

Invited Review

***Toxoplasma gondii* Recombinant Surface Antigen (SAG 1; P 30) and its Applications in Serodiagnosis and Vaccine Development for Toxoplasmosis**

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INTRODUCTION

Toxoplasmosis, caused by an intracellular protozoan parasite, *Toxoplasma gondii*, is widespread throughout the world. The disease is of major medical and veterinary importance, being a cause of congenital disease and abortion in humans and in domestic animals.^[1] In addition, it has gained importance recently due to toxoplasma encephalitis in AIDS patients.^[2]

T. gondii was discovered 100 years ago. Its identification was rapidly followed by the recognition that it was a human pathogen. During the past 100 years, the spectrum of diseases caused by this ubiquitous pathogen has expanded to include both congenital and acute infections as well as the recognition of diseases caused by this pathogen in the immune-compromised host. Recent data on behavioural changes in animals due to chronic toxoplasmosis is leading to research on the effect of this pathogen on the behaviour of humans.^[3] Experimental studies on *T. gondii* have resulted in it becoming a model organism for studies on host pathogen interactions. Integration of clinical and experimental data on *T. gondii* should continue to lead to important insights into improvements in diagnosis for clinical management and vaccine development for control of toxoplasmosis.

THE PARASITE

Toxoplasma gondii is a ubiquitous protozoan parasite that is estimated to infect one-third of the world's human population. It can infect many species of warm-blooded animals and is a significant zoonotic and veterinary pathogen. It has been 100 years since *T. gondii* was initially described in the tissues of *Ctenodactylus gundi*, a North African rodent, by Nicolle and Manceaux.^[4] Splendore, in Brazil, reported on the identification of this organism in the tissues of a rabbit.^[5]

The genus was named by Nicolle and Manceaux as *Toxoplasma* for its bow-like shape (from Greek: toxo = bow or arc; plasma = creature). Other forms of *Toxoplasma* including tissue cysts were recognised to exist by several researchers, but it was not until the 1960s and 1970s that the parasite was identified as a coccidian.^[6] The cat was identified as the definitive host by several groups working independently, including Frenkel *et al.*^[7] Further details of the history of the discovery of the pathogen are described in recent reviews.^[8, 9]

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T. gondii infects a wide range of intermediate hosts that include different species of mammals. Acute toxoplasmosis in these hosts is characterised by intracellular growth of the rapidly dividing tachyzoite stage in many tissues. As the immune response controls the acute infection, some of the tachyzoites become encysted, particularly in the muscles and brain, in a dormant stage termed the bradyzoite. These cysts contain hundreds of bradyzoites which are retained for years. If the tissue that contains these cysts is eaten by a non-feline carnivore, the bradyzoites are released in the intestine and again yield infection, characterised initially by rapid growth of tachyzoites and ultimately by the persistence of encysted bradyzoites. However, when encysted bradyzoites are eaten by a member of the cat family, the sexual cycle occurs in the intestine, resulting in the production of oocysts. When a sporulated oocyst (ovoid and measuring approximately $10 \times 12 \mu\text{m}$) is ingested by a mammal, the sporozoites are released and infect the intestinal epithelial cells. They become rapidly multiplying tachyzoites that first produce an acute and then a chronic infection with encysted bradyzoites, thereby completing the natural life cycle (Figure 1).

Tachyzoites are crescent shaped and about $6 \mu\text{m}$ long and $2 \mu\text{m}$ wide. They are coated with three unit membranes, which form the pellicle. The anterior end of the tachyzoite is marked by the presence of a conoid, a hollow, truncated cone of spirally wound fibres that are likely to be microtubules. The rhoptries are club shaped, densely osmophilic structures whose narrow ends terminate in the conoid. Micronemes are situated close to the rhoptries. Dense organs are distributed throughout the

