



Contents lists available at ScienceDirect

Research in Veterinary Science

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

Immunological changes induced by *Toxoplasma gondii* Glutathione-S-Transferase (TgGST) delivered as a DNA vaccine

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ARTICLE INFO

Article history:

Received 30 May 2014

Accepted 3 December 2014

Keywords:

Toxoplasma gondii

Glutathione-S-transferase

TgGST

DNA vaccines

ABSTRACT

In this study, a DNA vaccine (pTgGST) encoding *T. gondii* antioxidant glutathione-S-transferase (TgGST) inserted into eukaryotic expression vector pVAX I was constructed and the immune protective efficacy of intramuscular vaccination of mice with pTgGST was analyzed. Mice immunized with pTgGST elicited high titers of total IgG, IgG₁, IgG_{2a}, IgA and IgM antibodies, while IgE showed no changes. Also, significant cytokine production of IFN- γ , IL-4 and IL-17 was detected in mice immunized with pTgGST, but not TGF- β 1. CD8⁺ T cells subsets and MHC-I molecules showed significant increase in contrast to CD4⁺ subsets. Immunization with pTgGST significantly prolonged survival time (14 days) after challenge infection with the virulent *T. gondii* RH strain, compared with the control groups which died within 8 days. These results suggested that TgGST DNA vaccine could trigger strong humoral and cellular responses and induce partial protection against acute toxoplasmosis.

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1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan responsible for a zoonotic parasitic infection throughout the world in a wide range of hosts, including humans (Calderaro et al., 2006). In addition to causing debilitating disease in congenitally infected infants and children, *Toxoplasma gondii* has emerged as a major opportunistic pathogen of immunocompromised individuals, particularly those infected with the human immunodeficiency virus (Israelski and Remington, 1988; Luft and Remington, 1992). Until now, besides some available chemotherapeutics that provide partial benefits to infected patients (Bhopale, 2003; Rodriguez and Szajnman, 2012), no effective control tool has been developed against toxoplasmosis infection to suppress the disease burden and its manifestations.

Recently in animal models, alternative drugs and treatment schemes have shown promising results against toxoplasmosis. A variety of new drugs with high activity *in vitro* and fewer side effects have been developed (Araujo et al., 1991a, 1991b, 1992, 1994, 1996). However, insufficient passage through the blood–brain barrier (BBB) and/or insufficient bioavailability of these drugs has decreased their application chances in humans (Schöler et al., 2001). In the view of cost and

possible resistance to the drug, a better alternative would be the development of vaccines, which would either completely prevent the infection or arrest the development of the parasite at a certain stage of its life cycle, or even partially reduce its ability to multiply (Rathaur et al., 2008).

Biologically, *T. gondii* is known to be equipped with sets of systems that help the parasite to survive and also determine its virulence. One of the important systems this parasite possesses is the antioxidant system, which is responsible for protecting the parasite against oxidative injury (Callahan et al., 1988). Glutathione-S-transferase (GST) is a major phase (II) detoxification enzyme protecting parasites from oxidative burst (Gupta et al., 2005). It has been well documented as a promising vaccine candidate against many helminths diseases as well as a drug target (Capron et al., 2012; Gupta et al., 2005; Rathaur et al., 2008; Veerapathran et al., 2009).

The purpose of this research is to evaluate the immunological capacities of *T. gondii* GST protein formulated as a DNA vaccine against Toxoplasmosis infection.

2. Materials and methods

2.1. Animals and parasite

Six to eight week-old female Swiss Webster (SW) mice were purchased from Center of Comparative Medicine, Yangzhou University (Yangzhou, China) and maintained under specific-pathogen-free standard conditions. All the animal experiments were approved by the Animal Ethics Committee of Nanjing Agricultural University

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(Approval number 200709005). *Toxoplasma gondii* strain "RH" (Type I) was provided by the Laboratory of Veterinary Molecular and Immunological Parasitology, Nanjing Agricultural University, China. To maintain the parasite, mice were intraperitoneally infected with the tachyzoites of *T. gondii*. Every 3 days, the tachyzoites were recovered from peritoneal washings of infected mice to be used for re-infection.

2.2. Construction of the prokaryotic plasmid

Following a standard protocol, total RNA was extracted from *T. gondii* tachyzoites followed by construction of the cDNA. The open reading frame (ORF) Glutathione-S-transferase (GST) gene [XM_002369189.1] was amplified PCR using the following synthetic primers in which recognition sites were inserted as underlined below. GST: Forward primer: 5'-GCGGGATCCATGACAGACAAGAGG AATC-3' (*Bam*H I); Reverse primer: 5'-CGCAAGCTTTCAGTAACGATGA GGTGAT-3' (*Hind* III). Cloning vector pMD-18T (TaKaRa, Dalian, China) was utilized for sequence analysis and verification. Following confirmation the fragment TgGST was recovered by double digestion (*Bam*H I/*Hind* III) and sub-cloned into pET28a (+) prokaryotic system (Novagen, USA) creating the recombinant plasmid designated as pET28a/TgGST.

