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REVIEW ARTICLE

# *Toxoplasma gondii* vaccine candidates: a concise review

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**Abstract**
*Toxoplasma gondii* is an obligate intracellular parasite that causes toxoplasmosis. It has been shown that the severity of symptoms depends on the functioning of the host immune system. Although *T. gondii* infection typically does not lead to severe disease in healthy people and after infection, it induces a stable immunity, but it can contribute to severe and even lethal Toxoplasmosis in immunocompromised individuals (AIDS, bone marrow transplant and neoplasia). The antigens that have been proposed to be used in vaccine candidate in various studies include surface antigens and secretory excretions that have been synthesized and evaluated in different studies. In some studies, secretory antigens play an important role in stimulating the host immune response. Various antigens such as SAG, GRA, ROP, ROM, and MAG have been from different strains of *T. gondii* have been synthesized and their protective effects have been evaluated in animal models in different vaccine platforms including recombinant antigens, nanoparticles, and DNA vaccine. Four bibliographic databases including Science Direct, PubMed Central (PMC), Scopus, and Google Scholar were searched for articles published up to 2020.The current review article focuses on recent studies on the use and usefulness of recombinant antigens, nanoparticles, and DNA vaccines.

**Keywords** DNA vaccine · Nanoparticles · Recombinant antigens · *Toxoplasma gondii* · Vaccine

## Introduction

Toxoplasmosis is a parasitic disease caused by the intracellular protozoan *Toxoplasma gondii*. The infection is transmitted through drinking of water contaminated by definitive host's feces containing oocysts or through consumption of the definitive or intermediate hosts' tissues containing tissue cysts [1]. The parasite life cycle includes the following steps in summary: the oocysts sporulation step in the environment that makes them infective takes 1–5 days. The next step is the infection of intermediate hosts in nature (including sheep, pigs, cattle, birds, rodents, and humans)

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after ingesting soil, water, or plant material contaminated with oocysts. In this step, oocysts transform into tachyzoites shortly after ingestion [2].

Toxoplasmosis can present in two forms in human. The first form is asymptomatic and occurs by latent infections due to tissue cysts consumption. The second form is severe infections that occur in immunocompromised hosts (e.g., AIDS and organ transplant recipients) or in fetus or newborn with congenital toxoplasmosis [3].

The toxoplasmosis adverse consequences are due to the ability of the parasite to destroy host cells especially cells from critical organs such as brain and eye [4]. Furthermore, the parasite is capable of crossing the placenta; therefore, it can cause fatal or severe and debilitating morbidity in the fetus and newborn [5]. Infection in pregnant women especially during the first trimester can lead to spontaneous abortion, preterm labor, or severe congenital defects such as hydrocephalus, mental retardation, and chorioretinitis [6]. Toxoplasmosis and its prevention is also an important issue in the veterinary medicine and livestock industry as the infection not only decreases the meat production by causing abortion especially in sheep and goat but also can be both a source for human infections and a reservoir for the parasite [7]. Currently, toxoplasmosis control strategies are largely based on treatment by medications in the acute phase of the infection [8].

However, the current medications have drawbacks including toxic effects, limited accessibility, and high costs. Furthermore, reinfections can occur after treatment due to the complicated life cycle of the *T. gondii* and the presence of its infectious life stages in the environment [6, 8, 9]. In addition, the drug is not effective on the tissue cysts and is not applicable in the primary stages when the infected individuals are asymptomatic [10, 11]. The parasite exists in three forms depending on the stage in its life cycle: tachyzoites, bradyzoites, and sporozoites that are similar in ultrastructure but with differences in intracellular organelles [12]. The parasite life cycle can be divided into two phases: (1) the intestinal or isospora stage in definitive feline hosts. (2) Extra-intestinal stage in both the definitive and intermediate hosts [13]. The prevalence of congenital toxoplasmosis dramatically varies from 1 to 100 per 10,000 live births in different countries and even in different regions or communities in one country [14]. It is estimated that approximately 30–50% of the world population is infected by the *T. gondii* [15]. The severity of the disease by the parasite is determined by the host resistance, the parasite's variants and the antigenic variations. The main antigens of the *T. gondii* are membrane, cytoplasmic, and soluble antigens that the latter results from a combination of cytoplasmic shedding, active secretion by the parasite, and lysis due to the immune system responses [16]. The parasite secretory antigens that are produced by three parasite's organelles microneme, rhoptry, and dense granule comprise 90% of the soluble antigens [17, 18]. The *T. gondii* DNA vaccine studies are mainly focused on four families of molecules. These are surface antigens (SAGs), microneme antigens (MIC), rhoptry antigens (ROP), and dense granules antigens (GRA) [19, 20]. Identification of the molecules that are crucial in pathogenesis and immune protection is a bottleneck in efficient vaccine development. Excretory/secretory antigens (ESAs) produced by *T. gondii* in tachyzoites and bradyzoites forms have an important role in immune system stimulation [21]. These antigens are mainly GRA that is suggested as a candidate antigen for vaccine development [22]. The microneme secretions contain cell surface adhesion molecules that are involved in the first step of parasite adhesion and invasion to the host [23, 24]. The rhoptry antigens are secreted into the expanding parasitophorous vacuoles during the parasite invasion [24]. The immune response to the *T. gondii* depends on the clinical presentation of the infection. The CD4 + and CD8 + cells are crucial for protection against the infection [25]. These cells are involved in protection by secretion of inflammatory cytokines such as IFN-γ, TNF-α, IL-1, and IL-6. Toxoplasmosis can induce CD8 + cytotoxic lymphocytes in both human and mice that can destroy the infected cells [26]. It has been found that all the mice strains can develop a strong T helper cell type 1 (Th1) immune response against the *T. gondii*. Furthermore, macrophages, natural killer (NK) cells, dendritic cells (DCs), antibodies, and other immune effector factors are involved in the prevention of the infection [27]. So far, a variety of *T. gondii* antigens have been identified by different methods and the molecular characteristics of many of them have been evaluated to be used in diagnostic, therapeutic, immunization, and vaccine development applications [28].

In people with a healthy immune system, the symptoms of the infection usually are similar to mild flu symptoms, while in immunocompromised patients, it can cause severe and even life-threatening complication such as encephalitis and severe ocular complications. In addition, in pregnant women, toxoplasmosis may cause abortion or congenital toxoplasmosis with manifestations such as neurological or ocular in the fetus [6, 29]. It is estimated that approximately 50% of untreated maternal infections are transmitted to the fetus in which approximately 60% are subclinical, 30% have severe damage such as hydrocephalus, intracerebral calcification, retinochoroiditis (Classical triad) and mental retardation and 9% are fatal for the fetus [30]. Generally, in immunocompetent women with one experience of *T. gondii* infection related abortion or fetal infection [31], the subsequent pregnancies are safe regarding *T. gondii* reinfection and manifestations; however, there is occasional reports implying transmission of congenital toxoplasmosis by immunocompetent women infected before conception [32, 33]. The available therapeutics for the treatment of

toxoplasmosis are not completely safe and effective [6, 20]. Recently considerable progress has been made in designing toxoplasma vaccine candidates that can efficiently stimulate the immune responses [34]. In the current study, we reviewed the *T. gondii* candidate vaccines that include a heterogeneous collection of studies with different methodologies including recombinant antigens, micro/nanoparticles displaying the antigens and DNA vaccine.

## Methods

### Database search

Four bibliographic databases including Science Direct, PubMed Central (PMC), Scopus, and Google Scholar were searched for articles published up to 2020. The following MeSH (Medical Subject Headings) keywords were considered in the initial search strategy: "Toxoplasmosis," "nanoparticles -based vaccines," "Recombinant vaccines," and "DNA vaccines of *T. gondii*" with employing the Boolean operators 'OR' and/or 'AND.'