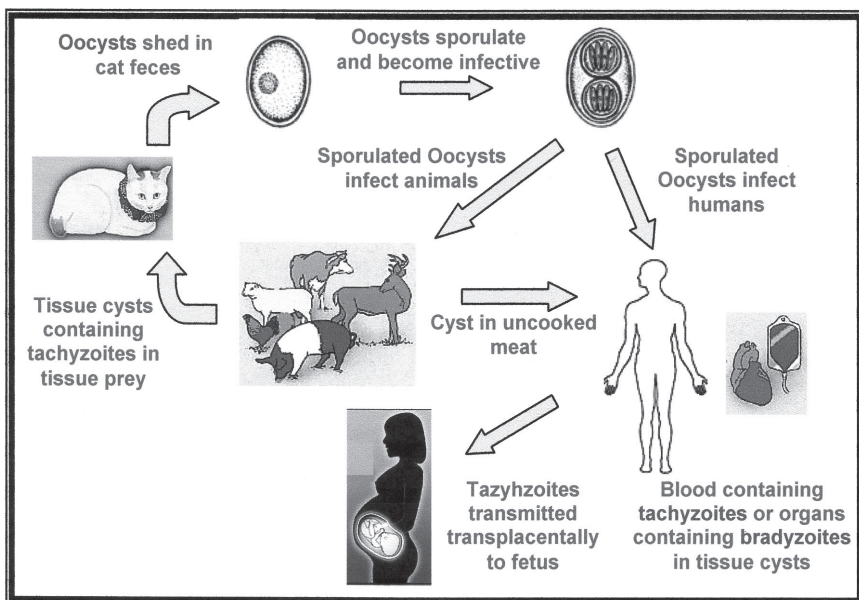


Figure 1. Life cycle of *Toxoplasma gondii*

Source: Modified from: <http://www.omafr.gov.on.ca/english/livestock/swine/facts/04-055.htm>

cytoplasm of tachyzoites. Proteins from the dense granules are released into the external environment as excreted/secreted antigens. Bradyzoites differ from tachyzoites in their higher content of micronemes, amylopectin granules and stage-specific antigens.^[10]

Sexual forms of the parasite are found in the intestinal epithelium of definitive hosts such as domestic cats which then transform into oocysts which are subsequently shed in the environment. Oocysts, remarkably stable environmentally, are transmitted to other hosts through inadvertent ingestion. Humans acquire *T. gondii* through ingestion of undercooked meat, contact with feline faeces and rarely through drinking contaminated water or through transplantation of a contaminated organ.^[11]

For clinical purposes, toxoplasmosis can be divided for convenience into five infection categories: (i) those acquired by immuno-competent patients; (ii) those acquired during pregnancy; (iii) those acquired congenitally; (iv) those acquired by or reactivated in immuno-deficient patients; and (v) ocular infections. In any category, clinical presentations are not specific for toxoplasmosis, and a wide differential diagnosis must be considered. Furthermore, methods of diagnosis and their interpretations may differ for each clinical category.^[1,12,13,14]

RECOMBINANT *T.GONDII* SURFACE 1 (SAG1; P 30)

The immuno-dominant surface antigen of *T. gondii*, surface antigen 1 (SAG1; previously named P30), is commonly expressed in *Escherichia coli* as a fusion protein containing the mature part of the SAG1 protein supplied with six histidyl residues in the N-terminal end. The construction can be produced in large amounts, is easy to purify, and is recognised by anti-SAG1 monoclonal antibodies, *Toxoplasma*-specific acute phase immunoglobulin M (IgM) antibodies and chronic-phase IgG human antibodies. Native mature SAG1 protein is presumed to be post-translationally modified by removal of the signal sequence and the C terminus, the latter upon addition of the GPI anchor.^[15,16] Santoro *et al.* demonstrated that SAG1 antigen express on the surface of intra- and extra-cellular tachyzoites.^[17]

Rodriguez *et al.* suggested that SAG1 is the most immunogenic constituent of tachyzoites, and that a single region of this molecule contains most of the immunogenic activity.^[18] Some epidemiological studies were carried out by researchers using this antigen and in some experimental studies, this antigen was used for vaccination. Others used this antigen for diagnosis of congenital toxoplasmosis. In conclusion, the 30 kDa protein gene of *Toxoplasma* tachyzoites surface antigen (SAG1) that was cloned in expression vector and the expressed protein (30 kDa) has been widely used for diagnosis of toxoplasmosis using the ELISA system.