2.3. Expression and purification of the recombinant TgGST (rTgGST)

The recombinant plasmid pET28a/TgGST was used to transform *E. coli* BL21 (DE3) bacteria, and protein expression was induced with 0.8 mM Isopropyl- β -D-thiogalactopyranoside (IPTG, BIOSHARP). After incubation at 37 °C for 7 hr, the bacterial cell were harvested and pelleted by centrifugation. The pellets were lysed using lysozyme (10 μ g/ml), followed by disruption with sonication. Expression of the protein was analyzed by 12% (w/v) Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

The recombinant protein was purified with Ni²⁺-nitrilotriacetic acid (Ni²⁺/NTA) column (GE Healthcare, USA) according to the manufacturer's instructions. Purity of the protein was detected by 12%SDS-PAGE, followed by determining the concentration according to the Bradford procedure using bovine serum albumin (BSA, sigma-Aldrich) as a standard. The purified protein was used to develop anti-TgGST sera by injecting rats while the remaining was stored at -20 °C for later applications.

2.4. Western blot analysis of native TgGST and rTgGST

T. gondii lysates were prepared as previously described (Hassan et al., 2014a), with minor modifications regarding this current experiment. Briefly, tachyzoites of *T. gondii* RH strain were obtained from the peritoneal washings of infected mice. The exudates were passed twice through a 27-gauge needle and then through 5 μ m filter membranes to remove debris and host cells. Parasites were then washed, re-suspended in phosphate-buffered saline (pH 7.4), and disrupted by sonication on ice.

T. gondii lysates and purified rTgGST were mixed with SDS loading buffer and electrophoresed on 12% SDS-PAGE gel and electro-transferred to PVDF (Immobililon, Millipore, USA) with a Trans-Blot SD system (Bio-Rad), respectively. Non-specific binding sites were blocked by immersing the membranes in blocking buffer (5% skim milk in Tris-buffered saline containing 0.1% Tween-20) for 2 h at 37 °C. The membranes were then washed 5 times (5 min each) in Tris-buffered saline containing 0.1% Tween-20 (TBST). Subsequently, the membranes were incubated with the primary antibody (rat anti-rTgGST polyclonal antibodies) overnight at 4 °C (dilutions 1:100 in TBST). After being washed 5 times in TBST, the membranes were then incubated with HRP-conjugated goat anti-rat IgG (SouthernBiotech, USA) for 1 h at 37 °C (diluted 1:6000 in TBST). Finally, the immunoreaction was visualized using freshly

prepared diaminobenzidine (DAB, Sigma) as a chromogenic substrate after 5 min.

2.5. Construction of the eukaryotic plasmid

TgGST fragment was recovered by double enzymatic digestion (*Bam*H I and *Not* I), and the target gene DNA fragment was directionally sub-cloned into the eukaryotic vector pVAX1 (Invitrogen, Life Technologies, USA) which was previously linearized with the same enzymes. Recombinant eukaryotic plasmid designated as pTgGST was verified and confirmed by sequencing in both directions as well as with double enzyme digestion. Plasmids were then purified from transformed *E. coli* DH5 (JM109) cells by anion exchange chromatography (EndoFree Plasmid mega Kit, Qiagen Sciences, USA) following the manufacturer's instructions, dissolved in sterile endotoxin-free H₂O and stored at -20 °C until use. The concentration of pTgGST was determined by spectrophotometer at OD₂₆₀ and OD₂₈₀.

2.6. In vivo translation pTgGST eukaryotic plasmid

Two groups, each with 5 mice, were used to verify the *in vivo* expression of pTgGST. One week after injection with the pTgGST and empty pVAX1, the mice were sacrificed and the quadriceps muscles from both groups were collected, grinded and treated on ice with RIPA (Beyotime, China) lysis Buffer (50 mM Tris pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% Sodium deoxycholate, 0.1% SDS) and centrifuged. The supernatant from each group was collected, and electrophoresed in 12% SDS-PAGE gel and western blot analysis performed as described in Section 2.4., using rat anti-rTgGST polyclonal antibodies as the primary antibody and goat anti-rat IgG (SouthernBiotech, USA) as the second antibody.

2.7. Mice immunization and challenge

Three groups of mice (25 per group) were injected intramuscularly (i.m.) two times with a two-week interval with 100 μ l diluted pTgGST DNA vaccine (1 μ g/ μ l), PBS or empty pVAX1 plasmid (1 μ g/ μ l), respectively. Another group of 25 mice was kept as blank control without injection. Blood samples were collected before vaccination (as negative control), as well as at the time of the 2nd injection and 2 weeks after the 2nd injection. The sera were stored at -20 °C

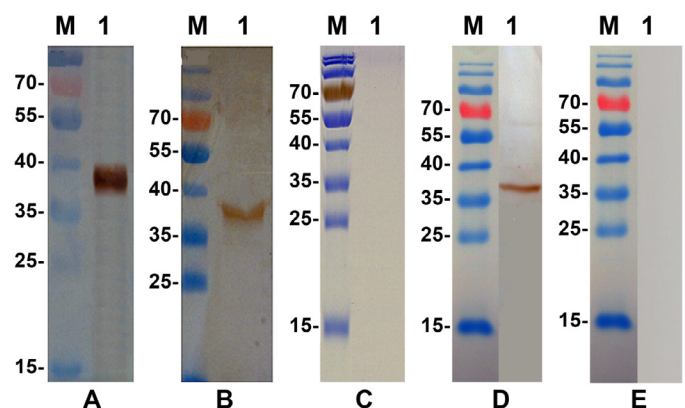


Fig. 1. Western blot analyses of recombinant proteins and native TgGST protein: (A) rTgGST recombinant protein probed with sera of rats experimentally immunized with rTgGST; (B) *in vivo* translation of pVAX1/TgGST (mice muscles lysates) probed with rat anti-rTgGST antibodies; (C) Negative control lysates of mice muscles transfected with empty pVAX1 plasmid, no band equivalent to TgGST protein was exposed; (D) Lysates of *T. gondii* tachyzoites probed with rat anti-rTgGST antibodies; (E) Lysates of *T. gondii* tachyzoites probed with rat normal serum. (M) Represents prestained protein marker.