### Vaccine design based on recombinant antigens

Toxoplasmosis in a healthy host that is immunocompetent stimulates a lifelong protective immunity that prevents reinfection. The immunogenic proteins that can induce these protective responses have been identified that are candidates for vaccine development for *T. gondii*. These immunogenic proteins include surface antigens (SAG), dense granule antigens (GRA), rhoptry proteins (ROP), and microneme proteins (MIC) [35]. The use of recombinant antigens as a candidate vaccine against toxoplasmosis has been considered since the 1990s [36]. At the beginning, the SAG 1 antigen, which is on the surface of the parasite, as well as the (GRA)1 antigen were studied [37]. Subsequently, after 2000, other secretory antigens such as GRA7, ROP2, MICs, and other proteins such as heat shock proteins considered as candidates of vaccines. In recent years, more than 10 genes have been cloned into eukaryotic and bacterial expression systems, as follows: ROP 18 [38, 39], GRA4 [40–42], and ROP4 [40, 41, 43, 44], ROP2 [41–46], SAG1 [41, 45, 47, 48], actin depolymerizing factor (ADF),GRA6 [49, 50], ROP5 [48], GRA2 [50, 51], SAG2 [52], *Toxoplasma gondii* Hsp70 (TgHSP 70) [53], *Toxoplasma* tissue cyst matrix protein (MAG1) [44], *T. gondii* serin protease inhibitor-1 (TgPI-1) [42], GRA5 [51]. Many of these antigens have been used to detect specific antibodies in the serum of mice, pigs, and cats and to assess the immune response in the host. The results of some studies have shown that immunization using these recombinant antigens responds well and effectively induce the immune responses. Table 1 shows the immunogenicity of recombinant antigens produced in different hosts such as mice, pigs, and cats.

### Vaccine design based on DNA genome of antigens

The DNA vaccines are a new strategy to prevent infectious diseases to help reduce the antibiotics use and diseases spread. The DNA vaccines can be used in oral or injective forms to induce rapid immunization against a diverse range of diseases that are hard to be overcome by antibiotics or traditional vaccines. A typical DNA vaccine is composed of a plasmid with a strong viral promoter and the gene of interest that is expressed and induces specific immune responses. The advantages of DNA vaccines include stability, cost effectiveness, and safety. However, the hurdle in DNA vaccine development against parasites is the complexity of the parasitic diseases.

The GRA and SAG antigens that are parasitic secretory and surface antigens are suitable candidate antigens to design DNA vaccines for immunity against toxoplasmosis. It has been demonstrated that DNA vaccination with the sequences of GRA1, GRA7, and ROP2 proteins can induce protection against infection with different virulent *T. gondii* strains in C3H mice but not in BALB/c and C57BL/6 mice. Furthermore, immunization of sheep with a DNA vaccine containing the GRA1, GRA4, GRA6, and GRA7 sequences formulated in liposome showed a significant immune response against *T. gondii* [54]. Table 2 lists the studies of antigens used as candidate DNA vaccines. Including SAG1 antigen from ME49, VEG strains [55], SAG1, ROP16, GRA14,MIC8,ROP54, *Toxoplasma gondii* calcium-dependent protein kinase 2 (TgCDPK2), *T. gondii* Myc regulation 1 (MYR1), Perforin-like proteins (PLP)1, ROP18, GRA2, GRA5, GRA17, GRA23, GRA7, ROP2, TgHSP60, ROP21, TgHSP-40,GRA16, Rhomboid 4(ROM4), ROP35,GRA8, GRA4, GRA24, GRA25, MIC6, SAG5-D for RH [42, 55–77] and ROP35, GRA8, ROP19, GRA24, GRA25, MIC6 for PRU strain [72, 74, 75].

### Vaccine design based on nanoparticles of recombinant antigens

Nanoparticles can be used to improve delivery of subunit vaccine in order to increase the immunogenicity of the pathogen proteins used in the vaccine design [78]. Furthermore, virus-like particles (VLPs) or nanoparticles have been used to design recombinant vaccines with promising safety and efficacy both in preclinical and clinical studies. VLPs display the antigens in a repetitive high-density manner similar to the proteins of viral surface proteins, which contribute to strong T-cell and B-cell immune responses against the vaccine antigens [34]. Nanoparticles can play an adjuvant role in the vaccine formulation and improve the humoral and

**Table 1** Recombinant Toxoplasma antigens based on vaccine studies

| Antigen | Toxoplasma strain | Expression system | Fusion type | Processing and purification method | Route of injection and the location and dose |
|---|---|---|---|---|---|
| **ROP18** | RH strain | pET30a system in *E. coli* BL21 | HIS fusion | HIS-sepharose columns (and then dialyzed) | Subcutaneously injected with 100 µg rROP18 and Re |
| **GRA 4 and ROP4 proteins** | RH strain of virulent *T. gondii* | pcDNA3<br>*Escherichia coli* strain M15 | Histidine-linked tag fusion | Nitrilotriacetic acid-Ni2_ columns | 10 µg of either rROP2, rGRA4, or a mix of 10 µg of each recombinant antigen adsorbed to 0.5 mg of Al(OH)3 by intramuscular injection into the hindquarters |
| **Recombinant ROP2 and SAG1 fusion protein (compared to their DNA vaccine)** | RH strain of virulent *T. gondii* | For recombinant protein: pET28b vector *E. coli* strain of BL21-Codon Plus (DE3)-RIL<br>For DNA vaccine: pcDNA3.1vector (for DNA vaccine)<br>*E. coli* strain DH5α | HIS tag | Ni2 + chelated affinity column | Subcutaneously with 2.5 µg rROP2-SAG1 protein mixed with equal volume of Freund's complete adjuvant (FCA) intramuscularly with 100 µg pcROP2-SAG1 DNA three times |
| **Recombinant SAG1 Protein** | *T. gondii* C56, a mildly virulent strain | *P. pastoris* (no more elaboration) | Not mentioned | Not mentioned | Subcutaneously with 10 mg (500 ml) of recombinant SAG1, formulated with the SBAS1 adjuvant |
| **Recombinant ROP2 and ROP4** | Low virulent DX strain (lysates from highly virulent BK strain) | *Escherichia coli* Top 10 (for gene cloning) TA vector (pGEM-Teasy)<br>*Escherichia coli* BL21(DE3) (for protein expression) poly histone vector | pHis tag | Affinity Ni2 + -chromatography using His-Bind columns | Subcutaneous injection either rROP2, rROP4 or a mix of 10 µg of each recombinant antigen emulsified with complete Freund's adjuvant |
| **ROP2, ROP4, GRA4 and SAG1** | Low-virulence DX *T. gondii* strain | *Escherichia coli* Top 10 (for gene cloning) TA vector (pGEM-Teasy)<br>*Escherichia coli* BL21(DE3) (for protein expression) poly histone vector | pHis tag | Affinity Ni2 + -chromatography using His-Bind columns | Subcutaneous injection 10 µg. of recombinant and antigen ncomplete Freund's adjuvant |
| **Actin depolymerizing factor (ADF)** | Challenge by *T. gondii* of highly virulent RH strain | *E. coli* Rosetta host bacteria cells, vector pET-28a (+) | Not mentioned | Centrifuged at 12,000 *g* Ni2 + column | Intramuscular injections rADF (100 µg/each) |
| **GRA2 and GRA6** | RH strain tachyzoites | *E. coli* BL21 (DE3) pLysS competent cells pUET1expression plasmid | 2 HisX6 tags | Centrifuged at 12,000 × *g*, the supernatants were passed through 0.2 µm filters, Ni2 + -NTA agarose column | Injected subcutaneously (s.c.) in their hind footpad with either 20 µg of rGRA2, 20 µg of rGRA6, or with a mix of 10 µg of each antigen, all formulated in 50 µl of monophosphoryl lipid A (MPL) adjuvant |

Table 1 (continued)