Although serological tests give satisfactory results, production of reliable and standard reagents continues to be a major constraint in serodiagnosis leading to inter-assay variability. Most conventional test kits employ *T. gondii* tachyzoites grown in mice or in tissue culture which are often contaminated with extra parasite material. Therefore, use of purified recombinant proteins in suitably designed serotests is the only alternative for specific and sensitive results. For diagnostic applications, SAG1 is a commonly used antigen because it is the most immuno-dominant and stage-specific, present in tachyzoite stage and not in bradyzoite stage.^[19]

We have generated this immuno-dominant surface antigen of *Toxoplasma gondii*, surface antigen 1 (SAG1), which was expressed in *Escherichia coli* as a fusion protein containing a majority of the SAG1 protein supplied with six histidyl residues in the N-terminal end. The recombinant protein was purified on a Ni-chelate column and then on a fast performance liquid chromatography column and was in a non-reduced condition. It was recognised by *T. gondii* specific human immuno-globulin G (IgG) and IgM antibodies as well as by a mouse monoclonal antibody (S13) recognising only non-reduced native SAG1. Antibodies induced in mice by the recombinant SAG1 recognised native SAG1 from the *T. gondii* RH isolate in culture. This recombinant SAG1 is suitable for use in detecting human anti-SAG1-specific IgG and IgM antibodies from sera of patients (Figure. 2). Assays based on the recombinant SAG1 are expected to be easier to standardise and to be more reproducible because only a single protein with a few immuno-dominant epitopes is used.

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Most workers therefore cloned only the sequence coding for the mature part of the protein, residues 49 through 323 (2), into an *E. coli* expression vector, pGH433 (12), using

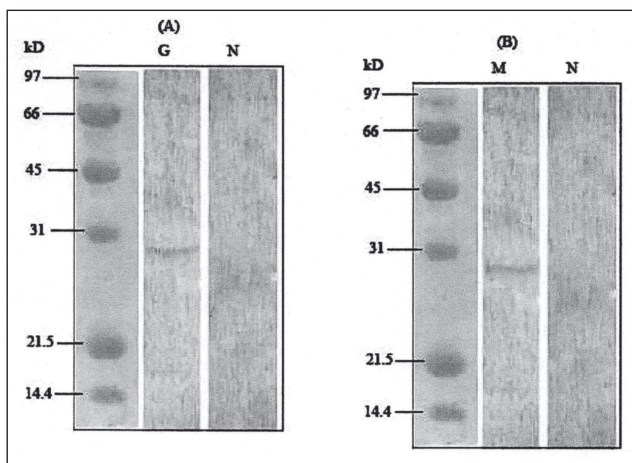


Figure 2. Reactivity of the immunodominant surface antigen of *Toxoplasma gondii*, surface antigen 1 (SAG1) mol. Wt 30 kDa with A. *T. gondii* specific human immunoglobulin G (IgG) and B. IgM antibodies from sera of patients. Recombinant SAG1 is suitable for use in diagnostic systems for detecting anti-SAG1-specific IgG and IgM antibodies.

Source: Data came from work funded by a grant from the Ministry of Science, Technology and Innovarion Science Fund 02-01-04-SF0801

PCR. An oligo-nucleotide encoding six histidyl residues and a factor Xa cleavage site (13) was cloned into the vector in frame with the SAG1-coding region (pDH26), giving rise to a recombinant SAG1 (rSAG1).

The B- and T-cell epitopes of the SAG1 protein have been studied by several groups and it has been found that SAG1 contains one immuno-dominant region in the middle of the protein, with repetitive epitopes containing all of the B-cell epitopes.^[21,22] The B-cell epitopes are conformational since human *T. gondii*-immune sera do not recognise the reduced SAG1. The SAG1 gene codes for 12 cysteine residues, and the immuno-genicity of the recombinant protein depends entirely on the correct folding. In *E. coli*, the formation of disulfide bonds takes place within the periplasm, while we presume that our rSAG1 is situated in the cytoplasm, since it does not contain any secretion signal sequence.