for antibody evaluation and cytokine measurement. Two weeks after the last injection, the mice in the four groups were challenged intraperitoneally (i.p.) with 1×10^4 tachyzoites of *T. gondii* virulent RH strain. The survival rates of the challenged groups were observed and recorded on a daily basis.

2.8. Determination of immunoglobulins by ELISA

The levels of total IgG and isotypes IgG₁ and IgG_{2a} as well as IgA, IgM and IgE in serum samples were determined using indirect enzyme-linked immunosorbent assay (ELISA). Microtiter plates (Costar, USA) were coated with 5 µg TgGST recombinant protein in 50 mM carbonate buffer (pH 9.6) and incubated at 4 °C overnight. After three washes, the plates were blocked with 2% skimmed milk for 1 h at 37 °C and subsequently incubated with the mouse sera diluted in the same blocking buffer (1:100) for 1 hr at 37 °C. HRP-conjugated goat anti-mouse IgG, IgG₁, IgG_{2a}, IgA, IgM and IgE (Santa Cruz Biotechnology) was used as the secondary antibody at ratio 1:1000. Finally, the immune complexes were developed by incubating the plates with 3,3',5,5'-Tetramethylbenzidine (TMB) (TIANGEN, China) for 30 min. The reaction was stopped by adding 2 M H₂SO₄, and the absorbance was measured at 450 nm with an automated ELISA reader (MULTISKAN FC, Thermo Scientific); all samples were run in duplicate.

2.9. Cytokine assay

To assay cytokine production levels, sera from each experimental group were obtained as described previously. Interferon Gamma (IFN-γ), Interleukin-4 (IL-4), Interleukin-17 (IL-17) and Transformation Growth Factor-β1 (TGF-β1) were measured using ready ELISA kits according to the manufacturer's instructions (Boster Systems, Wuhan, China). Cytokine concentrations were determined by reference to standard curves constructed with known amounts of mouse recombinant IL-4, IL-17, IFN-γ and TGF-β1. The analysis was performed with the data from three independent experiments.

2.10. Flow cytometry analysis of T cell subpopulations and MHC molecules

The percentages of T cell subpopulations CD4⁺ and CD8⁺, beside MHC-I and MHC-II molecules in the splenocytes of mice in the test groups, pTgGST and pVAX1, PBS and blank, were analyzed using flow cytometry technique. Five mice from each group were sacrificed and the spleens were collected. Single cells from spleens were prepared as described by (Sasai et al., 2000).

The spleens were gently pushed through a mesh screen and cells were washed with calcium and magnesium-free Hank's balanced salt solution (HBSS). Lymphocytes were centrifuged over with a

