Toxoplasma vaccines: appropriate end points and sample size in future human clinical trials

Expert Rev. Anti Infect. Ther. 7(8), 905–908 (2009)

Claudio Silveira
Clinica Silveira, Erechim, Rio Grande do Sul, Brazil
Tel.: +55 54 3321 1900
Fax: +55 54 3522 3088
silveira@itake.com.br

Nicola Gargano
R&D Area, Sigma-tau Industrie Farmaceutiche Riunite s.p.a., Rome, Italy
Tel.: +39 06 9139 4042
Fax: +39 06 9139 3988
nicola.gargano@sigma-tau.it

Aize Kijlstra
Department of Ophthalmology, University Hospital Maastricht, Maastricht, The Netherlands
Tel.: +31 320 238 095
Fax: +31 320 238 050
aize.kijlstra@wur.nl

Eskild Petersen, MD, DSc
Author for correspondence
Department of Infectious Diseases, Aarhus University Hospital, Skejby Aarhus, Denmark
Tel.: +45 8949 8307
Fax: +45 8949 8360
eskildp@dadlnet.dk

EXPERT REVIEWS

"...infection with *Toxoplasma gondii* is mostly a poverty-related disease where populations depending on surface water and fresh meat are at a particularly high risk."

Toxoplasma eye disease is a huge problem in certain parts of the world, especially South America and Africa [1,2]. In these regions, infection with *Toxoplasma gondii* is mostly a poverty-related disease where populations depending on surface water and fresh meat are at a particularly high risk [3]. *T. gondii* eye disease is prevalent in tropical Africa, and the data available point to nonarchetypal genotypes dominating in sub-Saharan Africa [4].

The very high prevalence in Brazil can be partly explained by more virulent *T. gondii* genotypes [5], but the infectious pressure may also be more pronounced, especially by a high *T. gondii* infection rate in meat [6]. Current findings indicate that most strains from Brazil do not fit the clonal pattern seen in North America [7]. Other studies suggest that clonal lineages II and III predominate in Africa as in Europe and North America [8,9], and most infections are acquired through food or water in these areas [10,11]. Control of *T. gondii* infections have not been realized by public health measures, ensuring *Toxoplasma*-safe water and meat.

There is a lack of efficient drugs and no new drugs are on the horizon. Most patients acquire the infection during childhood or early adulthood and there is therefore a possibility to immunize people before infection.

Much work has been done on developing vaccines against *T. gondii* [12], but Phase I or II trials in humans are not imminent, as *T. gondii* is not perceived as a large public health problem in industrialized countries, such as the USA and the EU, where congenital toxoplasmosis, but not eye disease, is a concern. Monitoring and treatment of pregnant women and newborns is the current medical practice in these countries [13].

"...there is an urgent need to develop tools that can prevent acquired *Toxoplasma* eye disease."

In Erechim, southern Brazil, 17.7% of the adult population has ocular toxoplasmosis, with 0.9% of children between 1 and 8 years of age being affected, increasing to 21.3% in individuals above 13 years of age [14,15]. These data show that out of a population in Erechim of approximately 2 million people, more than 400,000 will have *T. gondii* eye disease, of which 5% or 20,000 will have unilateral macular lesions casing unilateral blindness, and 1% or 4000 have bilateral macular lesions causing blindness.

In Africa, Toxoplasmosis has been shown to be the cause of uveitis in 52% of cases in Sierra Leone [16] and similar findings were already reported earlier from Nigeria [17]. A study from the London (UK) area showed that the incidence of ocular toxoplasmosis was 100-times higher in black immigrants born in west Africa as compared with subjects born in the UK [18]. Uveitis is a major cause of blindness in west Africa [16], ranking as the second course of blindness in Sierra Leone [19]. The contribution of ocular toxoplasmosis to blindness is high in west

 10.1586/ERI.09.81 ISSN 1478-7210

Africa and, although definitive proof is not yet available, most cases of *Toxoplasma* eye disease from west Africa are considered to be acquired [16].

Thus, there is an urgent need to develop tools that can prevent acquired *Toxoplasma* eye disease. It must be stressed that the available drugs are not able to prevent the development of eye disease as by the time the patient develops symptoms, the retina has already been destroyed.

> "...there has been considerable progress towards the development of *Toxoplasma* vaccines, whose feasibility was suggested by the long-term immunity induced by primary infection."

