Toxoplasma gondii Protein Disulfide Isomerase (TgPDI) Is a Novel Vaccine Candidate against Toxoplasmosis

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Abstract

Toxoplasma gondii is a ubiquitous protozoan parasite that can infect all warm-blooded animals, including both mammals and birds. Protein disulfide isomerase (PDI) localises to the surface of T. gondii tachyzoites and modulates the interactions between parasite and host cells. In this study, the protective efficacy of recombinant T. gondii PDI (rTgPDI) as a vaccine candidate against T. gondii infection in BALB/c mice was evaluated. rTgPDI was expressed and purified from Escherichia coli. Five groups of animals (10 animals/group) were immunised with 10, 20, 30, 40 μg of rTgPDI per mouse or with PBS as a control group. All immunisations were performed via the nasal route at 1, 14 and 21 days. Two weeks after the last immunisation, the immune responses were evaluated by lymphoproliferative assays and by cytokine and antibody measurements. The immunised mice were challenged with tachyzoites of the virulent T. gondii RH strain on the 14th day after the last immunisation. Following the challenge, the tachyzoite loads in tissues were assessed, and animal survival time was recorded. Our results showed that the group immunised with 30 μg rTgPDI showed significantly higher levels of specific antibodies against the recombinant protein, a strong lymphoproliferative response and significantly higher levels of IgG2a, IFN-gamma (IFN-γ), IL-2 and IL-4 production compared with other doses and control groups. While no changes in IL-10 levels were detected. After being challenged with T. gondii tachyzoites, the numbers of tachyzoites in brain and liver tissues from the rTgPDI group were significantly reduced compared with those of the control group, and the survival time of the mice in the rTgPDI group was longer than that of mice in the control group. Our results showed that immunisation with rTgPDI elicited a protective immune reaction and suggested that rTgPDI might represent a promising vaccine candidate for combating toxoplasmosis.

Introduction

Toxoplasma gondii is a ubiquitous protozoan parasite that can infect virtually all warm-blooded animals, including both mammals and birds. Epidemiological surveys have suggested that T. gondii infection has a wide distribution and a high prevalence in many areas of the world. Up to one-third of the world’s population is infected with this parasite [1,2]. Ingesting uncooked meat containing tissue cysts or food and water contaminated with oocysts from infected cat faeces, and transmission of tachyzoites to foetuses through placenta are the three primary routes of transmission of T. gondii. Most people infected with T. gondii show no symptom, but severe complications occur in pregnant women and immunocompromised individuals, such as recipient of organ transplant, AIDS patients and cancer patients undergoing chemotherapy [3].

In addition, infection of T. gondii in domestic animals represents a considerable threat to public health due to food-borne outbreaks leading to heavy economic loss worldwide [4]. Treatment of toxoplasmosis is difficult due to the severe side effects of the available drugs and re-infection may occur at any time [5,6]. Therefore, development of an effective vaccine against T. gondii or new anti-Toxoplasma drugs are of great importance in preventing both foetal infection and infection of immunocompromised patients, as well as reduce the economic loss due to abortion of farm animals [7].

In recent years, extensive efforts have been made to develop anti-Toxoplasma vaccine, including vaccines of inactivated, attenuated, subunit, genetically engineered and DNA vaccines [8]. However, little protection has been offered against this ubiquitous pathogen. At present, only an attenuated vaccine (Toxovax, Intervet Shering-Plough) based on the live attenuated S48 strain has been licensed for use in sheep in Europe and New Zealand for over two decades [9]. The drawbacks of this vaccine are short shelf-life, adverse effects, and the potential risk of reverting to a pathogenic strain. This vaccine is apparently not suitable for human [7]. Therefore, it is important to search for novel target antigens in order to stimulate potent immunoprotection via an optimal delivery strategy.

By using bioinformatics, genomics and proteomics strategies, more novel and effective vaccine candidates have been identified.


TgPDI: A Protective Antigen against Toxoplasmosis

Materials and Methods

Animals and parasites

The inbred-strain mice used in this study were 6-week-old BALB/c mice purchased from the Institute of Laboratory Animals, Chinese Academy of Medical Science, Beijing. T. gondii tachyzoites (RH strain) were kindly provided by Peking University Health Science Center and were maintained via serial intraperitoneal passage in BALB/c mice.

Ethics Statement

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of Shanxi Medical University. Mice were bred and maintained under conventional, non-SPF conditions at the Center of Laboratory Animals and were maintained via serial intraperitoneal passage in BALB/c mice.