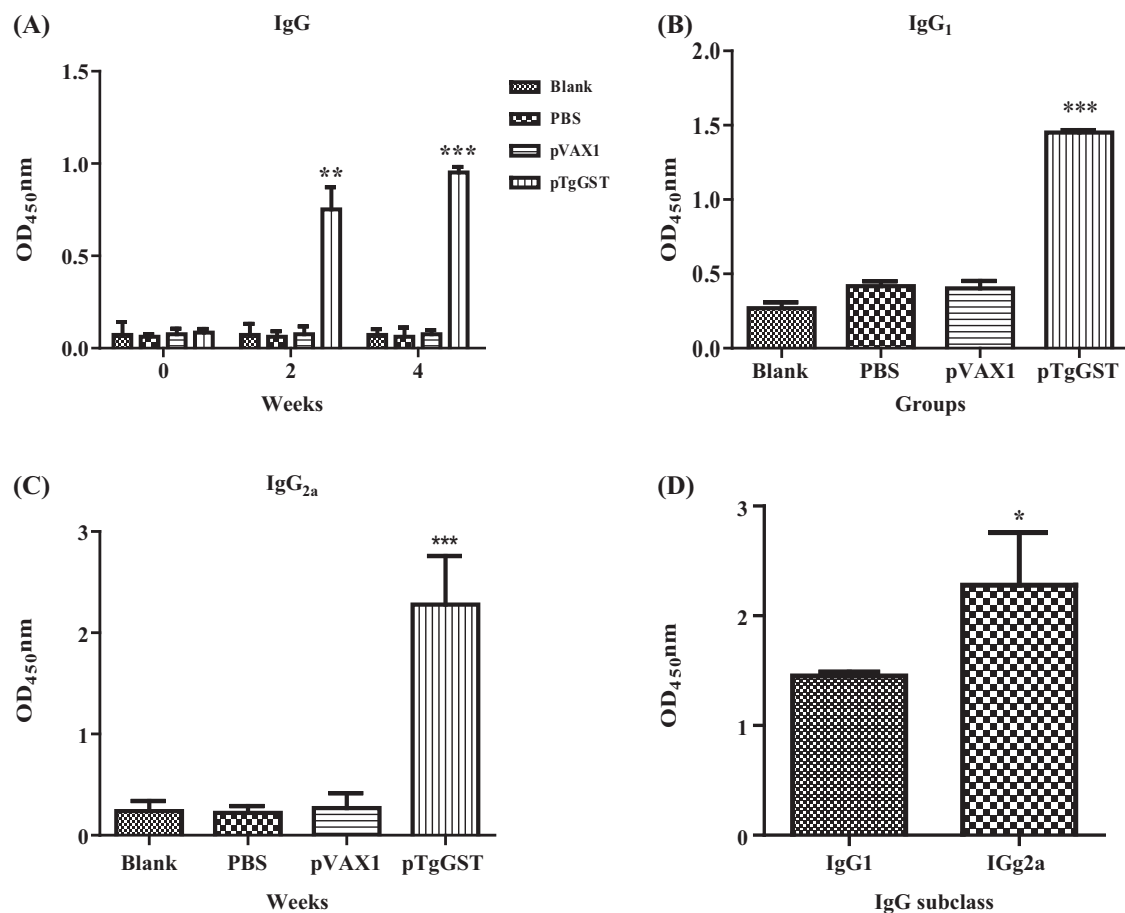


Fig. 2. IgG dynamics: (A) Levels of total IgG in sera of pTgGST, pVAX1, PBS and Blank groups were determined using indirect ELISA. Samples, before and after each immunization was collected, represent 3 timepoints marked as week 0, week 2 and week 4 (n = 5). (B) Levels of IgG₁ subclass, (C) levels of IgG_{2a} subclass, (D) comparison of the distribution levels of IgG₁ and IgG_{2a} subclasses in sera of pTgGST vaccinated group after immunization (n = 5). Results expressed as means $\pm$ SD of OD₄₅₀; the asterisk designates statistically significant differences between groups (P < 0.05).

separation solution (Nacalai tesque, Kyoto, Japan) according to the manufacturer's protocol. The cells at the interface were collected, washed twice and re-suspended in phosphate buffered saline (PBS), pH 7.4. Splenocyte suspensions (1×10^6 cells/ml) were dually stained with anti-mouse CD3e-FITC + anti-mouse CD8-PE, anti-mouse CD3e-FITC + anti-mouse CD4-PE, anti-mouse CD3e-FITC + anti-mouse MHC I-PE or anti-mouse CD3e-FITC + anti-mouse MHC II-PE (eBioscience) for 30 min at room temperature in the dark. Cell population analysis was conducted by FACScan flow cytometer with CellQuest software (BD Biosciences, Franklin Lakes, NJ, USA). A lymphocyte specific gating was set according to forward and side scatter profiles. The percentages of CD4⁺ and CD8⁺ T lymphocytes, MHC-I and MHC-II molecules in mice splenocytes were determined as described by (Song et al., 2010).

2.11. Statistical analysis

Data are expressed as mean $\pm$ the standard deviation (SD). One-way analysis of variance (ANOVA) was performed using the GraphPad Premier 5.0 (GraphPad Prism, USA) to compare the means. Significance was set at $P < 0.05$.

3. Results

3.1. Expression of TgGST recombinant plasmids

In vitro expression of the recombinant TgGST protein took place by adding IPTG followed by purification. The purified rTgGST was used to develop polyclonal antibodies (anti-rTgGST) and western blot technique was performed to verify the polyclonal antibodies (Fig. 1A). As for the eukaryotic plasmid (pTgGST), an *in vivo* experiment was

conducted to screen the expression. Using western blot technique, muscle lysates of the mice injected with the eukaryotic plasmid were immobilized on PVDF membrane and probed with anti-rTgGST polyclonal antibodies; successful expression of the protein was confirmed (Fig. 1B), while muscles injected with only pVAX1 showed no specific bands and remained negative (Fig. 1C). The native TgGST protein in lysates preparation of *T. gondii* tachyzoites was also detected using anti-rTgGST polyclonal antibodies showing a specific band (Fig. 1D).

3.2. Immunoglobulin response and isotype determination in immunized mice

The levels of specific antibodies IgA, IgM, IgE and total IgG, beside subclasses IgG₁ and IgG_{2a}, were detected by ELISA. As shown in (Fig. 2A), high levels of IgG antibodies were detected in the experimental group vaccinated with pTgGST, reaching the highest point after 2nd immunization (0.952 ± 0.03); a highly significant difference between pTgGST group and the three control groups was detected ($P < 0.01$). The distribution of IgG₁ and IgG_{2a} isotypes was analyzed two weeks after the last immunization. Both IgG₁ and IgG_{2a} were detected in the sera of mice immunized with pTgGST and considered highly significant ($P < 0.01$) compared with the three control groups (Fig. 2B and 2C), but there was a predominance of IgG_{2a} over IgG₁ (Fig. 2D).