Vaccination is the most obvious tool for immunizing the population before being infected, and we discuss here the hypothetical impact of a *Toxoplasma* vaccine, administered for instance between the age of 3 and 6 years, in terms of reduction of symptomatic eye disease, requirements of safety and efficacy, appropriate end points and sample size needed. However, for ethical reasons it may be preferred to use an older age group, for example, children 10–12 years of age, but that should be balanced with the problem that some children will be infected with *T. gondii* earlier and have already developed eye disease.

In the last decade, there has been considerable progress towards the development of *Toxoplasma* vaccines, whose feasibility was suggested by the long-term immunity induced by primary infection. However, no vaccines are presently available for humans, but much work has been done on different vaccine prototypes in animal models [12]. The end points of protection against *Toxoplasma* after animal vaccination have mainly been parasite load in tissues, survival and protection against congenital infection. Animal challenge models for vaccines against ocular toxoplasmosis have been limited to a few approaches with tissue cyst-deficient strains [20–22].

A commercially available attenuated vaccine strain is used in sheep to prevent abortion due to *T. gondii* infection and has shown a protective efficacy of approximately 75%, but many side effects occur and protection lasts approximately 18 months [23]. Sterile immunity has been obtained in pigs immunized with a cyst-deficient *T. gondii* strain [24], and a vaccine based on a *T. gondii* uracil pathway knockout strain showed 100% protection against lethal challenge in mice [25].

Subunit vaccines have the advantage of being pure, safe and well defined. Up to now, such vaccines have been able to induce up to 90% protective efficacy in mice against selected *Toxoplasma* strains, mostly of genotype II [12,26]. It has recently been shown that subunit vaccines having a high protective efficacy against an avirulent *T. gondii* genotype II showed no protection against a more virulent genotype III strain [26].

Subunit vaccines should demonstrate a high efficacy against all genotypes, but to date no vaccines have been shown to do so, and thus far subunit vaccines need to be much improved before they can enter clinical trials [25].

There is a need for preclinical development of *Toxoplasma* vaccines to achieve protection against ocular toxoplasmosis caused by south American and west African *T. gondii* isolates. However, no effective subunit vaccines have yet been developed against the apicomplexan parasites *T. gondii* and *Plasmodium falciparum*.

Given the problem with recombinant subunit vaccines, attenuated vaccines are more promising. In a model of ocular toxoplasmosis, vaccination with an attenuated ts-4 *Toxoplasma* strain could induce both ocular pathology and ocular protective immunity [23]. Attenuated vaccines may have more side effects compared with subunit vaccines and there is a theoretical risk of the vaccine strain reverting to wild-type parasites. Given the very high burden of disease, the theoretical risk of reversal to wild-type should be balanced by the very high burden of impaired eyesight in communities in southern Brazil and probably tropical Africa. A recent study of an attenuated *P. falciparum* vaccine showed excellent protection and this illustrates that attenuated vaccines can induce protective immunity in infections with apicomplexan parasites [27].

It should be taken into consideration that eliminating ocular disease might not necessarily mean a vaccine conferring 100% sterile immunity. A vaccine that can prime the immune system and reduce the cyst burden in the host may prevent *T. gondii* eye disease. The protective efficacy of a vaccine against *T. gondii* eye disease should thus be related to the burden of disease but not to the infection itself. Therefore, a new correlate of protection – an immunological threshold above which an individual is protected – would probably need to be defined for protection against ocular toxoplasmosis.

> "It should be taken into consideration that eliminating ocular disease might not necessarily mean a vaccine conferring 100% sterile immunity."

In particular, ELISAs based on the vaccine antigen and assays investigating cellular immunity induced by the vaccine would be valuable tools for the determination of vaccine uptake and evaluation of the induced cytokine profile, respectively. Although the presence of a humoral immune response is the usual marker for protection, immunity to *T. gondii* is intimately correlated with the generation of cellular immune responses, in particular IFN-γ-producing T cells.

Currently, the correlate of protection against ocular toxoplasmosis is not known. An ELISA based on the vaccine antigens would need to be developed, with a seroprotective cut-off in terms of IgG titer, to define the hypothetical antibody level that should confer protection. On the other hand, protection may be correlated to the presence and/or absence of certain cytokines produced by Th1, Th2, regulatory T cells or Th17 cells [28–30].