Alignment of the amino acid sequence of TgPDI

The amino acid sequence of PDI from three strains of T. gondii was obtained from internet (http://www.ncbi.nlm.nih.gov). The protein accession number is CAC28361 for RH strain, type I; XP_002371293 for ME 49 strain, type II; EEE29915 for VEG strain, type III respectively. The alignment was performed by DNAMAN software (Lynnon, Quebec, Canada).

Recombinant protein expression in E. coli and purification

Total RNA was extracted from 5 x 10⁸ tachyzoites according to the instructions of the manufacturer of the TRIZol reagent (CoWin Biotechnology, Beijing, CN). First-strand cDNA was synthesised using the HiFi-MMLV cDNA Kit (CoWin Biotechnology), and the coding region of TgPDI was amplified via reverse transcription polymerase chain reaction (RT-PCR). Primers for amplification of the ORF of the TgPDI gene were designed considering the TgPDI sequence of the RH strain of T. gondii (GenBank accession No: AJ306291.2). The forward primer was 5'-ACGCCGATG-CATGGACGCGGTTTTCGTGGT-3' and the reverse primer was 5'-ACGCICGACAGTCTCTTCAACCTTTGTCGTCG-3', which contained BamH I and Xho I restriction sites (underlined), respectively. PCR was carried out according to a conventional protocol.

For recombinant protein expression in E. coli, the TgPDI cDNA fragment was cloned into the pET-30a(+) vector (Novagen, USA) to form pET-30a(+) - TgPDI. This pET-30a(+) - TgPDI plasmid was then transformed into Rosetta (DE3) host bacteria cells (Transgen, CN), and recombinant protein expression was induced with 0.1 mM IPTG under continuous culture at 25° C for 8 h.

The recombinant cells were harvested via centrifugation, and the obtained pellets were resuspended in lysis buffer (50 mM Tris pH 7.7, 500 mM NaCl, 10 mM imidazole, 1 mM PMSF, 2 mM DTT) and homogenised via sonication on ice. The lysate was then centrifuged to separate the supernatant and cell debris. The level of rTgPDI expression was evaluated through 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue R-250 staining. A western blotting assay with a His primary antibody, rabbit anti-T. gondii serum or human anti-T. gondii serum (which was provided by professor Suo Xun, China Agricultural University) was performed to confirm the expression of rTgPDI. Moreover, native TgPDI from soluble tachyzoite antigen (STAg) was detected by Western blotting using the mouse anti-rTgPDI serum which was generated in this study.

All of the subsequent purification steps were performed at 4° C. The clear supernatants were mixed with Ni²⁺-NTA agarose (Qiagen, Germany) and incubated for 1 h with gentle shaking. The resultant slurry was transferred to a chromatography column, washed with 10 bed volumes of wash buffer (50 mM Tris pH 7.7, 500 mM NaCl, 20 mM imidazole), and the target protein was eluted with elution buffer (50 mM Tris pH 7.7, 500 mM NaCl, 150 mM imidazole), then pooled and transferred to desalting buffer (25 mM Tris pH 8.5, 80 mM NaCl, 2 mM DTT) using a Gravity DeSalting Column (Shanghai Sangon, CN). The desalted samples were concentrated to 5 mg/ml in sterile PBS using a 300,000 molecular weight device (Sartorius Stedim Biotech), and the purified protein was analysed via SDS-PAGE.

rTgPDI immunisation

The immunisation experiments were performed in 6-week-old BALB/c mice, half of which were male, and half were female. Five groups of mice (10 per group) were intranasally immunised with 10, 20, 30 or 40 µg of rTgPDI dissolved in 20 µl of sterile PBS, or with PBS as a control. Each dose of immunogen (10 µl/nostril) was introduced into the nostrils of mice with a micropipette within a period of 5 minutes to reduce the distress of the mice. The mice were immunised using the same protocol on days 0, 14, and 21. Two weeks after the final inoculation (on day 35), the mice were anesthetised with sodium pentobarbital (1.5%, 0.1 ml/20 g bodyweight) and sacrificed. At 24 h post-infection (p.i.), mice were challenged intraperitoneally with 2 x 10⁵ tachyzoites of the RH strain.
were expressed as the stimulation index (SI), which is the ratio of the counts to the control. Counting Kit-8 (Beijing Dojindo, CN) was used, and the results were examined in triplicate. All samples were measured in an ELISA reader (Epoch Multi-Volume Spectrophotometer System, Biotek, USA) at 450 nm. All samples were terminated by adding 1 M H2SO4. The optical density (OD) was revealed by incubation with orthophenylene diamine (Sigma) and 0.15% H2O2 for 30 min, and the enzyme reaction was then stopped by the addition of 1 M H2SO4. The optical density (OD) was measured in an ELISA reader (Epoch Multi-Volume Spectrophotometer System, Biotek, USA) at 450 nm. All samples were examined in triplicate.