Studies on immunisation of mice with a fusion protein consisting of glutathione *S*-transferase and SAG1 produced in *E. coli* have shown decreased *T. gondii* infection of macrophages, but the immunogenicity of rSAG1 was not reported. The fusion products showed the same immunological phenotypes, since rabbits immunised with either of the two fusion proteins elicited significant antibody responses against rSAG1, but only rabbits immunised with rSAG1 expressed in eukaryotic cells displayed serological reactivity against a tachyzoite lysate.^[23]

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SERODIAGNOSIS OF TOXOPLASMOSIS

Diagnosis of *T. gondii* infection or toxoplasmosis in humans is made by biological, serological, histological, or molecular methods or by some combination of these methods. Clinical signs of toxoplasmosis are non-specific and are not sufficiently characteristic for a definite diagnosis. In fact, toxoplasmosis mimics several other infectious diseases. Detection of *T. gondii* antibodies (mainly immunoglobulin G [IgG] and IgM) in patients may aid diagnosis. IgG antibodies usually appear within 1 to 2 weeks of acquisition of the infection, peak within 1 to 2 months, decline at various rates, and usually persist for life.^[25,26]

IgM antibodies may appear earlier and decline more rapidly than IgG antibodies, so the detection of IgG antibodies may be helpful for diagnosis of chronically infected patients, if IgM antibodies are negative. An IgM test is still used by most laboratories to determine if a patient has been infected recently or in the distant past; because of the hurdles posed in interpreting a positive IgM test result, confirmatory testing should always be performed.^[27,28]

There are numerous serological procedures available for the detection of humoral antibodies; these include the Sabin-Feldman dye test, the indirect hemagglutination assay, the indirect fluorescent antibody assay, the direct agglutination test, the latex agglutination test, the enzyme-linked immunosorbent assay (ELISA), and the immunosorbent agglutination assay test.^[29]

Most of these immuno-diagnostic tests are not easy to apply in the field, for example, the ELISA or the indirect fluorescence antibody assay, since these techniques require special equipment and reagents. Performing any of these tests even in the laboratory generally takes time, sometimes with overnight incubation steps; otherwise, enzyme reagents would need a cold chain for delivery. In such situations, a rapid, simple, and inexpensive colorimetric assay with robust reagents and no instrumentation could have many diagnostic applications.

The choice of the appropriate diagnostic method(s) and its (their) interpretation may differ for each clinical category. The dot immuno-binding assay, using an nitrocellulose (NC) membrane as a test matrix, is becoming widely used in simple qualitative research applications.^[30] Colloidal gold-labeled antibodies are also used in dot blot assays to avoid use of the sometimes problematic enzyme-labeled detecting antibodies. Recently, some colloidal dye particles were screened to label antigen or antibody for the detection of antibody or antigen; compared with gold or enzyme; the colloidal dye is cheaper or easier to preserve.^[31] The colloidal dye particle technique utilises the concepts of ELISA, dot blot assays, colloidal dye-labeled antigen or antibody, and immuno-chromatography to produce an inexpensive, robust, NC based dipstick test for antibody or antigen detection. Since it requires no instrumentation for qualitative detection of antigen or antibody, it has many potential field applications.

Previously, our laboratory used monoclonal antibodies (MAbXC3) conjugated with a colloidal dye to detect *Brugia malayi* excretory antigens on a NC membrane dipstick based on immuno-chromatography (DIA). The DIA for detecting antigens in patients with brugian filariasis showed very high sensitivity, specificity, and positive predictive value. The assay is useful for detection of active cases of lymphatic filariasis in areas where the disease is endemic and is rapid, simple, cheap and effective.^[32]

Since the diagnosis of clinical toxoplasmosis is often difficult, serologic procedures have been employed to aid in diagnosis. These include the Sabin-Feldman dye test, the indirect hemagglutination (IHA) test, the indirect immuno-fluorescence (IFA) test and the enzyme-linked immunosorbent assay (ELISA).^[33] The two most used assays, the IFA test and ELISA, require expensive fluorescence microscopes or photometers. Recently, we successfully applied an ELISA using microlitre volumes of a monoclonal antibody (DiMab) onto nitrocellulose paper (Dot-ELISA) to detect antigenemia in canine dirofilariasis.^[34] This

dot-ELISA for canine dirofilariasis is highly versatile and a rapid microprocedure and is as sensitive and specific as ELISA. It does not require a photometer, and is field-portable. In addition, the Dot-ELISA can be easily standardised with respect to incubation times and temperatures, antigen and reagent stabilities, enzyme-conjugated antibody specificity, and reproducibility.^[35]

