



Toxoplasma gondii: Protection against colonization of the brain and muscles in a rat model

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ABSTRACT

The objective was to test immune protection against the formation of *Toxoplasma gondii* tissue cysts in rats. It has been previously shown that 50 *T. gondii* tissue cysts of strain Me49 are not pathogenic for CF-1 mice, whereas 1 *T. gondii* tissue cyst of strain M-7741, can be lethal for mice 11–13 days after subcutaneous or oral administration. In the present study, ten rats were fed *T. gondii* oocysts of strain Me49 and after a further 30 days they were each orally challenged with *T. gondii* oocysts of strain M-7741. Thirty days after this, they were euthanased and brain and muscle samples inoculated subcutaneously or orally dosed, respectively, to mice for bioassay. None of the mice died, whereas all the mice that were inoculated with brain homogenates or were fed muscle samples from four non-immunized rats that had been inoculated with *T. gondii* oocysts of strain M-7741, died. These results encourage further research towards achieving vaccinal protection against the formation of *T. gondii* tissue cysts in meat animals and people.

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1. Introduction

Toxoplasmosis can cause fetal damage in humans and abortion in sheep, goats, pigs, and rabbits following a primary infection during pregnancy (Dubey and Beattie, 1988; Remington and Desmonts, 1990). The incidence of human congenital toxoplasmosis has been shown to be 1–6/1000 births. Although most infected newborns are asymptomatic at birth, adverse sequelae may develop later in life in a large proportion of affected patients (Remington and Desmonts, 1990). In addition, reactivation of a latent *Toxoplasma gondii* infection from tissue cysts in the brain, is often fatal in patients who become immunosuppressed as a result of developing acquired immunodeficiency syndrome (AIDS), during cancer therapy or after organ transplantation (Luft and Hafner, 1990).

Sheep meat is a potential source of toxoplasma infection for people, if eaten after insufficient cooking. Viable toxoplasma tissue cysts were found in edible muscle tissue in all of 8 lambs examined, and they were shown to be viable after at least 15 days frozen (Dubey and Kirkbride, 1989). In another experiment in which sheep were orally dosed with toxoplasma oocysts, tissue cysts were shown to be present in muscle for at least four months (Dubey and Sharma, 1980). There are at least two reviews of isolation of *Toxoplasma* from mutton in several countries (Freyre and Falcon, 1989; Dubey and Beattie, 1988). *Toxoplasma* has been isolated from muscles of sheep with specific antibodies, naturally infected with

the parasite (Jacobs et al., 1963). In another study the prevalence of toxoplasma antibody in 1613 ewes from 18 farms in Uruguay was 28.7%, with a range of 1.6–72.7% (Freyre et al., 1999), and this is in keeping with the observation that infection of sheep with *Toxoplasma* is common worldwide (Dubey and Beattie, 1988; Freyre and Falcon, 1989).

Pork is also considered to be a potential source of *Toxoplasma* for humans. Viable tissue cysts in swine may be widely disseminated and persist in tissues for more than two years (Dubey et al., 1984), and the infection in this species is widely disseminated: Dubey et al. (1991) found, in a study on the seroprevalence of toxoplasma antibodies in swine in several states in the USA, that about 25% of the herds studied had been exposed to the parasite. More detailed information on the toxoplasma infection of pigs in the US has been given more recently (Dubey et al., 2002). A quarter of the world swine herd is located in Brazil, where toxoplasmosis prevalence varies from 9.6% to 54% (Suaréz-Aranda et al., 2000) and in a survey in Uruguay 70% of pigs were seropositive for *Toxoplasma* (Freyre et al., 1991).

In a vaccination study in pigs, after challenge with oocysts of *T. gondii*, the parasite was recovered from samples of the brain, liver, retina and tongue of four of nine control non-immunized animals, and from none of nine pigs given an experimental toxoplasma-IS-COM vaccine, and from one of nine animals inoculated with the RH strain of the parasite before challenge (Freire et al., 2003). More recently, Garcia et al. (2005) recovered *Toxoplasma* from heart, diaphragm, masseter and tongue from all of 10 control non-immunized pigs, from three of 10 pigs immunized with the RH strain and

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from eight of 10 pigs vaccinated with crude rhoptry toxoplasma proteins incorporated in ISCOMs, after challenge with 4×10^4 oocysts of the VEG strain by the oral route. In previous experiments of premunition against acquired toxoplasmosis in a rat model (Freyre et al., 2006), protection conferred by two strains of *Toxoplasma* against the development of tissue cysts in the brain was tested, and a significant reduction was obtained with one of them. However, tissue cyst numbers in muscle tissue was not sought (Freyre et al., 2006).

Aside from these isolated experiments, to date no vaccination strategy exists for preventing *T. gondii* tissue cyst formation in humans or in animals. This sort of protective effect is highly desirable in order to protect immunosuppressed patients and to diminish the availability of meat infected with *Toxoplasma* for the population in general. Non-infected humans would not succumb to toxoplasmic encephalitis, since most cases of toxoplasmic encephalitis develop as a consequence of the reactivation of a previous latent infection with the parasite, rather than as a consequence of a primary toxoplasmic infection during immunodepression (Luft and Hafner, 1990).

