.Y. Huang, Y. Xu, J. Chen, J.L. Wang, W.P. Tian, et al., Evaluation of immune responses in mice after DNA immunization with putative Toxoplasma gondii calcium-dependent protein kinase 5, Clin. Vaccine Immunol. 21 (2014) 924–929.
[20] N.Z. Zhang, Y. Xu, M. Wang, E. Petersen, J. Chen, S.Y. Huang, et al., Protective efficacy of two novel DNA vaccines expressing Toxoplasma gondii rhomboid 4 and rhomboid 5 proteins against acute and chronic toxoplasmosis in mice, Expert Rev. Vaccines 14 (2015) 1289–1297.
[21] G. Zhang, X. Huang, D. Boldbaatar, B. Battur, B. Battsetseg, H. Zhang, et al., Construction of Neospora caninum stably expressing TgSAG1 and evaluation of its protective effects against Toxoplasma gondii infection in mice, Vaccine 28 (2010) 7243–7247.
[22] A.V. Machado, B.C. Caetano, R.P. Barbosa, A.P. Salgado, R.H. Rabelo, C.C. Garcia, et al., Prime and boost immunization with influenza and adenovirus encoding the Toxoplasma gondii surface antigen 2 (SAG2) induces strong protective immunity, Vaccine 28 (2010) 3247–3256.
[23] H. Cong, M. Zhang, Q. Xin, Z. Wang, Y. Li, Q. Zhao, et al., Compound DNA vaccine encoding SAG1/SAG3 with A2/B subunit of cholera toxin as a genetic adjuvant protects BALB/c mice against Toxoplasma gondii, Parasites Vectors 6 (2013) 63.
[24] X.N. Wu, J. Lin, X. Lin, J. Chen, Z.L. Chen, J.Y. Lin, Multicomponent DNA vaccine-encoding Toxoplasma gondii GRA1 and SAG1 primes: anti-Toxoplasma immune response in mice, Parasitol. Res. 111 (2012) 2001–2009.
[25] E. Hiszczynska-Sawicka, G. Oledzka, L. Holec-Gasior, H. Li, J.B. Xu, R. Sedcole, et al., Evaluation of immune responses in sheep induced by DNA immunization with genes encoding GRA1, GRA4, GRA6 and GRA7 antigens of Toxoplasma gondii, Vet. Parasitol. 177 (2011) 281–289.
[26] M. Xue, S. He, Y. Cui, Y. Yao, H. Wang, Evaluation of the immune response elicited by multi-antigenic DNA vaccine expressing SAG1, ROP2 and GRA2 against Toxoplasma gondii, Parasitol. Int. 57 (2008) 424–429.
[27] X.M. Sun, J. Zou, A.E. A, W.C. Yan, X.Y. Liu, X. Suo, et al., DNA vaccination with a gene encoding Toxoplasma gondii GRA6 induces partial protection against toxoplasmosis in BALB/c mice, Parasites Vectors 4 (2011) 213.
[28] W.S. Li, Q.X. Chen, J.X. Ye, Z.X. Xie, J. Chen, L.F. Zhang, Comparative evaluation of immunization with recombinant protein and plasmid DNA vaccines of fusion antigen ROP2 and SAG1 from Toxoplasma gondii in mice: cellular and humoral immune responses, Parasitol. Res. 109 (2011) 637–644.
[29] B. Zheng, S. Lu, Q. Tong, Q. Kong, D. Lou, The virulence-related rhoptry protein 5 (ROP5) of Toxoplasma Gondii is a novel vaccine candidate against toxoplasmosis in mice, Vaccine 31 (2013) 4578–4584.
[30] Z.G. Yuan, X.X. Zhang, X.H. He, E. Petersen, D.H. Zhou, Y. He, et al., Protective immunity induced by Toxoplasma gondii rhoptry protein 16 against toxoplasmosis in mice, Clin. Vaccine Immunol. 18 (2011) 119–124.
[31] D. Qu, J. Han, A. Du, Evaluation of protective effect of multiantigenic DNA vaccine encoding MIC3 and ROP18 antigen segments of Toxoplasma gondii in mice, Parasitol. Res. 112 (2013) 2593–2599.