However, serodiagnosis may also become complicated by inapparent past infection with *Toxoplasma*. In the United States, serological incidence of toxoplasmosis increases to between 30% and 50% in the over-20 age group; incidence varies with geographic area and with age.^[36] Thus, isolated positive serum IgG titres may only indicate past exposure to *T. gondii*, complicating the interpretation of sero-diagnostic findings. Postitive IgM titres are more indicative of acute infection with *T. gondii*. In terms of detecting *Toxoplasma*-specific antibodies in the population, the Dot-ELISA, ELISA, and IFA tests are equivalent. The three assays varied by <5% in detection of IgG and <3% in detecting IgM antibodies. The significantly higher geometric mean titres observed in the Dot-ELISA are comparable to those of the ELISA and IFA tests but do not necessarily result in greater assay sensitivity. This phenomenon has been observed in the Dot-ELISA for sero-diagnosis of human visceral leishmaniasis and indicates that this assay regularly shows higher titres than other serologic tests. Nevertheless all of the assays detected significant levels of IgM antibodies.

Although serological tests give satisfactory results, production of reliable and standard reagents continues to be a major constraint in sero-diagnosis leading to inter-assay variability. Most conventional test kits employ *T. gondii* tachyzoites grown in mice or in tissue culture which are often contaminated with extra parasite material. Therefore, use of purified recombinant proteins in suitably designed sero-tests is the only alternative for specific and sensitive results. For diagnostic applications, several target genes of *T. gondii* have been cloned and expressed. Among the *T. gondii* antigens, surface antigen 1 (SAG1) is the choice because it is the most immuno-dominant and stage-specific, present in tachyzoite stage and not in bradyzoite stage, while granule protein 7 (GRA7) produces very strong antibody response in the acute phase of infection.^[37] Despite potential advantages of these recombinant antigens in sero-diagnosis, only limited studies have considered more than one antigen in enzyme-linked immunosorbent assays (ELISAs). The current paradigm in sero-diagnosis is the use of two recombinant proteins, SAG1, a tachyzoite stage-specific protein and GRA7 separately and/or as a cocktail of these proteins in ELISA format to replace the whole tachyzoite proteins as antigens for specific detection of *T. gondii* infection.

VACCINATION PROSPECTS AGAINST TOXOPLASMOSIS

Treatment of this disease is difficult due to toxic effects of available drugs, and reinfection occurs rapidly. Under the present scenario, development of either new antitoxoplasma drugs or a vaccine is an attractive alternative.

In the last few years, there has been considerable progress towards the development of a vaccine for toxoplasmosis, and a vaccine based on the live attenuated S48 strain was developed for veterinary uses.^[38] However, this vaccine is expensive, causes side effects and has a short shelf life. Furthermore, this vaccine may revert to a pathogenic strain and therefore is not suitable for human use. Various experimental studies have shown that it

may be possible to develop a vaccine against human toxoplasmosis. This section of this review highlights the protective immune response generated by *T. gondii* as well as the current status of development of a vaccine for toxoplasmosis.

In recent years, significant progress has also been made in the identification of vaccine candidates which can induce a protective immune response (Table 1). Most of the work has focused on surface antigens of tachyzoites. SAG1 (30 kDa), SAG2 (22 kDa), SAG3 (43 kDa), which are major surface antigens of tachyzoites.^[39] Amongst them, SAG1 is the predominant vaccine candidate. Excretory secretory antigens of *T. gondii* also play an important role in the stimulation of the protective immune system.^[40, 41] These antigens are expressed by both tachyzoites and encysted bradyzoites. The major components of excretory secretory antigens are GRA molecules.^[42] GRA1 (23 kDa), GRA4 (40 kDa) and GRA7 (29 kDa) have also been identified as vaccine candidates. Recently, ROP2 (56 kDa) antigen (expressed by tachyzoites, bradyzoites and sporozoites) has also been proposed as a vaccine candidate against toxoplasmosis.^[43]

Humans are generally infected by ingesting oocysts released in cat faeces or consuming meat from infected animals containing the long-lived tissue cysts. After ingesting infective tissue cysts or oocysts, tachyzoites are released which first invade and multiply in intestinal epithelial cells. From the gastro-intestinal tract, tachyzoites are disseminated to other organs of the human body. In the lumen of the gut, intra-epithelial lymphocytes are located among the epithelial cells.^[44, 45] They participate in modulating host immunity through the release of various cytokines, most apparently interferon- γ (IFN- γ) and induce immunity.^[9] Similarly to intra-epithelial lymphocytes, mesenteric lymph node lymphocytes may migrate to the gut and prevent parasite invasion.^[46]