| Antigen | Toxoplasma strain | Expression system | Fusion type | Processing and purification method | Route of injection and the location and dose |
|---|---|---|---|---|---|
| **ROP5 alone or in combination with rSAG1** | Lethal challenge with the *T. gondii* RH strain | *Escherichia coli* BL21 (DE3) cells Into pHis vector | His tag | Ni2 + -NTAagarose columns | Subcutaneous injection 100 µg of recombinant antigens |
| **SAG2** | Orally challenged with 3,000 sporulated oocysts of the Me49 strain of *T. gondii*, or orally with 10 tissue cysts of the C56 strain | *Escherichia coli* pGEX-2 T vector | Glutathione-S-transferase (GST) fusion protein | Affinity to glutathione agaros | Subcutaneously in a volume of 0.2 ml either 0.65 mg v22 iscom or 0.8 mg GST iscom combind with approximately 10 mg Quil-A |
| **TgPI-1, ROP2 and GRA4 proteins** | Orally challenged with 20 ME49 strain tissue cysts (sublethal dose) | *Escherichia coli* Strain E15 pQE | Six-histidine tag | Ni2 + -NTAagarose columns | Intradermally 10 µg each of rTgPI-1, rROP2, rGRA4 Ags with 0,125 mg of aluminum (Al(OH)3, or intranasally with 10 µg of CpG-ODN 1826 |
| **TgHSP70** | TgHSP70 from T. gondii RH TgHSP70 gene oral challenge ME-49 T. gondii infection | *E. coli* pGEx-4 T-2 plasmid | Fusion with GST tag | Purified by glutathione and polimixin B affinity chromatography | Injected subcutaneously with 10 µg of rTgHSP70 dissolved in 100µL of Alum adjuvant (Alhydrogel 2%) |
| **ROP18 (both protein and DNA vaccine)** | 76 K strain of *T. gondii* (type II) for experimental infection | Drosophila Schneider 2 cells (S2 cells) expression vector pMT/BiP/V5-HisA the DNA vaccine plasmid was cloned in *Escherichia coli* DH5α | His tag | HisTrap HP affinity column | Immunized either with ROP18S2 (15 µg) emulsified in Montanide™ ISA 71 combined with 10 µg poly I:C by subcutaneous injection (or with ROP18S2 (15 µg) plus 1 µg cholera toxin intranasally<br>For DNA immunization, mice received three intramuscular injections at 2-week intervals of pROP18 (100 µg) without or with adjuvant, IL-12-containing plasmid (pIL12) at 100 µg/mouse |
| **GRA2 and GRA5** | Challenge by *T. gondii* virulent RH strain | *Escherichia coli*, BL21 (DE3) pLysS | Not mentioned | Affinity purification | Subcutaneously with final protein dose of 10 mg |
| **ROP2 + ROP4 + SAG1 + MAG1** | Low-virulence *T. gondii* DX strain for challenge highly virulent RH and BK strains of *T. gondii* as a source of native antigen and DNA template for cloning | *E. coli* BL21(DE3) pJET1.2/ blunt vector pHis expression vector | His tag | Purified by Ni(2 +) affinity chromatography on His-Bind columns | Subcutaneous injection 30 µg of each antigen supplemented with 10 µg of MPL and Alhydrogel® 2% |
| **GRA2** | The gene sequence of gene sequence of RH strain strain | *Escherichia coli* pUET1 expression plasmid | His tag | $Ni^{2+}$-nitrilotriacetic acid resin | Injected subcutaneously in their hind footpad of 20 µg of GRA2 in a volume of 50 µl |

**Table 1** (continued)

| Antigen | Toxoplasma strain | Expression system | Fusion type | Processing and purification method | Route of injection and the location and dose |
|---|---|---|---|---|---|
| **rROP2 (administered intranasally)** | RH strain for obtaining DNA to amplify rop2 gene and ME-49 strain was used for tissue cyst production for challenge | *Escherichia coli* DH5-α *Escherichia coli* Rosetta 2 pTrcHis | His tag | Ni–NTA Superflow resin | Intranasal 100 µg of rROP2 plus Quil-A (20 µg) (100 µl of final solution was administrated in each animal per nostril) |
| **booster** | **A combination of recombinant antigens** | **adjuvants** | **Immune response elicited by vaccine** | **Animal used for vaccine response evaluation** | **Year of publication** |
| Boosted with the same components at the same dosage 2 weeks later | No | Ginsenoside Re (Re), the major ginsenoside in the ginseng radix | Elevated IgG1 and IgG2a in sera<br>Increased in vitro proliferation and IL-4 and IFN-g production in splenocyte following incubation with Ag<br>Increased survival time of immunized mice | ICR mice | 2013 |
| Boosted with the same dose three more times in a period of 2 or 8 weeks | Yes | Alum | The C57BL/6 lower brain cyst burden (only in rGRA4 or combination of both)<br>In C3H mice Lower brain cyst burden in immunizationby both Ags or their combinations *gra4* gene DNA vaccine lower brain cyst burden (like rGRA4 protein + alum) humoral response All immunized C57BL/6 and C3H mice showed high IgG titers against rGRA4 and rROP2 splenocytes: specific proliferative response (all immunized groups)<br>Cytokine: change only in immunized C57BL6 mice ROP2 stimulation: high IL-4<br>Gra4 stimulation high IFN-g | C57BL/6 and C3H mice | 2004 |

**Table 1** (continued)

| booster | A combination of recombinant antigens | adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|---|
| Boosted Twice with the same dose for the peptide vaccine<br>For the DNA vaccine boosted twice with the same dose (100 µg) of DNA or 2.5 µg of rROP2-SAG1 peptide mixed with equally volume of Freund's incomplete adjuvant (FIA) | Yes as a hybrid combination | Freund's complete adjuvant (FCA) | Specific IgG (IgG1 twice the IgG2a) in the serum of group immunized by hybrid protein (but not DNA)<br>Ag-specific proliferation of splenocyes was increaseed especially in the group immunized by hybrid protein<br>IFN-g production was increased in the immunized groups (pro and DNA) | BALB/c mice | 2011 |
| Twice at 3-week intervals with the same dose | No | SBAS1 adjuvant | High titers of immunoglobulin in the serum<br>Recombinant SAG1 vaccination provided protection against maternofetal transmission | Dunkin-Hartley Guinea Pig | 2000 |
| Twice at 2-week intervals the same dose with the incomplete Freund's adjuvant | Yes a combination of ROP2 & ROP4 | Complete Freund's adjuvant ($1^{st}$) and incomplete Freund's adjuvant ($2^{nd}$ and $3^{rd}$) | In the mice received combination vaccine:<br>Significant reduction in brain cyst load<br>Increased IgG1 (higher) and IgG2a specific to both Ags<br>Elevated Ag-specific proliferation and IFN-g and IL-2 production in splenocytes | C3H/HeJ mice | 2009 |

**Table 1** (continued)

| booster | A combination of recombinant antigens | adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|---|
| Twice the same dose at 2-week intervals | Yes a combination three of the following antigens: ROP2, ROP4, GRA4 and SAG1 | Complete Freund's adjuvant | The best result by the following combination: "rROP2 and rROP4" + "rGRA4" or "rSAG1" immunization by trivalent vaccines induced significant reduction in the brain cyst number vaccination resulted in antigen-specific proliferative response in splenocytes elevated IFN-g production (not by rROP2 + rGRA4 + rSAG1 mixture) elevated IL-2 production (by all three mixtures) All recombinant proteins used in the vaccines induced a IgG1 and IgG2a with (higher IgG1) | BALB/c mice (H-$2^d$) | 2012 |
| Boosted twice with 2-week intervals with the same dose | No | Freund's complete adjuvant ($1^{st}$ week) Freund's incomplete adjuvant ($2^{nd\ week}$) | Reduced number of brain cyst Increased survival time Specific serum IgG antibody Elevated percentage of CD4 + cells in the spleen | BALB/c mice | 2012 |
| Boosted twice times, at weeks 3, and 6 with the same dose | Yes a combination of GRA2 and GRA6 | Monophosphoryl lipid A (MPL) adjuvant | Strong IgG response high ratio of IgG2a to IgG1 rGRA2 immunization induce high levels of IFN-g and IL-2 (Th1 cytokines) Reduced number of brain cysts (in rGRA2 immunized or combination) | CBA/J mice | 2007 |

**Table 1** (continued)

| booster | A combination of recombinant antigens | adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|---|
| Boosted twice by at 2-week intervals | Yes<br>ROP5 alone or in combination with rSAG1 | Complete Freund's adjuvant (1st immunization) and incomplete Freund's adjuvant (2nd and 3rd) | High levels of IgG (Both IgG1 and IgG2a); A predominance of IgG2a over IgG1 single rSAG1 group, (rSAG1 elicited aTh1-type response)<br>High splenocyte proliferation after specific Ag activation (the highest rROP5 + rSAG1, followed by rROP5) The highest IFN-g and IL-2 concentrations were detected in the group of rROP5 + rSAG1<br>rROP5 + rSAG1 or rROP5 induces mice immunized with rROP5 + rSAG1 or rROP5 produced specific amounts of IL-10 (and low levels of IL-4): a mixed Th1/Th2 response in rROP5 + rSAG1 or rROP5 groups<br>Increased survival time in all the immunized groups (the most prolonged: rROP5 + rSAG1) | BALB/c mice | 2013 |
| There were five immunization Episodes (4 boosters), with intervals comprising 4 weeks between the 1st and the 2nd,6 weeks between the 2nd and the 3rd, 2 weeks between the 3rd and the 4th, and 6 weeks between the 4th and the 5th episode | No | ISCOM (SAG2 was expressed as a glutation-s-transferase fusion protein) | Elevated titers of SAG2 antibodies, however, "decreased" survival time!! | Swiss-Webster mice | 1997 |