Lymphocyte proliferation assays

Two weeks after the last immunisation, the spleens were collected from the mice in each group under aseptic conditions in Hank’s balanced salt mixture (Solarbio, CN), then minced using a pair of scissors and passed through a 41.6 μm mesh sieve to produce a homogeneous cell suspension. The lymphocytes were obtained using lymphocyte isolation solution (Biyuntian, CN). Then, 2x10⁵ cells/well were cultured in 96-well plates in triplicate in RPMI-1640 containing 10% FCS and either stimulated with 10 μg/ml of rTgPDI or not for 72 h at 37°C. After washing three times (each time for 5 min) with PBS, individual sera (100 μl/well) diluted in 1% BSA-PBST (1:150, 1:50 for IgA, IgG1 and IgG2a) were transferred to the wells, followed by incubation for 1 h at 37°C. After the plates were washed, bound antibodies were detected by adding 50 μl of horseradish peroxidase-conjugated goat anti-mouse IgA, IgG, IgG1 or IgG2a (ProteinTech Group, Inc, USA) diluted 1:2000. After the plates were washed in PBS, immune complexes were revealed by incubation with orthophenylene diamine (Sigma) and 0.15% H₂O₂ for 30 min, and the enzyme reaction was then terminated by adding 1 M H₂SO₄. The optical density (OD) was measured in an ELISA reader (Epoch Multi-Volume Spectrophotometer System, Biotek, USA) at 450 nm. All samples were examined in triplicate.

Cytokine assays

For these assays, 1.5x10⁵ cells/well were seeded into 24-well plates in triplicate and stimulated with 10 μg of rTgPDI. Cell-free supernatants were harvested and assayed to determine IL-2 and IL-4 activities at 24 h; IL-10 activity at 72 h; and interferon-gamma (IFN-γ) activity at 96 h. The concentrations of IL-2, IL-4, IL-10 and IFN-γ were evaluated using a commercial ELISA kit (PeproTech, USA) according to the manufacturer’s instructions. All assays were performed in triplicate. Cytokine concentrations were determined in reference to standard curves constructed with known amounts of mouse recombinant IL-2, IL-4, IL-10 or IFN-γ. The sensitivity limits of IL-2, IL-4, IL-10 and IFN-γ detection were 16, 16, 47 and 25 pg/ml, respectively.

Challenge infection

According to the results regarding the doses of rTgPDI that elicited different immune responses, 30 μg of rTgPDI was selected as the dose for chronic and acute challenge infection. A total of 52 mice (half male and half female) were randomly divided into two groups (the PBS and 30 μg dose groups; 26 mice/group, 10 subjected to chronic infection and 16 to the acute assay) and challenged via intragastric administration of 1x10⁴ tachyzoites for the chronic assay or 4x10⁴ tachyzoites for acute infection on the fifteenth day after the last immunisation. The numbers of tachyzoites in the brains and livers of the mice were measured to assess the results of the chronic challenge infection assay. For survival analysis, the infected mice were monitored at 8 am, 2 pm and 8 pm daily regarding their physical appearance, such as displaying rough coat, decreases in appetite, weakness/inability to obtain food or water and depression. When these conditions were observed, a mouse would be moved to an isolated cage for further husbandry; if obvious suffering, such as struggling or whining was observed, the mouse would be sacrificed through ether inhalation. No obvious suffering was observed in this study. To assess the protective effect of rTgPDI in the infected mice, the time to death and survival were recorded and assessed for one month after parasite challenge.

Quantitation of T. gondii tachyzoites in murine brains and livers

At two weeks post-vaccination with PBS or 30 μg rTgPDI, 10 mice from each group were challenged orally with 1x10⁴ tachyzoites from the T. gondii RH strain. During the challenge, no suffering was observed. Four weeks later, the challenged mice were killed using an overdose of sodium pentobarbital, and the intact brains and partial livers were collected from each mouse and homogenised in 2 ml of PBS. For each brain and liver suspension, the mean number of tachyzoites was determined from four samples (25 μl each) in a hemocytometer. The tachyzoite load was estimated on the basis of the average quantity of tachyzoites per gram brain or liver tissue.