T. gondii induces a potent cell-mediated immune response. Tachyzoites stimulate macrophages to produce interleukin-12 (IL-12) and tumour necrosis factor- α (TNF- α).^[47, 48] IL-12, in turn, activates natural killer cells and T cells to produce IFN- γ , which is crucial for resistance.^[11] IFN- γ and TNF- α act synergistically to mediate killing of tachyzoites by macrophages.^[11] The combination of these two cytokines results in the enhanced production of free radicals and nitric oxide (NO), which also can affect parasite killing.^[49, 50] NO is produced as a result of activation of inducible nitric oxide synthase (iNOS). iNOS is induced in *T. gondii* replication in murine cells but not in human macrophages. In an *in vivo* study with iNOS-deficient mice, the protective role of NO against *T. gondii* infection was tissue specific rather than systemic.^[51] Other *in vitro* studies demonstrated that IFN- γ -induced antitoxoplasma activity in human cell depends on the induction of indoleamine 2,3 dioxygenase (IDO), which is the rate-limiting enzyme for the L-tryptophan-L kynurenine pathway. In the absence of tryptophan, parasitic growth becomes restricted. Thus, IDO and iNOS are involved in the immuno-modulatory role of IFN- γ . Recent studies suggest that there is an anti-toxoplasma mechanism of cross-regulation between iNOS and IDO and that the expression of the main antiparasitic effector mechanism for iNOS or IDO may vary among mouse tissues. Among the T cell population, CD8⁺ T cells are considered to be the major effector cells responsible for protection against *T. gondii*, with CD4⁺ T cells playing a synergistic role. Furthermore, the depletion of the total T cell population abrogates protective immunity against toxoplasma challenge in the immunised animals.^[52] CD8⁺ T

Table 1. Protection afforded by immunization with recombinant vaccine candidates against *T. gondii*

Vaccine candidate	Adjuvant	Immunisation	Challenge infection	Result
r SAG-1 <i>E. coli</i>	Alum	Mice, four doses, 2-week intervals, 20 µg SAG1 + 0.5 mg Alum RH strain,	Forty-five days after last dose RH strain,tachyzoites—2 weeks after last dose, cysts SS1119 strain, cysts ^b	Significantly increase in survival days
r SAG-1 <i>E. coli</i>	r-Interleukin-12	Mice, twice a week for 2 weeks, 4 µg r-SAG1 + 4 µg IL-12 ^b	Four weeks after last dose, PRU strain, cysts ^c	Reduced the brain parasitic load by 40%
r-SAG1 <i>P. pastoris</i>	SBAS1	Guinea pigs, three times at 3-week intervals, 10 µg. r-SAG1 + SBAS1 ^b	Three weeks after mating, C56 strain, tachyzoites ^b	Significant protection against materno-foetal transmission
r-P30 Baculovirus	ISCOM	Mice, 0.1, 1.0 and 10.0 µg, r-P30 + ISCOM ^b	After 6 weeks, ZSI strain, tachyzoites ^a	Prolonged the survival period
Plasmid encoding SAG1 gene		Mice, two doses at 0 and on third week, 50 µg ^d	Five weeks after last dose, RH strain tachyzoites	Induced effective protection
Plasmid encoding SAG1	Freund's	Mice, three doses at 0, 3 and 6 weeks, 50 µg at base of tail,	Eight weeks after first immunisation, ME49 strain Cysts ^c	Increased the survival time
		Rats, three doses at 0, 4 and 8 weeks, 50 µg ^d	One hundred and twenty-two days after the first immunisation, veg strain oocysts ^c	Reduced in number of brain cysts

Table 1. Continued on page 10

Table 1. Continued

Vaccine candidate	Adjuvant	Immunisation	Challenge infection	Result
Plasmid encoding GRA4 gene	pGM-CSF or r-interleukin-12	Mice, three doses at 0, 14 and 28 d, 50 µg PGRA4 plus 25 µg	Two weeks after last dose, 76 k strain, cysts ^e	Increased survival time
Plasmid encoding GRA1, GRA7, ROP2		Mice, three times at 3-week intervals, 100 µg ^d	Six or 9 weeks after third dose, IPB or 76 k strain, cysts ^{c or a}	Partial protection
r-BCG producing GRA1 antigen	Freund's	Mice, 5 × 10 ⁷ r-BCG ^a , boosted with r-GRA1-GST ^b with emulsions of 12.5 and 25 µg antigen in Freund's adjuvant	For first and second boosts respectively. 8 weeks after last dose, 76 k, strain, cysts ^c	Conferred limited degree of production

^a Intraperitoneal. ^b Subcutaneous. ^c Oral. ^d Intramuscular.