**Table 1** (continued)

| booster | A combination of recombinant antigens | adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|---|
| Boosted 3 times with 2-week intervals | Yes a combination of TgPI-1, ROP2 and GRA4 proteins | Aluminum (Al(OH)3, Sigma) (intradermally) or 10 µg of CpG-ODN 1826 (intranasally) | Elevated IgG<br>rTgPI-1 induced significant production of both isotypes IgG1 and IgG2a<br>rROP2 predominantly IgG1 (Th2)<br>rGRA4 1 elicited only IgG1 (low levels)<br>Elevated IFN-γ, IL-2 (cellular immune response)<br>**Mucosal response**: IgA after immunization by combination of the 3 antigens MLN lymphocytes significantly proliferated in vitro after stimulation<br>**Cellular response**:<br>rTgPI-1 + rGRA4 or rTgPI-1 + rROP2 + rGRA4 induced a response with a mixed Th1/Th2 profile while splenocytes from mice immunized with rTgPI-1 + rROP2 showed no cytokine secretion | C3H/HeN mice | 2018 |
| Boosted 2 more times at weeks 2 and 4 by the same dose | No | Alum adjuvant | Reduction of cerebral parasitism and less cyst numbers<br>Induces high antibody titers (high IgG1) Immunization **did not** alter the production of IL-2, IL-4, IL-6, IL-10, IL-17a, IFN-g, nor TNF enhanced Nitric oxide (NO) production by peritoneal macrophages<br>Increased Number of iNOS + Cells in the Brain | C57BL/6 and Swiss-Webster mice | 2017 |

**Table 1** (continued)

| booster | A combination of recombinant antigens | adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|---|
| Boosted twice at intervals of 2 weeks The same doses as the prime | No | **Adjuvant**: Montanide™ ISA 71 combined with poly I:C) subcutaneous (or cholera toxin) intranasally( | Significant brain cyst reduction in intranasal group<br>Higher IgG in the subcutaneous immunized group<br>Both IN and SC Predominantly IgG1 over IgG2a indicating predominant Th2 responses<br>Splenocytes produced IFN-g and IL-2 following antigen stimulation indicating cell-mediated immunity induced by vaccine. IL-5 and IL-10 (non-significantly), which are in favor of Th2 responses, both produced significantly by splenocytes after activation. Both routs induced a mixed Th1/ Th2 type cellular immune response<br>DNA vaccine group: high IgG response (IgG2a higher than IgG1——>Th1 predominant) Splenocytes produced IFN-g and IL-2 following antigen stimulation with no increase in IL-5 or IL-10 | CBA/J mice (H-$2^k$) | 2017 |

**Table 1** (continued)

| booster | A combination of recombinant antigens | adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|---|
| Twice at 2-week intervals | Yes a combination | Complete/incomplete Freund's adjuvant (C/IFA) | Strong humoral immune response<br>Both IgG2a and IgG1 (Th1 andTh2 responses)<br>Increased Ag-specific proliferation of T lymphocytes<br>Higher level of IFN-g and IL-2 compared, In contrast, relatively low levels of IL-4and IL-10 (indicative of skewed responses to Th1 responses) | BALB/c mice | 2016 |
| Boosted twice at 2-week intervals | Yes a combination | MPL (monophosphoryl lipid A from Salmonella enterica serotype minnesota Re 595; Sigma-Aldrich) and alum adjuvant | Reduced brain cyst burden<br>Significant serum level of Ag-specific IgG (both IgG1&IgG2a) | C3H/HeOuJ mice | 2018 |
| Boosted twice at weeks 3 and 6 by the same dose | No Yes a combination | MPL adjuvant | Strong IgG response produced preferentially IgG1 antibodies in response, although IgG2a/c antibodies were also produced at substantial amount (mixed Th1/Th2 response) high amount of IFN-g decreased brain cyst production | C57BL/6 mice | 2018 |
| Boosted three times at 3-week intervals (weeks 3, 6, and 9 after prime immunization) | No | Quil-A adjuvant | IgG and IgA levels above cut-off level only in rROP2<br>Immunized group fewer oocysts shedding however not statistically significant | Domestic cats | 2017 |

**Table 2** *Toxoplasma* antigens based on DNA vaccines studies

| Antigen | Toxoplasma strain | Plasmid and the cloning organism | Processing and purification method | Route of injection and the location and dose |
|---|---|---|---|---|
| **SAG1 DNA** | Mice challenged with 80 tissue cysts of ME49 strain<br>Rats with VEG strain oocysts resulted (brain cysts)<br>highly virulent RH strain tachyzoites for mice challenge and also to derive the SAG1 cDNA clone | pCMVInt expression vector | By double-banding on CsCl | Intramuscular<br>Hindquarters 100 mg of pCMVToxo or pCMVInt in 100 mL of PBS with 25% sucrose + 50 mg of pGM-CSF |
| **ROP16 DNA** | Highly virulent RH strain of *T. gondii*<br>The DNA sequence of the Ag from *T. gondii* RH strain | pVAX➔ pGEM | Not mentioned | Intramuscularly (i.m.) with 100 µg of plasmid DNA suspended in 100 µl sterile PBS, 100 µl in each thigh skeletal muscle, |
| **MIC8** | Challenge by RH strain of *T. gondii*<br>genomic DNA of *T. gondii* RH strain | pGEM-T easy vector➔ pVAX vector | Not mentioned | Intramascular, pVAXMIC8 plasmid or empty pVAX I vector into each anterior tibial muscle (final plasmid concentration, 100 µg/100 µl) |
| **GRA4** | Oral challenge by 76 K T. *gondii* RH strain cysts | MLPIX➔ pcDNA3 expression vector | All the plasmids were purified from transformed E. coli DH5a by anion exchange chromatography | The tibialis anterior (TA) muscles of both hind legs each 50 mg pGRA4 in 50 ml PBS |
| **GRA14 DNA (boosted by recombinant protein)** | Highly virulent<br>RH strain of *T. gondii* for challenge | Cloning<br>vector pTG19-T➔ eukaryotic expression vector pcDNA3 | Plasmids were purified by EndoFree Plasmid Giga Kit | Intramuscularly with 100 µg pcGRA14 into thigh skeletal muscle the first time |
| **ROP54** | Intraperitoneally *T. gondii* RH (acute infection) and oral Prugniaud (PRU) strains tissue cists for chronic | pVAX I plasmid | Not mentioned | Route, location, and dose<br>100 µg of plasmid DNA dissolved in 100 µl sterile PBS intramuscular injection into the quadriceps |
| **TgCDPK2** | *T. gondii*<br>RH strain (type I) for the DNA sequence and challenge intraperitoneally | pMD18-T➔ vectorpVAX I vector | Anion<br>exchange chromatography | Bilateral<br>intramuscular injection into the quadriceps with 100 mL (1 mg/mL) |
| **MYR1 DNA** | *T. gondii* RH strain for the DNA sequence and challenge | pMD19-T ➔vector pVAX1 vector | End o-free plasmid giga kit | Subcutaneous injection with 100 mL of sterile PBS containing 100 mg pVAX1-MYR1 |
| **PLP1 and ROP18 DNA** | The RH (used to produce the PLP1 and ROP18 clones) 59 and PRU strains (used to challenge mice with tissue cysts) | PIRESneo➔ pVAX | Not mentioned | Eight groups of mice (30 mice/group) were vaccinated intramuscularly with 100 µg of 82 plasmid dissolved in 100 µl of PBS |
| **GRA2 and GRA5 DNA (separate groups, not in combination)** | The virulent *T. gondii* RH strain for lethal challenge | pcDNA 3.1C | Not mentioned | Intramuscular at tibialis anterior muscle of both leg with 100 µL (50 µL in each leg) of PBS containing 100 µg of pcGRA2, or 100 µg of pcGRA5 |