Regarding other immunoglobulin classes and compared to control groups, the vaccinated group showed significant ($P < 0.01$) OD values of IgA (1.395 ± 0.48) and IgM (1.62 ± 0.34), while no significant change was detected in class IgE (0.235 ± 0.024); dynamics of these classes are illustrated in (Fig. 3A, 3B and 3C).

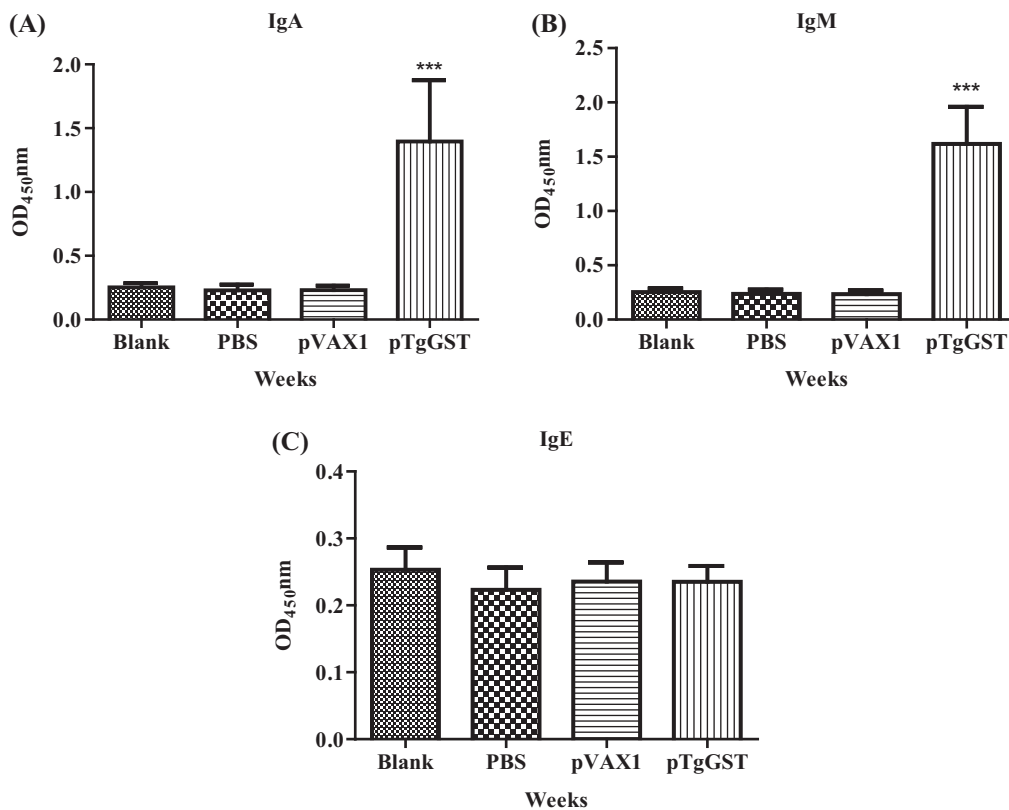


Fig. 3. (A) Class IgA, (B) class IgM and (C) class IgE. Sera samples ($n = 5$), at week 4, of mice in groups, pTgGST, pVAX1, PBS and Blank, were determined using ELISA; results expressed as means $\pm$ SD of OD₄₅₀. The asterisk designates statistically significant differences between groups.

3.3. Cytokine production

Sera samples collected at weeks 0, 2 and 4 were used to measure the amounts of IFN- γ , IL-4, IL-17 and TGF- β 1 produced in the different experimental groups. As shown in (Fig. 4A), mice vaccinated with pTgGST generated significant levels of IFN- γ ($P < 0.01$) compared to mice in the control groups; production peak was reached 2 weeks after the last immunization (757.67 ± 16.39). IL-4 and IL-17 of pTgGST group also showed a significant difference ($P < 0.05$) against the control groups (Fig. 4B and 4C). The peak of production was also at 2 weeks after the booster dose (76.6 ± 8.84) for IL-4 and (60.2 ± 4.83) for IL-17. However, TGF- β 1 (Fig. 4D) displayed no significant ($P > 0.05$) activity after immunization or when compared with the control groups.

3.4. T lymphocyte subsets and MHC molecule changes

Following immunization with pTgGST, no recruitment of CD4⁺ T cell subsets occurred, as there was no significant ($P > 0.05$) change detected (Fig. 5A); however, CD8⁺ T subset recruitment showed significant difference ($P < 0.05$) after 1st (7.28 ± 0.28) and 2nd (11.47 ± 0.40) immunizations, while pVAX1, PBS and blank control group remained at low levels as (2.83 ± 0.27), (3.14 ± 0.27) and (2.80 ± 0.17) respectively (Fig. 5B).

After both 1st and 2nd immunizations, MHC-I molecules of immunized group displayed significant readings (33.89 ± 1.83) and (36.22 ± 1.98) in a fashion similar to its associate CD8⁺; this increase came in contrast to pVAX1 (18.04 ± 1.34), PBS (17.85 ± 1.98) and Blank (17.56 ± 1.78) groups (Fig. 5C). Concerning MHC-II molecules, no antigen was presented through this pathway, as no difference at ($P < 0.05$) was detected after immunization or among the experimental groups (Fig. 5D).