Source: Girish MB. Development of a vaccine for toxoplasmosis: current status. Microbes and Infection 2003; 5: 457 - 462.

cells from TS-4 vaccinated animals are able to proliferate and secrete IFN- γ in response to recall antigen for a longer duration than the CD4 $^{+}$ T cells from the same mice. Immune CD8 $^{+}$ T cells from both infected mice and humans secrete IFN- γ and exhibit *in vitro* cytotoxicity towards infected cells. The CD4 $^{+}$ Th1 cells exert their protective effect through the production of IFN- γ and IL-2. Conversely, the cytokines produced by CD4 $^{+}$ Th2 cells, such as IL-4, IL-5 and IL-10, are associated with down-regulation of protective cell-mediated immune responses. These T cell subsets are able to cross-regulate their activity. The host immune response blocks the multiplication of tachyzoites, resulting in the formation of cysts containing bradyzoites in all the tissues. A variety of stress conditions include pH shock, heat shock, mitochondrial inhibitors, chemical stress and NO induced bradyzoite formation.^[53]

Control of multiplication of tachyzoites is largely dependent on endogenous INF- γ , with partial involvement of TNFRp55 and iNOS. In contrast, induction of bradyzoite-specific antigen expression and cyst formation during toxoplasmosis seem to be dependent on INF- γ , but independent of TNFRp55 and iNOS functions. The development of large numbers of cysts are controlled by major histo-compatibility complex class I gene, *Ld*, in mice. However, toxoplasma strain-specific differences have been observed. Recurrence of *T. gondii* infection and consequent disease is common in congenitally infected individuals. Immuno-suppressive therapy favours disease reactivation. Murine studies have found that neutralisation of IFN- γ or TNF- α or inhibition of NO production leads to relapse of chronic infection, resulting in active disease with numerous tachyzoites.^[54] Detailed studies are therefore required for the determination of the mechanism of conversion from bradyzoite to tachyzoite.

T. gondii mutant strains, viz., S48, TS-4 and T-203, have been identified as vaccine candidates.^[55] The live tachyzoites of strain S48 have been used as the commercial vaccine for ovine toxoplasmosis. Following subcutaneous inoculation of naive sheep (sero-negative for *T. gondii*) with the commercial vaccine, the parasite multiplies in the local draining lymph node, causing a mild fibrile response. Peak titres of antibody are reached by 6 weeks. The immunity induced by this vaccine is likely to involve both CD4 $^{+}$ and CD8 $^{+}$ T cells and IFN- γ . However, such a vaccine is not suitable for human use due to reactivation to the pathogenic form.

The natural site of infection for *T. gondii* is the mucosal surface of the intestine. Protective immunity obtained after natural infection with *T. gondii* points to the importance of developing a vaccine that stimulates mucosal defence. When lysate of tachyzoites with cholera toxin (mucosal adjuvant) was administered orally, significant protection was noted in mice. The intranasal route of administration of vaccine is more effective than intragastric immunisation, as it generates an earlier, strong mucosal response. When SAG1 plus cholera toxin was administered intranasally, it provided significant protection and reduced the cyst burden. However, cholera toxin cannot be included in vaccine formulation for use in humans due to its toxicity. LTR72 and LTK63 are the two non-toxic mutants of heatlabile enterotoxin which have been used with bacterial antigens and showed protection against different infections after intragastric or nasal vaccination. Mice immunised with SAG1 plus these mucosal adjuvants had significantly fewer cysts. These combinations induced strong

systemic and mucosal humoral response. Salbutamol, currently used as an anti-asthmatic drug, was also tested for its potential adjuvant activity for nasal vaccination.