Table 2 (continued)

| Antigen | Toxoplasma strain | Plasmid and the cloning organism | Processing and purification method | Route of injection and the location and dose |
|---|---|---|---|---|
| **GRA17 and GRA23 DNA** | Challenge infection with the highly virulent RH strain of *T. gondii*<br>DNA sequence from RH strain of *T. gondii* | pMD18-T➔ pVAX | Not mentioned | Intramuscular injections 100 ml (100 mg) of pVAX-TgGRA17, pVAXTgGRA23, and pVAX-TgGRA17 + pVAX-TgGRA23 |
| **GRA7 and ROP2 DNA (each alone or in combination)** | DNA sequence from the *T. gondii* RH strain<br>Challenge by | pTOPO➔ pcDNA3.1 plasmid<br>Mass replication in (*E. coli*), strain TOP10 | Mass replication was extracted from the bacteria using endotoxin-free plasmid extraction kit | Intramuscularly with 100 µg of plasmid DNA in 100 µl PBS, 50 µl in each thigh skeletal muscle |
| **tgHSP60** | Two *T. gondii* strains RH and PRU for acute and chronic disease challenge, respectively | pMD18-T vector➔ pVAX | Not mentioned | Thigh muscle, 100 µg pVAX-HSP60 |
| **ROP21 DNA** | Challenged with tachyzoite cells of RH *T. gondii* and cysts of *T. gondii* PRU strain | pMD19-T vector ➔ pVAX plasmid<br>*Escherichia coli* DH5a | Purification by A commercial kit (TianGen, Beijing, China) | Intramuscular injection containing 100 mg of recombined plasmids |
| **GRA14 DNA** | RH strain tachyzoites for challenge and DNA sequence | pTG19-T➔ pcDNA3 | End o-free plasmid mega kit | Intramuscularly (anterior tibial muscle). Concentration 100 µg/100 µl plasmid DNA |
| **TgHSP-40** | DNA sequence from T. *gondii* RH tachyzoites challenge | pMD18-T linear vector➔ pVAX1 | Not mentioned | intramuscularly (i.m.)<br>100 µL of PBS containing 100 µg pVAX1-HSP40 |
| **SAG1 DNA** | Virulent *T. gondii* RH for challenged DNA sequence from tachyzoites of *T. gondii* RH | pTZ57 R/T cloning vector➔ pVAX1 *E. coli* DH5α | Anion exchange chromatography (end-free plasmid mega kit, Qiagen) | Intramuscular, each thigh skeletal muscle 100 µg of plasmid DNA, and different adjuvants suspended in 100 µl sterile PBS |
| **SAG1 and SAG3 DNA** | *T. gondii*, strain RH | pcDNA3<br>*E. coli*, strain TOP10 | Plasmid purification kit (Qiagen) | Intramuscularly with 100 µg of plasmid DNA suspended in 100 µL sterile PBS bilateral biceps |
| **GRA16 DNA** | Challenge by *T. gondii* RH (acute) and PRU (chronic) strain | pVAX<br>*E. coli* DH5α cells | Anion exchange chromatography (EndoFree Plasmid Giga Kit, Qiagen | Intramuscularly injected with pVAXGRA16 plasmids 100 µl (1 µg/µl) |
| **ROM4 DNA (alone or in combination with a peptide derived from its gene) Or SAG1 DNA as control** | Challenge by *T. gondii* RH and PRU strain | pEASY-T1 vector pEGFP-C1 expression plasmid<br>*Escherichia coli* DH5α | Endotoxin-free mega kit following the manufacturer's instructions (Qiagen) | Intramuscular route, location,and dose |
| **ROP35 DNA** | Virulent *T. gondii* RH strain and PRU strain for challenge | pMD19-T vectorpVAXI vector<br>*E. coli* DH5α cells | A commercial kit (TianGen, Beijing, China) to isolate the plasmid and to eliminate endotoxin contamination | Intramuscular injections containing 100 µg of recombined plasmids (1 µg/ µl) |

**Table 2** (continued)

| Antigen | Toxoplasma strain | Plasmid and the cloning organism | Processing and purification method | Route of injection and the location and dose |
|---|---|---|---|---|
| **GRA8 DNA** | DNA sequence from RH strain tachyzoites<br>Challenged with highly virulent *T. gondii* GFP-RH strain | pGEM-T Easy<br>vectorression plasmid pEGFP-C1HEK-293 T cell pDsRed2-N1 vector *Escherichia coli* DH5α | Endotoxin-Free mega kit according to the manufacturer's instructions (Qiagen) | 50 µg pDsRed2-GRA8 into the tibialis anterior muscles of both hind legs (100 µg/per mouse) |
| **ROP19 DNA** | DNA sequence from *T. gondii* PRU strain tachyzoites<br>Challenge by *T. gondii* strain PRU cysts | pEASY-T1<br>vector ➔ expression plasmid pEGFP-C1 HEK-293 T cells | The endotoxin-free mega plasmid kit (Qiagen) | Intramuscullar, buttocks injection 100 µl |
| **TgGRA24, TgGRA25 and TgMIC6 DNA** | DNA sequence from T. *gondii* RH strain challenge with the *T. gondii* RH and Pru strains | pMD-18 T Vector➔ pVAX I vector | Not mentioned | Intramuscular injection into the quadriceps, 100 µL (1 µg/µL) of pVAXGRA24 |
| **SAG5D DNA** | DNA sequence from T. *gondii* (RH strain)<br>Challenge by *T. gondii* RH strain | pEASY-T1 vector➔ pEGFP-C1 HEK 293-T cells | Endotoxin-free mega kit according to the manufacturer's instructions (Qiagen) | Intramuscularly, pEGFP-C1-SAG5D 100 µg/ each (1 µg/µl) |

| Booster | Adjuvants sequence or chemical | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|
| Boosted with the same dose at 3 and 6 weeks after the first injection | A plasmid encoding murine GM-CSF (pGM-CSF) | SAG1 specific AbA Th1 dominant response (in contrast to the peptide vaccine)<br>Splenocytes produce IL-2 and IFN-g but not IL-4 following specific Ag re-stimulation Increased survival and reduced brain cyst | C57BL/6 mice and Sprague–Dawley rat | 1999 |
| With the same protocols at weeks 2 and 4 | No additional adjuvant, The DNA CpG sequence itself | In the pVAX-ROP16 group: Significant anti-rop16 Ab production splenocytes significant proliferative response and high degree of CTL activity higher IFN-g, IL-2, IL-4, and IL-10 (cell-mediated immunity) and lower pro-inflammatory cytokines IL-6 and IL-12<br>Increased survival time | Kunming mice | 2011 |
| The same protocol 2 and 4 weeks after vaccination | No additional adjuvant, The DNA CpG sequence itself | In the VAXMIC8 group high levels of specific IgG splenocytes proliferative response to MIC8 significant increase in IFN-γ, IL-2, IL-4, and IL-10<br>Increased survival time | Kunming mice | 2010 |

**Table 2** (continued)

| Booster | Adjuvants sequence or chemical | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|
| The mice were boosted in the same way on days 14 and 28 | GM-CSF plasmid adjuvant (also pIL-12) | In the pGRA4 or pGRA4 + pGM-CSF group: strong antibody response significant proliferative response of splenocytes<br>Increased IFN-g and IL-10 (& low amounts of IL-2) (a modulated involvement of Th1 response)<br>pGRA4 mixed pIL-12-, which intensifies the Th1 response, dramatically decreases the survival rate | C57BL/6 | 2000 |
| Boosted by 20 µg rGRA14 two times (pcGRA14 + rGRA14 or pcGRA14 + rGRA14 + nanoadjuvants). at … | Alum and calcium phosphate (CaPNs) for the recombinant boost | Increased survival time in immunized mice<br>CaPNs adjuvanted DNA prime-protein boost vaccination induce both humoral and Th1 type cellular immune responses and high levels of total IgG, IgG2a isotype and IFN-γ (a Th-1 type response) and reduced brain parasitic load<br>Alum adjuvanted DNA<br>prime-protein boost vaccination:<br>predominance of IgG1 over IgG2a and increased IL-4 (a Th-2 type response) | BALB/c mice | 2019 |
| Boosted twice with the same dose at 2-week intervals | No additional adjuvant, The DNA CpG sequence itself | In the pVAX-ROP54 group: High levels of IgG antibodies higher ratio of IgG2a/ IgG1(and higher IgG2a) higher level of IFN-γ, IL-2, and IL-12 (p70) and slighter increase in IL-4 and IL-10 (collectively, mixed Th1/Th2 with Th1 predominance) elevate splenocyte proliferation prolonged survival time reduced brain cyst number | Kunming mice | 2017 |