3.5. Protection of vaccinated mice against challenge with *T. gondii* RH strain

Vaccinated and control mice groups were challenged with lethal *T. gondii* tachyzoites in the 2nd week after last immunization. Mortality was observed daily until all the mice died and survival curves of different groups were generated as shown in Fig. 6. Significantly longer survival time (14 days) was observed for mice immunized with pTgGST against the control groups, which died within 8 days post infection ($P < 0.05$).

4. Discussion

DNA vaccines have shown promise for defense against toxoplasmosis for their ability to induce long-term specific humoral and cellular immune responses (Chen et al., 2014; Hassan et al., 2014b; Xu et al., 2014). In this study, we demonstrated that DNA vaccine encoding TgGST of *T. gondii* could elicit significant immune response as well as provide considerable level of resistance leading to partial protection against acute toxoplasmosis infection.

Specific antibodies can inhibit the attachment of the parasite to the host cell receptors, and kill intracellular parasites (Kang et al., 2000; Sibley et al., 1993). In this study, significant antibody titers were obtained within 4 weeks of immunization in the vaccinated group. Our data demonstrated that, IgG₁, IgG_{2a} and IgG₃ subclasses were involved after immunization with TgGST, with IgG_{2a} being primarily expressed. This result suggested that TgGST could induce strong antibody responses. IgA, IgM and IgE were also reported to contribute to the immunological responses against *T. gondii* infection; that is, they could neutralize and block the dissemination of *T. gondii* parasite (Couper et al., 2005; Macpherson et al., 2008). In the present research, both IgA and IgM were positively recruited

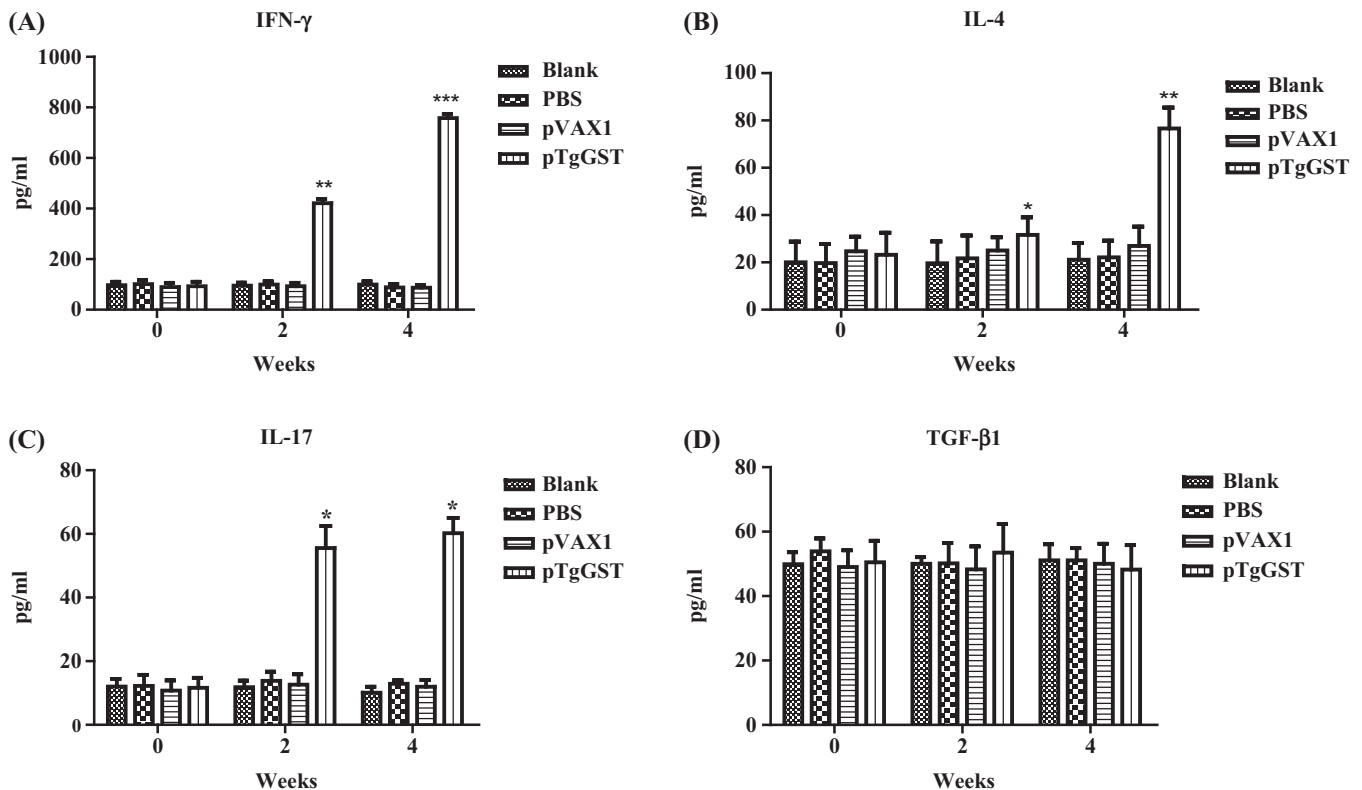


Fig. 4. Cytokine production: (A) IFN- γ , (B) IL-4, (C) IL-17 and (D) TGF β 1. Cytokine levels determined by antibody-captured ELISA in sera samples ($n = 5$) collected at weeks 0, 2 and 4. Results were expressed as means $\pm$ SD of pg/ml. The asterisk designates statistically significant differences ($P < 0.05$) between groups. Data presented here resulted from three independent experiments.