From studies carried out in Erechim, it is known that 99% of the Brazilian population is seronegative before 8 years of age and that 21.3% has symptomatic eye disease above 16 years of age [14,15]. Also, an estimated 2% of the population develops *T. gondii* eye disease every year, after 8 years of age, reaching a plateau of approximately 20% along adulthood. Consequently,

immunization before the age of 8 years with an effective vaccine with long-lasting protection would prevent symptomatic eye infection in 20% of the population.

In a hypothetical model of a clinical trial enrolling 1000 seronegative children under 8 years of age and following them along childhood and adolescence, without any intervention, 20 subjects will develop *Toxoplasma* eye disease every year for the next 8 years until reaching a prevalence of 200 per 1000 with symptomatic eye disease at 16 years of age. Immunizing 1000 seronegative children under 8 years of age with a 100% effective vaccine would therefore prevent 20 cases of *T. gondii* chorioretinitis per year per 1000 population.

Table 1 shows that immunizing 1000 persons and assuming that 2% develop *Toxoplasma* eye disease per year, a study enrolling 1000 subjects in each group (intervention and control) has the power to detect a 45% reduction in *Toxoplasma* eye disease (20 cases in the control group and 11 cases in the intervention group).

With a vaccine regimen at young age to protect against ocular toxoplasmosis, we may hypothesize that a protection of approximately 10 years of age would ideally be followed by a booster regimen at approximately 12 years of age to extend protection in individuals up to 20 years of age. In south American and west African countries, seropositivity can be as high as 90% and it is thus likely that the majority of pregnant women are protected at child-bearing age. Therefore, the primary focus of the protective capacity of the vaccine will be on ocular disease in adolescents, and may be followed by protection against congenital disease in women who remained free of *Toxoplasma* infection.

Table 1. Sample size calculation.

n	P1	P2	SD	p
500	10	2	1.62	0.052
750	15	4	2.21	0.013
1000	20	5	3.01	0.001
1000	20	10	2.01	0.022
1000	20	11	1.81	0.035
1000	20	12	1.61	0.053

n: Sample size in each group (immunization and control groups); p: p-value, assuming a binominal distribution; P1: Number of retinochoroiditis cases in the control group; P2: Number of retinochoroiditis cases in the intervention group; SD: Standard deviation.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

1 Jones JL, Muccioli C, Belfort R Jr *et al.* Recently acquired *Toxoplasma gondii* infection, Brazil. *Emerg. Infect. Dis.* 12, 582–587 (2006).

2 Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int. J. Parasitol.* 39(12), 1385–1394 (2009).

3 Bahia-Oliveira LM, Jones JL, Azevedo-Silva J *et al.* Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil. *Emerg. Infect. Dis.* 9, 55–62 (2003).

4 Ajzenberg D, Yera H, Marty P *et al.* Genotype of 88 *Toxoplasma gondii* isolates associated with toxoplasmosis in immunocompromised patients and correlation with clinical findings. *J. Infect. Dis.* 199, 1155–1167 (2009).

5 Khan A, Jordan C, Muccioli C *et al.* Genetic divergence of *Toxoplasma gondii* strains associated with ocular toxoplasmosis, Brazil. *Emerg. Infect. Dis.* 12, 942–949 (2006).

6 Belfort-Neto R, Nussenblatt V, Rizzo L *et al.* High prevalence of unusual genotypes of *Toxoplasma gondii* infection in pork meat samples from Erechim, southern Brazil. *An. Acad. Bras. Cienc.* 79, 111–114 (2007).

7 Khan A, Jordan C, Muccioli C *et al.* Genetic divergence of *Toxoplasma gondii* strains associated with ocular toxoplasmosis, Brazil. *Emerg. Infect. Dis.* 12, 942–949 (2006).

8 Velmurugan GV, Dubey JP, Su C. Genotyping studies of *Toxoplasma gondii* isolates from Africa revealed that the archetypal clonal lineages predominate as in North America and Europe. *Vet. Parasitol.* 155, 314–318 (2008).

9 Sousa S, Ajzenberg D, Vilanova M *et al.* Use of GRA6-derived synthetic polymorphic peptides in an immunoenzymatic assay to serotype *Toxoplasma gondii* in human serum samples collected from three continents. *Clin. Vac. Immunol.* 15, 1380–1386 (2008).

10 Cook AJ, Gilbert RE, Buffolano W *et al.* Sources of *Toxoplasma* infection in pregnant women: European multicentre case–control study. *Br. Med. J.* 321, 142–147 (2000).