The objective of the present investigation was to test whether immunity could be induced to prevent *T. gondii* tissue cyst formation in rat muscle. Muscle colonization in rats after oral inoculation with cysts and oocysts of strains Me49, M3 and M-7741 of *Toxoplasma*, was investigated. Me49 is the strain previously used by the authors to immunize rats (Freyre et al., 2006). Strains M3 and M-7741 of *Toxoplasma* were used later for heterologous challenge. In addition, it was determined that 50 cysts of the Me49 strain were not pathogenic for mice when subcutaneously (s.c.) (Freyre et al., 2006) or orally (present work) inoculated, and that 1 cyst of the M-7741 strain, of rat origin, was lethal for mice 11–13 days after s.c. or oral inoculation (present work). At least five cysts of strain M3 were necessary to achieve similar results by the s.c. (Freyre et al., 2006) or the oral (present work) routes. In these conditions, the results (survival or death) of the mice that received the brain homogenates and muscle of the rats immunized with strain Me49 and then challenged with strain M-7741 would indicate the rate of protection achieved. After the results of these previous experiments were known, a prototype experiment in rats was performed, to test muscular protection against *Toxoplasma*.

2. Materials and methods

2.1. Experimental design

Groups of 3–10 rats were fed cysts or oocysts of toxoplasma strains Me49, M3 or M-7741. After 30 days, the animals were killed. *Toxoplasma* tissue cysts present in one cerebral hemisphere from each rat, were counted. When cysts were not seen under the microscope, the other cerebral hemisphere was bioassayed in mice and one of the hindlimbs was skinned and fed to another group of mice for bioassay.

In the immunization assay with rats, ten rats were fed oocysts of strain Me49 of *Toxoplasma* and were orally challenged with oocysts of strain M-7741, 30 days later. Thirty days after challenge, the rats were killed and a homogenized cerebral hemisphere was inoculated in each of two mice and a hindlimb was fed to 4 mice per rat. Four rats that were not immunized but each received the same challenge inoculum by the same route, served as controls.

2.2. Animals

Sprague–Dawley rats, each weighing in the region of 150 g were purchased from Taconic, Hudson, NY, while CF-1 mice weighing around 20 g were sourced from Charles River Laboratories, Boston,

MA. Both mice and rats were judged to be free of toxoplasma infection, as they were seronegative for *T. gondii* by the direct agglutination (DA) test (Desmonts and Remington, 1980), using 1/64 as the threshold titer indicative of toxoplasma infection. The same test was used to look for toxoplasma seropositivity, in mice that survived after being given rat brain homogenate or muscle tissues.

2.3. Strains of *Toxoplasma*

We have used *T. gondii* strain Me49 in previous experiments, looking for protection against cerebral infection of rats with the parasite (Freyre et al., 2006). Strains M-7741 and M3 were used for the first time in this study to look for evidence of tissue cysts in muscle, to allow the use of heterologous challenge strains. Strain M-7741 is highly pathogenic for mice (1 zoite kills a mouse) and belongs to genotype III of the parasite (Howe and Sibley, 1995), and strain Me49 belongs to genotype II, as does strain M3 (M.L. Dardé, personal communication to A. Freyre), and the latter was isolated from a case of sheep abortion in Scotland (Buxton, 1991).

2.4. *Toxoplasma gondii* tissue cysts and oocysts

Toxoplasma tissue cysts and oocysts were maintained and prepared as already described (Freyre et al., 2001; Freyre et al., 2003, respectively).

2.5. Inoculation of rats and mice

Twenty-one rats were each fed with 10^3 cysts, and 8 and 20 rats were each fed 10^4 and 10^5 oocysts, respectively, of strain *T. gondii* Me49, respectively (Table 1). A further 6 and 10 rats each received orally 10^2 and 10^3 oocysts of strain M3, respectively, and another 9 rats each received 10^4 oocysts of strain M-7741 (Table 2).

2.6. Enumeration of brain cysts

To enumerate tissue cysts in the brain, a given cerebral hemisphere was divided into three portions. Squash preparations were made with each of the portions, between two glass slides 5×5 cm locally manufactured upon request (Vidriería Deolindo y G Bia, Montevideo). They were screened for tissue cysts under a magnification of $100\times$. When tissue cysts were not observed, the entire other hemisphere was used altogether for bioassay in mice.

2.7. Bioassays

A given cerebral hemisphere homogenized with 1 ml of sterile saline with 1000 IU penicillin and 0.1 mg streptomycin in a

Table 1

Brain and muscle colonization in rats after being fed cysts and oocysts of strain Me49 of *Toxoplasma*

Stage and dose	Brain colonization ^a	Muscle colonization ^a
10^3 cysts	14/21 ^b	17/21
10^4 oocysts	8/8 ^c	8/8
10^5 oocysts	20/20 ^d	20/20

^a No. of rats infected with *Toxoplasma*/total No. of rats studied. For No. of rats with brain *Toxoplasma* infection, positive results in the bioassays, of microscopically negative brains, were added to microscope observation of brain cysts.

^b No. of cysts: 4, 6, 4, 5, 58, 32, 180, 24, 4, 4, 60 (average: 32 cysts).

^c No. of cysts: 304, 100, 500, 12, 12, 200, 650, 150, 150, 80, 10, 6, 42, 0, 52, 2, 194 (average: 145 cysts).

^d No. of cysts: 490, 70, 140, 1800, 2070, 70 1120, 350 (average: 764 cysts).

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