Mice vaccinated with SAG1 with salbutamol showed significant decrease in cerebral cysts.^[56] Nasal delivery of the antigen SAG1 plus LTR72 as the adjuvant produced a cellular response in local (mesenteric lymph node) and systemic sites. This cellular response was important for protection against infection with *T. gondii*. Now these non-toxic mutants are the most attractive candidates for the development of mucosally delivered vaccine. One of the goals of a vaccination protocol is to be able to appropriately direct the T helper response. In naturally occurring *T. gondii* infection, the Th1 immune response is predominant. Therefore, a vaccination protocol that directs immune response to the Th1 type is desirable.

Significant protection was found with SAG1 with liposome. Liposomes are known to be a particularly effective stimulator of CD8+ T lymphocytes. Vaccination with r-SAG1 produced by *Escherichia coli*, *Pichia pastoris* or an insect cell culture system demonstrated significant protection in experimental animals.^[57]

We have successfully immunised mice against lethal challenge using *E. coli*-expressed r-SAG plus alum induces protective immunity in mice. Immunised mice survive longer than the non-immunised (Figure 3). However, further studies require an adjuvant enhancing specific CTL response, which is believed to be the main mechanism behind protective immunity to *T. gondii*. Immunisation with r-SAG1 plus r IL-12 directed humoral and cellular immunity towards a Th1 pattern and reduced the brain parasite load in mice. Immunisation with r-

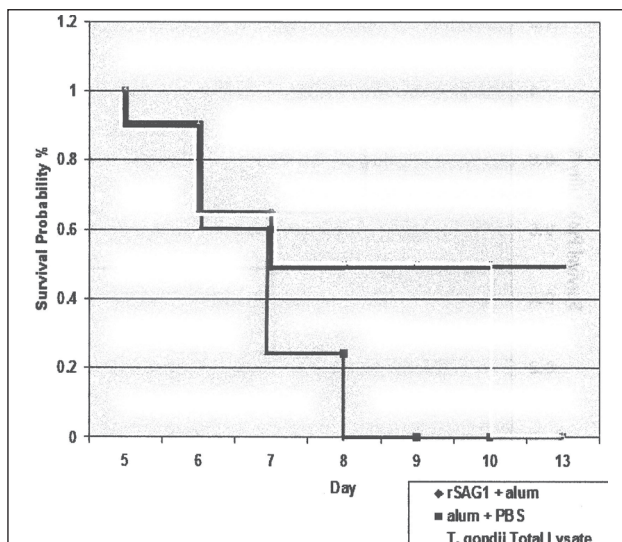


Figure 3. Survival analysis after lethal challenge with *T. gondii* tachyzoites in mice vaccinated with vaccine rSAG1-alum, *T. gondii* tachyzoite lysate and alum only as control.

Source: Data from work funded by Research University Grant Scheme RUGS 04-01-07-030.

SAG1 plus SBAs1 (Th1 response inducer adjuvant) induced significant protection against materno-foetal transmission in guinea pig.^[58] DNA vaccine has been shown to be a powerful method for the induction of specific humoral and cellular immune responses. It is a novel method involving the injection of the naked DNA plasmid into the host, whose cells express the encoded protein. Mice immunised with plasmid encoding the SAG1 gene (Pit SAG1) showed 80–100% protection. It influenced the immune response towards a Th1 type. Furthermore, DNA vaccine encoding SAG1 increased the survival time of animals and reduced the number of brain cysts in rodents.^[55] However, there was a slight increase in survival days of infected mice immunised with pGRA4 or plasmid encoding GRA1, GRA7 and ROP2 or BCG strain secreted GRA1.^[59] Recently, genetically modified skins have been developed, and these grafts produced some therapeutic proteins. The SAG1 gene-vaccinated mouse skin graft was effective for protection against challenge infection.^[60] However, the molecular basis of the gene vaccine effect against *T. gondii* infection remains to be determined.

In conclusion, these vaccine candidates (alone or in cocktail) demonstrated the development of significant protection in animal models. The route of administration of vaccine candidates and use of suitable adjuvants have been shown to be important in eliciting an immune response. Recent progress in characterising potentially protective defence mechanisms during naturally acquired and experimental toxoplasmosis will help to define the immune response which should be elicited by vaccination. Future vaccine design has to account for the stage-specific regulation of immunity against *T. gondii* to prevent abortion and damage as well as to reduce cyst formation in the intermediate host.

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