[32] E. Hiszczynska-Sawicka, H. Li, J. Boyu Xu, M. Akhtar, L. Holec-Gasior, J. Kur, et al., Induction of immune responses in sheep by vaccination with liposome-entrapped DNA complexes encoding Toxoplasma gondii MIC3 gene, Pol. J. Vet. Sci. 15 (2012) 3–9.
[33] R. Fang, H. Feng, M. Hu, M.K. Khan, L. Wang, Y. Zhou, et al., Evaluation of immune responses induced by SAG1 and MIC3 vaccine cocktails against Toxoplasma gondii, Vet. Parasitol. 187 (2012) 140–146.
[34] P.C. Sayles, G.W. Gibson, L.L. Johnson, B cells are essential for vaccination-induced resistance to virulent Toxoplasma gondii, Infect. Immun. 68 (2000) 1026–1033.
[35] Z. Liu, F. Yuan, Y. Yang, L. Yin, Y. Liu, Y. Wang, et al., Partial protective immunity against toxoplasmosis in mice elicited by recombinant Toxoplasma gondii malate dehydrogenase, Vaccine 34 (2016) 989–994.
[36] M. Wang, X.Y. Yang, N.Z. Zhang, D.L. Zhang, X.Q. Zhu, Evaluation of protective immune responses induced by recombinant TrxLp and ENO2 proteins against Toxoplasma gondii infection in BALB/c mice, BioMed Res. Int. (2016) 3571962.
[37] H.L. Wang, L.M. Wen, Y.J. Pei, F. Wang, L.T. Yin, J.Z. Bai, et al., Recombinant Toxoplasma gondii phosphoglycerate mutase 2 confers protective immunity against toxoplasmosis in BALB/c mice, Parasite 23 (2016) 12.
[38] Z. Liu, L. Yin, Y. Li, F. Yuan, X. Zhang, J. Ma, et al., Intranasal immunization with recombinant Toxoplasma gondii actin depolymerizing factor confers protective efficacy against toxoplasmosis in mice, BMC Immunol. 17 (2016) 37.
[39] A. Benson, S. Murray, P. Divakar, N. Burnaevskiy, R. Pifer, J. Forman, et al., Microbial infection-induced expansion of effector T cells overcomes the suppressive effects of regulatory T cells via an IL-2 deprivation mechanism, J. Immunol. 188 (2012) 800–810.
[40] L.C. Gavrilescu, B.A. Butcher, L. Del Rio, G.A. Taylor, E.Y. Denkers, STAT1 is essential for antimicrobial effector function but dispensable for gamma interferon production during Toxoplasma gondii infection, Infect. Immun. 72 (2004) 1257–1264.
[41] L.A. Lieberman, M. Banica, S.L. Reiner, C.A. Hunter, STAT1 plays a critical role in the regulation of antimicrobial effector mechanisms, but not in the development of Th1-type responses during toxoplasmosis, J. Immunol. 172 (2004) 457–463.
[42] T. Scharton-Kersten, H. Nakajima, G. Yap, A. Sher, W.J. Leonard, Cutting edge, Infection of mice lacking the common cytokine receptor gamma-chain (gamma(c)) reveals an unexpected role for CD4(+) T lymphocytes in early IFN-gamma-dependent resistance to Toxoplasma gondii, J. Immunol. 160 (1998) 2565–2569.
[43] X.Z. Li, L. Lv, X. Zhang, K.Y. Anchang, A.Y. Abdullahi, L.Q. Tu, et al., Recombinant canine adenovirus type-2 expressing TgROP16 provides partial protection against acute Toxoplasma gondii infection in mice, Infect. Genet. Evol. 45 (2016) 447–453.
[44] X.Z. Li, X.H. Wang, L.J. Xia, Y.B. Weng, J.A. Hernandez, L.Q. Tu, et al., Protective efficacy of recombinant canine adenovirus type-2 expressing TgROP18 (CAV-2-ROP18) against acute and chronic Toxoplasma gondii infection in mice, BMC Infect. Dis. 15 (2015) 114.