Western blotting

The STAgs were prepared as described previously [11]. The rTgPDI products expressed in E. coli Rosetta (DE3) and the soluble tachyzoite antigen were boiled at 95°C for 5 min, followed by centrifugation at 12,000×g for 10 min at room temperature. The supernatants were separated in 12% SDS-PAGE gels and then electrophoretically transferred to PVDF membranes (GE Healthcare). The membranes were blocked in 5% skim milk for 1 h at room temperature and then incubated at 4°C overnight with the anti-His primary antibody (1:1000), rabbit anti-T. gondii serum (1:200), human anti-T. gondii serum (1:200) or mouse anti-T. gondii serum produced by immunization with rTgPDI (1:200). The PVDF membrane was subsequently incubated with anti-mouse (rabbit or human) HRP-IgG (Beyotime, CN) for 1 h at room temperature, and chemiluminescence was detected using an ECL blot detection system (Ensgreen, CN).

Statistical analysis

All data, including results of the lymphocyte proliferation assays, antibody responses, cytokine levels and the tachyzoite loads, were statistically analysed by one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) for Windows, version 13.0. P values of less than 0.05 were considered statistically significant. The survival times for the vaccinated and control groups were compared using the Kaplan-Meier method.

Results

TgPDI is a conserved protein

The alignment of the amino acid sequence of TgPDI shows a 100% homology among three strains which represent different
Genotypes of T. gondii (Fig. 1). The results indicate the PDI is a conserved protein in evolution of Toxoplasma.

rTgPDI was expressed and purified successfully

The cDNA sequence corresponding to the TgPDI ORF was cloned into the prokaryotic expression vector pET-30a(+) and transformed into the Rosetta (DE3) bacterial host. After induction with 0.1 mM IPTG at 25°C, the rTgPDI proteins were successfully expressed in E. coli and the molecular weight was approximately 57 KDa (Fig. 2A). The isolated protein was water soluble and showed 95% purity based on SDS-PAGE analysis (Fig. 2B). Western blotting analysis indicated that the rTgPDI band was able to react with the rabbit anti-T. gondii serum polyclonal antibody and anti-His antibody (Fig. 2C). Moreover, rTgPDI was recognised by anti-Toxoplasma human serum (Fig. 2D) and native TgPDI contained in the soluble tachyzoite antigen was recognized by the antibodies elicited from mouse immunised with rTgPDI (Fig. 2E).

Humoral immune responses were induced by rTgPDI

To investigate whether humoral immune responses were induced in mice immunised with different doses of the rTgPDI protein, collected sera were tested by ELISA. A total IgG antibody response was observed in all of the groups, except the PBS-treated control group. There was a significant antibody response detected in immunised group compared to the control (P<0.01) (Fig. 3A). The results also showed that 30 μg of rTgPDI elicited the maximum IgG antibody values among the tested doses (P<0.05). However, there was no significant difference in IgG responses observed between the groups immunised with 30 or 40 μg of rTgPDI (P>0.05).

The levels of the IgG1 and IgG2a humoral isotype responses in immunised BALB/c mice were evaluated by ELISA. A mixed IgG1/IgG2a response, involving predominant IgG2a production was detected in the sera of mice immunised with rTgPDI (Fig. 3B). These results indicated a shift toward a Th1-type response. Moreover, immunisation of mice with 30 μg of rTgPDI elicited higher levels of IgG2a compared to the control and other dosage groups (P<0.05).

Total IgA antibody levels were also measured. There was a significant antibody response in the 20 μg and 40 μg dose groups compared to the control group (P<0.05) (Fig. 4). Similarly, 30 μg of rTgPDI elicited the highest IgA antibody levels compared with the control group (P<0.01). These findings revealed that a mucosal immune response was elicited by intranasal rTgPDI immunisation.
rTgPDI stimulated lymphocyte proliferation

Splenocytes from mice immunised with different doses of rTgPDI or PBS were prepared to assess cell-mediated immune responses. The lymphocyte proliferative response assay was performed using 10 mg rTgPDI as a stimulus. As shown in Fig. 5, the specific proliferative response was significantly stronger in mice immunised with 20, 30 or 40 mg of rTgPDI (P < 0.05) than in mice immunised with PBS. However, there was no significant difference between the 10 mg rTgPDI and PBS groups (P > 0.05).