**Table 2** (continued)

| Booster | Adjuvants sequence or chemical | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|
| Twice at<br>2-week intervals the same dose | No additional adjuvant, The DNA CpG sequence itself | In the pVAX-TgCDPK2 plasmids immunized mice: significant IgG response higher levels of IgG1 and IgG2a (elevated IgG2a/ IgG1)➔ mixed Th1/Th2, with a predominant Th1<br>higher proliferation of splenocytes<br>increased % of CD4 + and CD8 + T cells in the splenocytes<br>Increased IFN-g, IL-12(p70) and IL-10 but not IL-4 in spleen cell cultures<br>longer survival time of the mice | Specific pathogen-free (SPF) female BALB/c | 2017 |
| Twice with 2-week intervals | No additional adjuvant, The DNA CpG sequence itself | In the group immunized with pVAX1-MYR1:<br>Specific IgG and IgG Isotypes (high IgG2a at first)<br>Th1 response at 2 weeks after vaccination and a mixed Th1/Th2 immune response at 6 weeks after vaccination<br>Higher proliferation of splenocytes<br>significantly higher levels of IFN-g, IL-12, and IL-10 but not IL-4 higher levels of CD4 + and CD8 + T cells in the splenocytes<br>Increased Expression of p65 and<br>T-bet in spleen lymphocytes mRNA<br>Increase in CTL activity<br>Increased survival time | BALB/c mice | 2019 |
| Boosted twice at 2-week intervals | pVAX/IL-18 adjuvant | Significantly increased serum IgG (and IgG2a levels), lymphocyte counts and Th1-type cytokine (IL-2, IL-12, and IFN-γ) levels longer survival times | Kunming mice | 2018 |
| 2 booster injections at 3-week intervals | No additional adjuvant, The DNA CpG sequence itself | In both the vaccinated groups: predominant Th1-like response➔ cellular-mediated immune response with significantly higher levels of interferon-gamma, interleukin-2 (IL-2), IL-4, and IL-10<br>Increased splenocyte Ag-specific proliferation slightly prolonged survival<br>No elevation of IgG was detected | BALB/c mice | 2017 |

**Table 2** (continued)

| Booster | Adjuvants sequence or chemical | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|
| Booster immunizations 2 and 4-week later | No additional adjuvant, the DNA CpG sequence itself | Specific humoral and cellular responses, with higher level of IgG antibody, increased levels of Th1-type cytokines IFN-g and IL-12 (p70), and CD3þCD4þCD8, and CD3þCD8þCD4 T cells, as well as prolonged survival time | BALB/c mice | 2017 |
| Twice with 3-week intervals | No additional adjuvant, The DNA CpG sequence itself | In the vaccinated groups after specific Ag re-stimulation: Increased IFN-γ levels<br>And decreased IL-4 expression level<br>Increased spleen lymphocyte proliferation<br>Significant high levels of IgG in the serum<br>Predominance of the levels of IgG2a over IgG1 increased survival time | BALB/c mice | 2018 |
| 2 times with 2-week intervals | No additional adjuvant, The DNA CpG sequence itself | In the HSP60 DNA-immunized mice: increase of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells in spleen increased levels of IL-2, IL-4, IL-10, IL-12p70, and IFN-γ<br>Increased proliferation of splenocytes<br>higher levels of specific antibodies in sera<br>Increased survival time (in the acute infection)<br>Decreased brain cyst (in the chronic infection) | Specific pathogen-free (SPF) grade Kunming mice | 2018 |
| Twice at $2^{nd}$ and $4^{th}$ weeks | No additional adjuvant, The DNA CpG sequence itself | In the pVAX-ROP21 vaccinated animals: increased levels of IgG, IgG1, and IgG2a (IgG2a predominant)<br>IFN-g was significantly increased (while no significant changes were detected in IL-2, IL-4, and IL-10)<br>prolonged survival time (virulent *T. gondii* RH strain challenge)<br>The number and size of brain cyst decreased | BALB/c mice | 2018 |

**Table 2** (continued)

| Booster | Adjuvants sequence or chemical | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|
| Boosted at days 14 and 28 with the same protocol | Calcium phosphate nanoparticles | Increased levels of level of IgG1 and IgG2a (IgG2a predominance)<br>Increased proliferation of splenocytes<br>Increased IFN-g levels<br>Increased survival time<br>Reduced tissue parasite load | BALB/c mice | 2017 |
| Boosted twice at a 2-week intervals | No additional adjuvant, The DNA CpG sequence itself | In immunized mice:<br>increase in T lymphocyte subclasses ($CD3^+CD4^+$ T and $CD3^+CD8^+$ T lymphocytes) in splenic tissues<br>reduction in the parasite cyst<br>burden in the brain<br>Pru strain–infected mice<br>No difference in survival time in challenge with the virulent RH strain<br>No difference in the level of antibodies, lymphocyte proliferation and concentration of cytokines (IFN-g, IL-2, IL-4, IL-10, and IL-12p70) | Kunming mice | 2018 |
| Boosted using the same protocol twice at 3-week intervals on days 21 and 42 and | FliC of Salmonella typhimurium plasmid (Toll-like receptor 5 agonist)<br>and<br>(alum and saponin) | The pVAX1-SAG1 + pVAX1-fliC group (compared to both traditional adjuvants and controls):<br>Higher IgG with a predominance of IgG2a over IgG2b and IgG1<br>higher levels of IFN-γ, IL-12 and IL-10 cytokines and low levels of IL-4 production<br>higher splenocyte proliferation response<br>increased survival time | BALB/c mice | 2019 |
| Boosted twice 3-week intervals | Alum and MMT | Cocktail<br>DNA group:<br>higher total IgG and the isotypes of IgG1 and IgG2a<br>higher levels of IFN-γ (the immune response was shifted toward Th1)<br>increase antigen-specific lymphocyte proliferation of splenocytes<br>increased survival time and rate | BALB/cmice | 2019 |

**Table 2** (continued)

| Booster | Adjuvants sequence or chemical | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|
| Boosted twice with 2-week interval (the same dose) | No additional adjuvant, The DNA CpG sequence itself | The pVAX-GRA16 group:<br>higher levels of specific IgG antibody<br>high Ag-specific proliferation of spleen lymphocytes<br>increased levels of IFN-γ, IL-2, IL-4, and IL-10 cytokines<br>higher percentages of CD4 + and CD8 + T cells<br>reduced numbers of tissue cysts<br>no change in the survival time | Specific pathogen-free (SPF) grade inbred Kunming mice | 2017 |
| Boosted three times 2-week intervals (in the pROM4/peptide group the first 2 times by plasmid and the second 2 times by peptide) | No additional adjuvant, The DNA CpG sequence itself | The vaccinated groups: high levels of IgG, IgG2a (predominant), and interferon (IFN)-γ, IL-12, and IL-2. (IgG, IgG2a, and IFN-γ, IL-12, and IL-2 levels were highest in the pROM4/peptide group)<br>Prolonged survival times and reduced numbers of brain cysts (especially those in the pROM4/peptide group) | BALB/c mice | 2017 |
| Boosted twice at 2nd and 4th week | No additional adjuvant, The DNA CpG sequence itself | In the pVAX-TgROP35 group:<br>Higher IgG (both IgG2a and IgG1)<br>IFN-γ, IL-2, and IL-10 levels were significantly increased, while there were no significant differences in IL-4 expression<br>increased survival time<br>Reduced brain cysts number and size | BALB/c mice | 2018 |
| Booster immunizations 2 and 4 weeks later | No additional adjuvant, The DNA CpG sequence itself | Higher IgG (both IgG2a and IgG1 increased predominant IgG2a)<br>Higher splenocyte proliferation<br>Increased IL-10, IL-12 (p70), IFN-γ, and TNF-α but not IL-4<br>Increased survival time | Specific pathogen-free (SPF) female BALB/c mice | 2018 |
| Boosted with the same protocoltwice with 2-week intervals | No additional adjuvant, The DNA CpG sequence itself | Higher levels of IgG antibodies<br>higher levels of IFN-g<br>reduced brain cysts | BALB/c mice | 2016 |

**Table 2** (continued)

| Booster | Adjuvants sequence or chemical | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|---|
| Boosted twice with 2-week intervals | No additional adjuvant, The DNA CpG sequence itself | In the immunized groups (more apparently in the multi-antigenic groups):<br>Increased IgG titer<br>higher IgG2a to IgG1 ratio<br>Increased IL-2, IFN-g, IL-12 and IL-23 levels (but not IL-4 and IL-10)<br>Increased percentages of $CD3^+$ $CD4^+CD8^-$ and $CD3^+CD8^+CD4^-$ T lymphocytes<br>Increased survival time<br>Increased spleen lymphocytes proliferation<br>Decreased brain cyst | Kunming mice | 2019 |
| Boosted twice at 2-week intervals pEGFP-C1-SAG5D 100 μg/ each (1 μg/μl) with α-GalCer at the 3rd time | Alpha-Galactosylceramide (α-GalCer) (2 μg/mouse) | In both pEGFPC1-SAG5D or α-GalCer/pEGFP-C1-SAG5D groups:<br>increase of IgG (IgG2a over IgG1)<br>higher level of IFN-γ<br>higher IL-4 (only in α-GalCer-treated groups)<br>longer survival time | BALB/cmice | 2014 |