11 Bahia-Oliveira LM, Jones JL, Azevedo-Silva J *et al.* Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro state, Brazil. *Emerg. Infect. Dis.* 9, 55–62 (2003).

12 Jongert E, Roberts CW, Gargano N, Förster-Wald E, Petersen E. Vaccines against *Toxoplasma gondii*: challenges and opportunities. *Mem. Inst. Oswaldo Cruz.* 104, 252–266 (2009).

13 SYROCOT Study Group. Individual patient data meta-analysis of prenatal treatment effect for congenital toxoplasmosis. *Lancet* 369, 115–121 (2007).

14 Glasner PD, Silveira C, Kruszon-Moran D *et al.* An unusually high prevalence of ocular toxoplasmosis in southern Brazil. *Am. J. Ophthalmol.* 114, 136–144 (1992).

15 Silveira C, Belfort R Jr, Muccioli C *et al.* A follow-up study of *Toxoplasma gondii* infection in southern Brazil. *Am. J. Ophthalmol.* 131, 351–354 (2001).

16 Ronday MJ, Stilma JS, Barbe RF *et al.* Aetiology of uveitis in Sierra Leone, west Africa. *Br. J. Ophthalmol.* 80, 956–961 (1996).

17 Ayanru JO. The problem of uveitis in Bendel State of Nigeria: experience in Benin City. *Br. J. Ophthalmol.* 61, 655–959 (1977).

18 Gilbert RE, Stanford MR, Jackson H, Holliman RE, Sanders MD. Incidence of acute symptomatic toxoplasma retinochoroiditis in south London according to country of birth. *Br. Med. J.* 310, 1037–1040 (1995).

19 Ronday MJ, Stilma JS, Barbe RF, Kijlstra A, Rothova A. Blindness from uveitis in a hospital population in Sierra Leone. *Br. J. Ophthalmol.* 78, 690–693 (1994).

20 Gazzinelli RT, Brézin A, Li Q, Nussenblatt RB, Chan CC. *Toxoplasma gondii*: acquired ocular toxoplasmosis in the murine model, protective role of TNF-α and IFN-γ. *Exp. Parasitol.* 78, 217–229 (1994).

21 Lu F, Huang S, Hu MS, Kasper LH. Experimental ocular toxoplasmosis in genetically susceptible and resistant mice. *Infect. Immun.* 73, 5160–5165 (2005).

22 Lu F, Huang S, Kasper LH. The temperature-sensitive mutants of *Toxoplasma gondii* and ocular toxoplasmosis. *Vaccine* 27, 573–580 (2009).

23 Buxton D, Thomson K, Maley S *et al.* Vaccination of sheep with a live incomplete strain (S48) of *Toxoplasma gondii* and their immunity to challenge when pregnant. *Vet. Rec.* 129, 89–93 (1991).

24 Kringel H, Dubey JP, Beshah E *et al.* CpG oligodeoxynucleotides enhance porcine immunity to *Toxoplasma gondii*. *Vet. Parasitol.* 123, 55–66 (2004).

25 Fox B, Bzik D. *De novo* pyrimidine biosynthesis is required for virulence of *Toxoplasma gondii*. *Nature* 415, 926–929 (2002).

26 Rosenberg C, De Craeye S, Jongert E *et al.* Induction of partial protection against infection with *Toxoplasma gondii* genotype II by DNA vaccination with recombinant chimeric tachyzoite antigens. *Vaccine* 27, 2489–2498 (2009).

27 Roestenberg M, McCall M, Hopman J *et al.* Protection against a malaria challenge by sporozoite inoculation. *N. Engl. J. Med.* 361, 468–477 (2009).

28 Bahia-Oliveira LM, da Silva JA, Peixoto-Rangel AL *et al.* Host immune response to *Toxoplasma gondii* and *Ascaris lumbricoides* in a highly endemic area: evidence of parasite co-immunomodulation properties influencing the outcome of both infections. *Mem. Inst. Oswaldo Cruz* 104, 273–280 (2009).

29 Lahmar I, Abou-Bacar A, Abdelrahman T *et al.* Cytokine profiles in toxoplasmic and viral uveitis. *J. Infect. Dis.* 199, 1239–1249 (2009).

30 Garweg JG, Candolfi E. Immunopathology in ocular toxoplasmosis: facts and clues. *Mem. Inst. Oswaldo Cruz* 104, 211–220 (2009).