Th1-type cellular immune responses were strongly elicited by rTgPDI

The cell-mediated immunity induced in the immunised mice was evaluated by measuring the levels of cytokines (IFN-γ, IL-2, IL-4 and IL-10) released in the supernatants of cultures of rTgPDI-stimulated spleen cells. The levels of IFN-γ, IL-2 and IL-4 secreted from the spleen cells of all of the rTgPDI-immunised mice, except those in the 10 μg dose group, were increased compared with the PBS group, and 30 μg of rTgPDI provoked cell-mediated immunity more efficiently compared to the other dosages (P < 0.01). Nevertheless, the level of IL-10 among the
rTgPDI was significantly longer (Fig. 4). Administration of 30 μg of rTgPDI stimulated significantly higher IgA production compared with the other rTgPDI doses and PBS. *P<0.05 and **P<0.01 compared with control group.

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rTgPDI reduced the burden of T. gondii tachyzoites

Lower brain and liver tachyzoite burdens (P<0.05) were observed in mice immunised with 30 μg of rTgPDI protein compared with the control group (Fig. 7). These findings suggested that the immunity elicited by rTgPDI was able to efficiently prevent the invasion or proliferation of tachyzoites in host tissues.

rTgPDI prolonged the survival of lethally challenged BALB/c mice

To evaluate whether the rTgPDI protein could induce a protective effect against a lethal T. gondii infection, mice were orally infected with 4×10⁴ tachyzoites on the fifteenth day after the last immunisation. The resultant survival curves are shown in Fig. 8. The survival time for mice immunised with 30 μg of rTgPDI was significantly longer (P<0.05) compared to the control group. All mice in the control group died from 5 to 8 days after challenge, whereas the immunised mice showed protection from infection. Immunisation of the mice with 30 μg of rTgPDI significantly increased their survival rate on the 30th day after challenge by approximately 31% compared with control group (P<0.05). Interestingly, one of survived mice was female and others were male.

Discussion

Recently, proteins responsible for parasite moving junction development, gliding motility, and adhesion to host cells and proteins that serve as essential determinants of parasite virulence and invasion have been investigated as vaccine candidates for combating T. gondii infection, including surface proteins, microneme proteins, rhoptry proteins [26–30] and dense granule proteins [31,32]. Most of previous studies focused on using DNA vaccines or DNA-cocktail vaccines, with few protein vaccines being tested. It is well known that protein vaccines elicit strong humoral responses by inducing antigen-specific antibodies. DNA vaccines though induce cellular immune responses [33] and priming of antigen-specific memory B-cells [34], are still suffering from low efficacy in immunogenicity. Therefore, protein vaccines should be emphasised in attempting to develop a vaccine against toxoplasmosis.

It has been revealed that the majority of T. gondii strains compromises three distinct clonal lineages [35,36] with a genetic difference of 1% or less [37]. These genotypes have highly different phenotypes in the laboratory mice, with type I strains being acutely virulent, type II strains intermediate virulence, and type III strains essentially avirulent [38]. PDI is highly expressed in T. gondii and acts as chaperones to aid protein folding by forming a bridge between two cysteines [39], as well as modulates the tachyzoite-host cell interaction [22,23]. In addition, PDI is a conserved protein in evolution of Toxoplasma as the alignment of the amino acid sequence of PDI revealed a 100% homology among three strains of T. gondii (Fig. 1). Theoretically, the protection elicited by TgPDI immunization can protect against all three strains.

In present study, the plasmid pET-30a(+)–TgPDI was constructed, and the rTgPDI protein, which could be recognised by anti-T. gondii serum, was expressed in E. coli and purified using Ni²⁺-NTA agarose. It is well established that IFN-γ, IL-2 and IgG2a expression is correlated with a Th1-type response, while IL-4, IL-10 and IgG1 favour a Th2-type immune response [40,41]. Most importantly, IFN-γ plays a critical role in protective immunity against T. gondii [42]. Our dose-dependence analyses indicated that 30 μg of rTgPDI evoked a significant Th1-type immune response, as demonstrated by high levels of IFN-γ, IL-2 and IgG2a (P<0.01), while no obvious changes in IL-10 of Th2-type immune response. In addition to cellular immune responses, both humoral immunity, associated with increased total IgG antibody levels, and mucosal immune responses, resulting in IgA production, are important in controlling T. gondii infection [43,44]. Our results showed that the administration of
30 µg of rTgPDI resulted in higher levels of IgG and IgA in sera compared with the other treatments. The findings described above are consistent with previous studies examining subunit vaccines for toxoplasmosis, which showed a mixed Th1/Th2 but predominantly Th1-type immune response by immunisation with recombinant proteins [45].