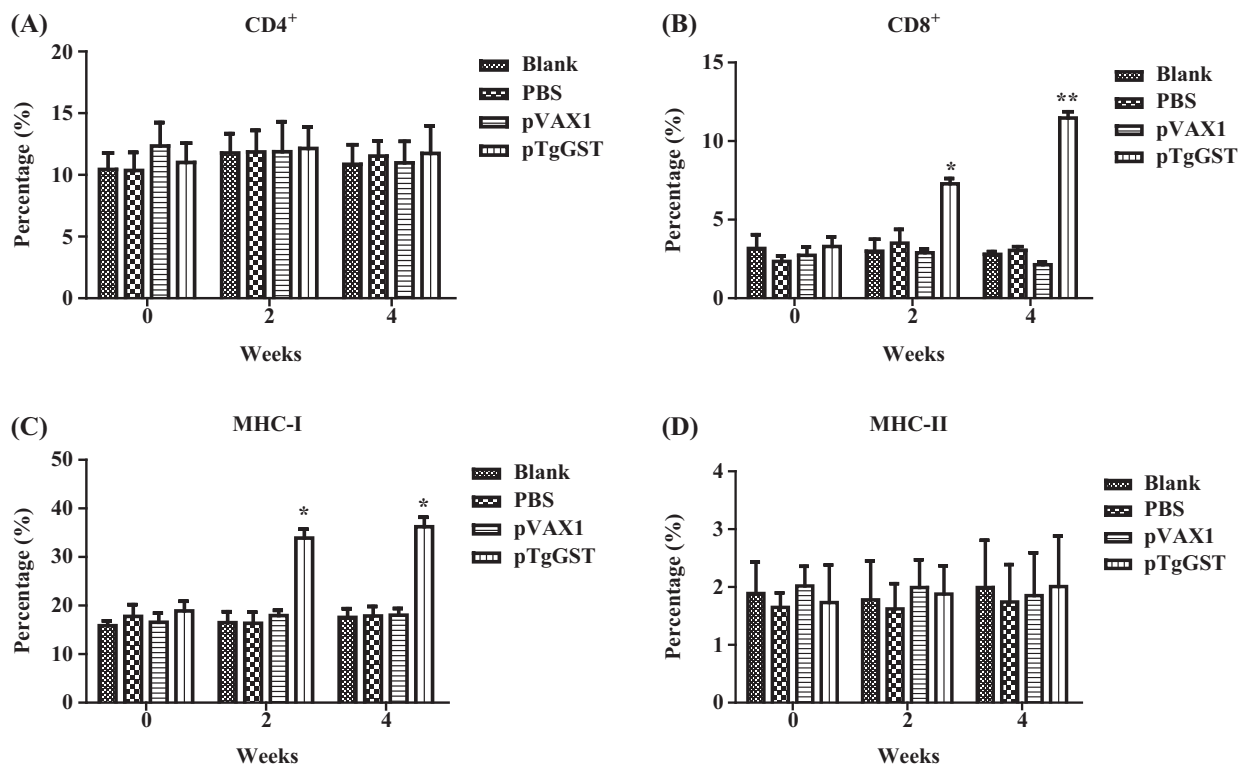


Fig. 5. Flow cytometry analysis of (A) CD4⁺ T lymphocytes subset, (B) CD8⁺ T lymphocytes subset, (C) MHC-I molecules and (D) MHC-II molecules. Representing samples collected at points marked as week 0, 2 and 4; data were represented as means $\pm$ SD (n = 5). The asterisk designates the significant differences between test groups. Results presented here were from three independent experiments.

after TgGST immunization. This result agrees with many reports concerning the roles of IgA and IgM during toxoplasmosis infection (Chardes et al., 1990; Pinon et al., 1990). However, in this study, IgE levels were not changed in the vaccinated groups. Absence of IgE could possibly be explained based on the route of delivering the antigen, since only orally infected mice were found to show IgE response (Godard et al., 1990).

IFN- γ was a typical cytokine of Th1 cells and was recognized for its important role during resistance against *T. gondii* (Janssen et al., 2002; Silva et al., 2009). It could inhibit and interfere *T. gondii*

persistence and reproduction (Dupont et al., 2012). In this study, remarkable levels of IFN- γ were detected in the immunized groups. This indicated that TgGST activated Th1 cells and resulted in Th1 response. Th2 immune response represented by IL-4 was also investigated in the trial. Recognized as B cell regulator and key cytokine of Th2 cells (Khan et al., 2005), IL-4 showed a relatively significant release in the immunized group compared with the control groups ($P < 0.05$). This positive increase comes in consistency with previous reports highlighting the implication of this cytokine during vaccination trials against toxoplasmosis (Alexander et al., 1998; Roberts et al., 1996). This result, together with the increase of IgG₁ subclass, indicated that TgGST could activated Th2 cells and stimulated Th2 response.