**Table 3** Particulate vaccines (nanoparticle, viral and quasi-viral, microparticle, bacterial or yeast) Toxoplasma

| Antigen | Toxoplasma strain | Particle type | Route of injection and the location and dose | Booster |
|---|---|---|---|---|
| **rSAG1, rSAG2 and rGRA1** | N/A | Muramyl dipeptide (MDP) microparticle | Intramuscularly in the dorsal neck region<br>300 µg Ag in 1 ml PBS | Boosted once, 6 weeks after the initial one |
| **Microneme protein 16 (TgMIC16)** | Challenged by virulent *T. gondii* RH strain tachyzoites | Yeast<br>*S. cerevisiae* EBY100 strain (containing pCTCON2 plasmid) | Intraperitoneally with heat-killed transfected yeast or orally with live transfected yeast<br>100 µl (4× 107 cells) | Boosted twice at weeks 2 and 4 after the initial one |
| **ROP2** | Protein sequence from *T. gondii* RH strain challenge by T. gondii RH strain | *M. bovis* BCG, sub-strain Pasteur pMV262 vector | Subcutaneous 0.1 ml (107 cfu/ml) BCG/<br>pMV262-ROP2 | Boosted once after 4 weeks by the same dose |
| **ROP18** | N/A | Poly (lactideco-glycolide) (PLGA) nanoparticle | Intraperitoneally (ip) with 10 µg rROP18 | Bossted twice in 2-week intervals |
| **Microneme protein 8 (MIC8)** | Challenge by highly virulent *T. gondii* (RH) (oral challenge) | Virus-like particles (derived from baculovirus + influenza matrix protein 1 {M1}) | Intranasal immunization (IN), intramuscular immunization (IM) with<br>75 µg of total MIC8 VLP protein per mouse | Boosted once 4 weeks later |
| **MIC3, ROP9, and SAG2** | Challenge by lethal *T. gondii* RH strain | Recombinant adenoviruses | 50 µL purified recombinant adenoviruses ($10^9$ PFU) intramuscular injection at 2-week intervals | Boosted once, 2 weeks later |
| **rSAG1** | Tachyzoites of *T. gondii* RH strain for challenge | PLGA | Subcutaneously (s.c) immunized in the right-hind footpad<br>20 µg of rSAG1-adsorbed<br>PLGA nanoparticles and rSAG1-encapsulated PLGA nanoparticle | Once, 3 weeks later by the same dose |
| **Rhoptry protein 18 (ROP18) and microneme protein 8 (MIC8)** | Intraperitoneally (IP) with tachyzoites of GT1 strain or orally challenged with *T. gondii* ME49 strain | Virus-like particle | Intranasally (IN) with 60 mg of ROP 18VLPs or MIC8 VLPs or a mixture of 30 mg ROP18<br>VLPs and 30 mg MIC8 VLPs (combination VLP vaccine) | Once, 4 weeks later by the same protocol |
| **SAG1** | N/A | Polymeric nanospheres | Intraperitoneal (i.p.) injections 10 mg rSAG1 protein + montanide or rSAG1 + PLGA intranasally | Boosted twice with 2-week intervals |
| **SAG1** | Challenged by *T. gondii* RH | Virus-like particle | Intramuscular administration with SAG1-VLPs<br>(120 µg) | Boosted once after 4 weeks |
| **Apical membrane antigen 1 (AMA1)** | Challenged with *T. gondii* ME49 | Virus-like particle | Intranasally immunized with 100 µg of VLPs | Boosted once after a 4-week interval |

**Table 3** (continued)

| Adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|
| rSAG1, rSAG2 and rGRA1 were covalently conjugated to 1 µm MDP microparticles (10 mg MDPmicroparticle/1 mg recombinant protein) | Increased IFN-g in the rGRA1<br>Immunization with recombinant proteins rSAG1, rSAG2 and rGRA1 alone or as a cocktail vaccine elicited IgG2 and a weak IgG1 response | Sheep | 2017 |
| The yeast itself as an adjuvant | In both intraperitoneally orally vaccinated groups:<br>Higher serum Ab concentration (dominant IgG2a over IgG1)<br>Higher lymphocyte proliferative response<br>Higher percentage of CD4 + and CD8 + T cells<br>increased levels of IL-2 and IFN-g (but not IL-4 or IL-10)<br>increased survival time | BALB/c mice | 2018 |
| The BCG itself as an adjuvant | Increased survival time<br>Elevated total Ab (humoral immunity)<br>Increased IFN-g and IL-2 production<br>Higher percentage of CD4 + cells (cellular immunity) | BALB/c mice | 2007 |
| Montanide adjuvant or PLGA nanoparticle | Both adjuvant group and PLGA demonstrated elevated IgG<br>IgA levels was significantly higher in PLGA + ROP18 group<br>In the PLGA group the IgG2a was dominant while in the adjuvant group the IgG1 was dominant | Swiss-Webster mice | 2017 |

**Table 3** (continued)

| Adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|
| None | IN mice group showed higher levels of *T. gondii*-specific IgG antibody response compared to IM mice group<br>IN group (IgG1 predominance {Th2})<br>IN induced higher levels of systemic and mucosal antibody responses<br>IM group no effective Ab response<br>Higher CD4 T cell, CD8 T cell and germinal center B cells in both IN and IM groups<br>Following parasite challenge higher levels of IFN-γ and IL-6 were detected in Naïve and IM groups compared to IN group (IN group reduced inflammatory reaction but higher humoral)<br>100% survival of IN group and 60% survival of IM group, 100% mortality in the control group | BLAB/c mice | 2017 |
| None, the adenovirus particle itself can have adjuvant effects | In the mice immunized with the recombinant adenoviruses group: extremely significantly higher *T. gondii*-specific IgG antibody Levels<br>Increased production of IL-6, TNF-a, IL-22, IFN-g, IL-17A and IL-10<br>Increased T lymphocytes (and activated Th lymphocytes) percentage in the spleen<br>Elevated survival time | BALB/c mice | 2019 |
| Adsorption or encapsulation of rSAG1 to PLGA nanoparticles can provide some adjuvant effects | Increased survival time in rSAG1 loaded PLGA groups<br>Total serum IgG and high IgG2a/IgG1 ratio in rSAG1 loaded PLGA groups<br>higher amounts of IFN-γ (but unchanged IL-10) in rSAG1 loaded PLGA groups after in vitro Ag re-stimulation of spleen cells | BALB/c mice | 2020 |

**Table 3** (continued)

| Adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|
| None | All three vaccine groups showed similar levels of IgG antibody responses which were significantly increased after boost immunization (combined ROP18 VLPs + MIC8 VLPs vaccine immunization showed a higher level of IgA antibody responses)<br>higher levels of CD4 + T cells, CD8 + T cells, and memory phenotypic T cells (combination *T. gondii* VLP immunization induces higher T cell responses after challenge)<br>Combination *T. gondii* ROP18 and MIC8 VLP immunization attenuates apoptotic cellular response after challenge<br>Combination VLP vaccine immune sera exhibit higher activity of controlling parasite loads in vivo<br>Combination VLP vaccination reduces pro-inflammatory cytokine (IFN-g and IL-6)<br>responses after challenge<br>Combined VLP vaccines improved protection against challenge infection with T. *gondii* via an oral or IP route | BALB/c mice | 2018 |
| Montanide adjuvant or PLGA nanoparticle | Both adjuvant group and PLGA demonstrated elevated IgG<br>IgA levels was significantly higher in PLGA + ROP18 group<br>In the PLGA group the IgG2a was dominant while in the adjuvant group the IgG1 was dominant | Swiss-Webster mice | 2018 |

**Table 3** (continued)

| Adjuvants | Immune response elicited by vaccine | Animal used for vaccine response evaluation | Year of publication |
|---|---|---|---|
| None | SAG1-VLP immunization:<br>Significant increase of the antibody (IgG, IgG1, IgG2a, and IgA) levels (IgG1 predominance) not only decreases the production of cytokines (IL-4, IL-12, and IFN-g) associated with the infection of pathogens in the host, but also effectively inhibits the inflammatory cytokines (IL-1<br>, IL-6, and TNF-a) after *T. gondii* infection<br>The survival rates of the immunized infection group were significantly increased compared to the non-immunized infection group | Balb/c mice | 2020 |
| None | In the AMA1 VLPs-immunized group:<br>higher levels of T. *gondii*-specific IgG and IgA<br>higher germinal center B cell populations<br>smaller cysts and lower cyst counts were detected from the brain, reduced body weight loss, higher survival rate | BALB/c mice | 2020 |

cellular immune responses. Among different type of nanoparticles, the calcium phosphate nanoparticle (CaPN) is a well-known member that has been used for many years as a delivery system in DNA vaccines and is approved to be utilized as the adjuvant.