For 30 µg of rTgPDI elicited the strongest cellular and humoral immune responses in this study, this dosage was used to evaluate the protective effect of rTgPDI against T. gondii challenge. Our data suggested that 30 µg of rTgPDI reduced tachyzoite loads in mouse brain and liver by 42% and 37%, respectively. Consider-
able levels of IgA antibodies were produced in the 30 μg of rTgPDI group, which is correlated with mucosal immunity and protection against pathogen infection of local mucous membranes, such as the intestinal mucosa. Therefore, we infer that the invasion or proliferation of tachyzoites in immunised host might be suppressed by the antibodies, cytokines and immune cells elicited by rTgPDI treatment, potentially through affecting the tachyzoite-host cell interaction [22,23] and proper folding of pathogenic proteins [39]. Furthermore, prolonged survival was observed in immunised BALB/c mice, with approximately 31% of the immunised mice remaining alive at the end of analysis period. These results demonstrated that rTgPDI treatment provided partial, but effective protection against both chronic and acute T. gondii infection.

As an indispensable part of vaccine, adjuvant plays a critical role in enhancing immunogenicity of weaker antigen and activating an appropriate immune response safely without multiple dose regimens. Monophosphoryl lipid A (MPL), oligodeoxynucleotides containing unmethylated CpG motifs and cytokines such as interleukin-12 (IL-12), acting as Th1-promoting immunopotentiators, exhibit desirable adjuvant activities that are essential for immunisation against T. gondii [26,46–48]. Therefore, to assess the degree of rTgPDI conservation, further studies should be performed involving different genotypes of T. gondii isolates, in conjunction with other antigens or appreciate adjuvants.

In this study, we used rTgPDI as antigen for a protein vaccine. The rTgPDI was expressed in soluble manner in E. coli ensuring proper processing of necessary functional groups of the recombinant protein. rTgPDI can be recognized by anti-Toxoplasma rabbit or human serum. Native TgPDI in the soluble tachyzoite antigen was recognized by antibodies elicited from mouse immunised with rTgPDI. These results show that TgPDI has antigenic property. Importantly, inoculation route and challenge manner in this study were intranasal immunisation and oral infection rather than traditional intramuscular injection and intraarterial infection [49–51]. Intranasal immunisation can induce mucosal immune responses which can inactivate the pathogen and contribute to pathogen elimination [52]. Oral challenge is more reasonable because T. gondii is an orally acquired apicomplexan protozoan parasite. In our study, about 31% infected mice immunised with rTgPDI were kept alive till the end of study period. Our results showed clear protective effects by rTgPDI in survival time and survival rate compared with other recombinant proteins such as recombinant Toxoplasma gondii actin depolymerizing factor and recombinant nucleoside triphosphate hydrolase-II. In their studies, all immunised mice all died within 9 or 14 days respectively [45,51].

BALB/c mice are commonly used for vaccine against Toxoplasma infection. In general, females tend to exhibit better humoral immunity than males. Males manifest greater levels of cellular immunity [33]. Female mice were more susceptible to acute infection, while surviving female mice with chronic infections harbored more cysts in their brains than did surviving males [54]. Taking these differences into account, both male and female mice were used in our study. Data from both male and female were averaged for eliminating gender immune differences. In acute infection assays, five mice (about 31%) survived, one was female and others were male. These results are in accordance with the observation that female mice are more susceptible and more likely to die from T. gondii infection than males [54,55]. A shortcoming in this study is that the kinetics and strength of the immune response in male and female rTgPDI-immunised BALB/c mice have not been observed. Therefore, the kinetics and strength of the immune response assays should be explored in future study using rTgPDI combined with appropriate adjuvants based on the present data.

In summary, the present study evaluated the immunogenicity and protective potency of rTgPDI as a protein vaccine. This protein was able to elicit significant humoral, mucosal and cellular immune responses of a Th1 type, which significantly reduced the tachyzoite loads in different tissues and increased the survival of BALB/c mice challenged with tachyzoites from the lethal T. gondii RH strain. This exploration demonstrated that PDI might represent a promising anti-toxoplasmosis vaccine candidate.

Author Contributions
Conceived and designed the experiments: G-RY. Performed the experiments: H-LW Y-QL MG. Analyzed the data: L-TY X-LM. Contributed reagents/materials/analysis tools: H-LJ J-JL. Wrote the paper: H-LW J-HZ.

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