Apart from the Th1/Th2 paradigm, a newly characterized lineage of helper T cells, Th17, has been characterized. This lineage produces proinflammatory cytokines such as IL-17, IL-21, and IL-22. These cytokines are known to induce the recruitment of neutrophils during inflammatory diseases (Chen and O'Shea, 2008; Miyamoto et al., 2003; Ye et al., 2001). Such cell-mediated response is essential for protection against infections like toxoplasmosis (Alexander et al., 1997; Kelly et al., 2005). In this investigation, after immunization, significant increase of IL-17 concentration was observed. This finding suggested that TgGST was capable of inducing inflammatory reaction through activating Th17 differentiation. This finding was consistent with previous report (Passos et al., 2010). TGF- β 1 is a typical cytokine of T_{reg} cells with inhibitory immune roles. It regulates many immune cell functions including survival, migration, differentiation and proliferation (Champs et al., 1995; Hayashi et al., 2003; Lin et al., 1995). In this study, TGF- β 1 demonstrated no significant changes after immunization with TgGST compared to the control groups. This indicated that TgGST did not stimulate T_{reg} cells. It also might reflect the antagonistic relationship between IFN- γ and TGF- β 1, which was highlighted by others (Bermudez et al.,

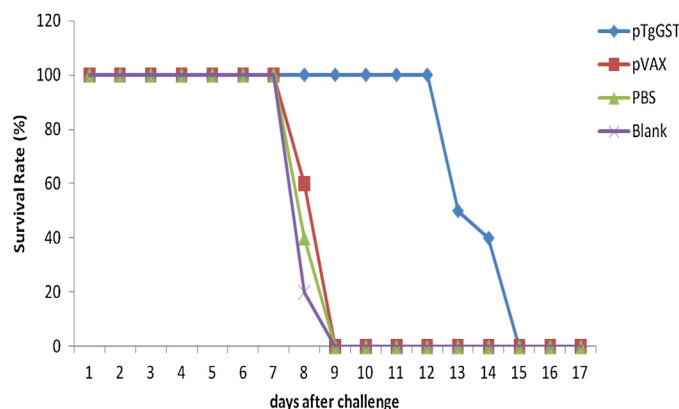


Fig. 6. Immune-protection of pTgGST. Survival time was monitored daily after lethal challenge with 2×10^4 tachyzoites of virulent *T. gondii* RH strain, 2 weeks after the last immunization. The mice immunized with pTgGST were all dead 14 days post challenge, showing an increased survival time compared with mice in the control groups (pVAX1, PBS, blanking controls), which died within 8–9 days after challenge ($P < 0.05$).

1993; Hunter et al., 1995; Schindler et al., 1998). Due to the characteristics of the intracellular fate of *T. gondii*, specific cellular immune response, in particular, strong CD8⁺ T cell mediated response, acts a key role in the control of development and spreading of *T. gondii* infection (Munoz et al., 2011). In the present study, mice vaccinated with pTgGST induced a significant increase of CD8⁺ T cells, suggesting that TgGST was able to drive the recruitment of this subpopulation type T cells, a finding similar to that previously reported (Hakim et al., 1991; Khan et al., 1991, 1994; Parker et al., 1991; Subauste et al., 1991). The increases of MHC I molecules in this study confirmed that TgGST could stimulate the MHC class I restricted antigen presentation pathways, which was required for the activation of cell mediated immunity. However, no significance was observed concerning CD4⁺ subpopulation, although this subset appeared to be crucial during Toxoplasmosis resistance in some reports (Araujo, 1992; Gazzinelli et al., 1991; Johnson and Sayles, 2002). This was contrary to the increases of some cytokines in the present study. Its reasons should be further studied.

To evaluate the protective efficacy of the pTgGST DNA vaccine, highly susceptible SW mice to *T. gondii* were intraperitoneally challenged with 1×10^4 tachyzoites of virulent *T. gondii* RH strain. Mice vaccinated with pTgGST showed significantly prolonged survival time, demonstrating moderately effective protective efficacy. It was similar to the results of previous studies employing several other single gene DNA vaccines (Xu et al., 2014; Zhao et al., 2013). Mice immunized with pTgGST showed stronger humoral and cellular immune responses, but all of the vaccinated mice showed signs of illness and were killed by day 14 post-challenge. The results indicate, therefore, that the DNA vaccines did not provide complete protection against *T. gondii* RH tachyzoite infection.

It is well known that *T. gondii* strains of different genotypes are responsible for different toxoplasmosis in humans and animals (Robert-Gangneux and Dardé, 2012; Sibley and Ajioka, 2008). In this study, we only observed the immune protection of TgGST against Type I strain of *T. gondii*, but not the other genotype strains. The immune protections of TgGST to other genotype strains needs further research.

5. Conclusion

In conclusion, the results of the present study showed that immunization with TgGST DNA vaccine induced strong humoral and cellular immune responses, and prolonged survival time against lethal challenge. Although TgGST elicited only partial protection against acute toxoplasmosis, it could be used as potential vaccine candidate in further studies of multi-component *T. gondii* vaccines against toxoplasmosis in the mice model. Further investigations on the mechanisms of the immune protection of this DNA vaccine might also help in understanding the mechanisms through which *T. gondii* relies on detoxifying enzymes during establishment of the disease.

Acknowledgements

This work was supported by the Special Fund for Public Welfare Industry of the Ministry of Agriculture of the People's Republic of China (200903036-04) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

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