Much research has been done on nanoparticles to design vaccine against *T. gondii*. Table 3 summarized the results of several different studies on nanoparticle vaccines, including the type of particle used for the SAG1, 2 and GRA1 antigen were muramyl dipeptide (MDP) microparticle [79], for MIC16 was yeast *Saccharomyces cerevisiae* EBY100 strain [68], and for ROP2, ROP18, MIC8, MIC3, ROP9, SAG2, SAG1ROP18, SAG1, SAG1, and AMA1 antigens were *Mycobacterium bovis*, Poly (lactideco—glycolide)(PLGA), virus-like particles (derived from baculovirus + influenza matrix protein 1), recombinant adenoviruses, PLGA, virus-like particle, polymeric nanospheres, virus-like particle, and virus-like particle respectively [80–88].

## Discussion

In recent years, progress has been made in designing a potential vaccine against *T. gondii*. Studies have also been performed using different types of *T. gondii* antigens, including recombinant vaccines, DNA vaccines, subunit vaccines, attenuated live vaccines, and nanoparticle vaccines [20]. Accordingly, significant advances have been made in characterization and isolation of antigens, gene cloning, antigen expression, and immunological methods. In addition to the prevention strategies, new options are now needed to develop effective vaccines as a way to prevent the toxoplasmosis [29]. Most of the *T. gondii* antigens are important for the virulence and immunogenicity of the parasite. However, future studies should focus on the quality and quantity of antigens and identify potential candidate antigens against *T. gondii* infection. In addition, more extensive studies are needed to identify recombinant vaccines, DNA vaccine performance, and evaluate recombinant nanoparticle vaccines. Many of the vaccine strategies against toxoplasmosis have been experiments in animal models; nevertheless, these experiments only resulted in relative protection against *T. gondii* infection.

Vaccines designed with recombinant antigens rely on the defined antigens to induce a host-specific immune system against pathogenic microorganisms, which can be expressed by plasmids in the bacterial and yeast hosts or delivered by viral vectors [89]. Recombinant antigen vaccines have advantages over classic methods. One of the problems with using live vaccines is that if the host's immune system is defective, it may cause the tachyzoite to return from an attenuated form to an active invasive form [29]. Another disadvantage is the complexity of obtaining sufficient amounts of purified immunogenic components of the antigen by the classical methods. The quality and effect of vaccination against *T. gondii* using recombinant antigens is very important, especially in pregnant women who may be at risk for the first time [6, 18, 20]. In the preparation of recombinant antigens, when the antigen is purified well, it can have far fewer side effects than raw antigens or live vaccines [90–93].

Studies on rodent animal models have shown that DNA vaccines can effectively induce both humoral and T-cell responses against a wide variety of candidate vaccine antigens [94]. Nevertheless, for unclear reasons, in primates and human's poor immune, responses have been observed to parasite DNA vaccines. Various adjuvants including cytokines and CpG oligonucleotides have been studied to improve the immune responses in these large animals [95].

Monomeric linear protein that can assemble into a nanoparticle is a new method for inducing immune responses against peptide epitopes of antigens from an infectious agent. As most of the infectious agents invade the host through mucosal surfaces, researchers are interested to design vaccines with the ability to mimic this aspect of the pathogens to induce an effective immune response; therefore, a better understanding of the mechanisms that the pathogens use to interact with cells and the biological fluids is required to design vaccines with adequate efficiency. Monomeric linear protein based nanoparticles vaccine against toxoplasmosis were in a study and effectively elicited T-cell-dependent cellular immune cells responses [35]. As the group SAG antigens are highly expressed on the *Toxoplasma gondii* tachyzoite, they are considered as one of the main candidates for toxoplasmosis vaccine design. The SAG1 is a 30 KDa beta-glycoprotein that can be extracted from tachyzoites and sporozoites of the *T. gondii* [96].

The SAG1 is the most immunogenic structure in tachyzoites and the first structure of the parasite that interact with the host cells. The gene encoding this protein is a single-copy gene and contains no introns. It is believed that SAG1 is the most promising candidate to develop an effective vaccine against *T. gondii* because it stimulates both the cellular and humoral immune responses [6, 20, 37, 79, 96]. According to the results of previous studies use of vaccines based on recombinant forms of the antigen and nanoparticle-based vaccines can induce stable specific immunity in hosts, including pregnant women (at risk of primary infection) and immunocompromised patients [79]. Previous studies indicate that the use of GRA1, GRA2, GRA6, GRA5, GRA4, and GRA7 antigens are good candidate for the design and production of DNA vaccines. For example, the GRA7 antigen is an acidic 29 KDa protein and comprise about 0.5% of all the *T. gondii* proteins. The Gra7 gene is composed of 1.3 Kbps and has no introns. The GRA7 antigen is present in the parasitophorous

vacuole in host cells infected by tachyzoites and also in the cytoplasm of host cells infected by bradyzoites. GRA7 is expressed in all of the stages of *T. gondii* infection and is a considerable candidate for the vaccine design [91, 93]. It can effectively induce both cellular and humoral immune responses against the *T. gondii* [29]. Another member of this family is GRA4 that is a 40 KDa protein secreted into the parasitophorous vacuole by the parasite [40, 41]. This protein strongly interacts with the milk IgA and to a lesser degree with the intestinal mucosal layer IgA [24]. The amino acid sequences 297–345 in the GRA4 are called C protein and can interact with the milk and intestinal mucosal IgA and serum IgG in mice infected by *T. gondii* and also serum IgG in human and sheep [24]. The GRA4 stimulates the mucosal T lymphocytes in BALB/C and CBA/J mice strains. The GRA4 can induce mucosal and systemic immune responses in mice after *T. gondii* ingestion [24, 29, 41]. Furthermore, GRA14 is a 47 KDa protein with 409 amino acids. The gene encoding this protein is consisting of 1227 bps. The GRA14 is present in the membranes of parasitophorous vacuole and intravacuolar network. This protein has a unique topology that is not seen in other proteins [29, 58, 97]. Due to the unique topology and its long length inside the vacuole system, this protein probably is a potential strong inducer of the immune responses. ROP proteins are the largest family of *T. gondii* serine-threonine kinases [24]. The evaluation of previous studies demonstrates that the ROP18 is the most interesting member of the ROP family in recombinant, nanoparticle, and DNA vaccine development studies [24, 64, 98]. It is probably due to the pre-formed presence of the antigen inside the rhoptry and as it is secreted into the parasitophorous vacuole during invasion to the host cells [64, 98, 99]. This antigen is one of the key virulence factors of *T. gondii* that protects the parasite from the host immune responses by its kinase activity [98]. The amino acids 243 to 539 are involved in the protein kinase activity of the protein. Another effect of this kinase activity is enhancing the parasite replication inside the host cells [99]. Previous studies have shown that GRA1, SAG1, SAG2, MIC1, MAG1, ROP18, GRA6, and GRA2 antigens are highly immunogenic. In addition, these antigens have been shown to stimulate specific antibodies in the host body or cytokines in vitro in the culture medium of splenocyte cells [6, 24, 29, 92]. ROP, GRA, and SAG antigens are the strongest candidates for the vaccine because they have been shown to contain relatively long antigenic fragments and regions, especially ROP, which appears to be a more suitable candidate than the other two antigens [6, 20]. It has also been shown that this antigen can elicit a strong protective immune response. DNA immunization of BALB/c mice with homogeneous mixtures of plasmids encoding short micronemic antigen fragments has been shown to enhance protective immunity, leading to an 85% reduction in the burden of *T. gondii* cysts [55].

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## Declarations

**Ethics approval** The protocol was approved by the Kermanshah University of Medical Sciences Ethics Committee [IR.KUMS.REC.1400.070].

**Conflict of interest** The authors declare no competing interests.

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