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Toxoplasma gondii: Cross-immunity against the enteric cycle

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Abstract

Eight of nine cats inoculated with strain ME-49 and challenged with three different strains of *Toxoplasma* were immune to oocyst shedding, as ascertained with bioassays of their feces. In a second experiment, only toxoplasma asexual stages were seen in H&E stained gut sections of cats treated with suppressive doses of sulfamerazine and pyrimethamine starting 2 days after oral inoculation with cysts of the strain ME-49 and killed 6 days later. In a third experiment, four cats were similarly inoculated and treated for 20 days. Six weeks later, the cats received an oral homologous challenge with cysts, and none shed toxoplasma oocysts. An acceptable level of cross-protection was achieved with strain ME-49, and therefore, it can be used as a candidate strain from which antigens could be tested for enteric protection.

Index Descriptors: Toxoplasma; Cross-immunity; Enteric cycle

1. Introduction

Toxoplasmosis can cause fetal damage in humans and abortion in sheep, goats, pigs, and rabbits if first contracted during pregnancy (Dubey and Beattie, 1988; Remington and Desmonts, 1990). The incidence of congenital human 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 the affected patients (Remington and Desmonts, 1990). In addition, reactivation of a latent *Toxoplasma gondii* infection from brain cysts is often fatal in patients who are immunosuppressed in the course of acquired immunodeficiency syndrome (AIDS), during cancer therapy or after organ transplantation (Luft and Hafner, 1990).

Toxoplasma gondii is a common protozoan parasite of cats, which are the definitive host of the parasite. When cats become acutely infected with *Toxoplasma gondii* by eating a chronically infected rodent or bird, raw mutton or pork, the sexual cycle begins in the gut. A few days later, many thousands or millions of oocysts are shed in the feces. After a few days in temperate climate, oocysts sporulate and form eight sporozoites, the infective stage. Oocysts can persist in the environment for up to 2 years and are a health hazard for both humans and animals (Dubey and Beattie, 1988).

There is a vaccine to prevent cats from toxoplasma oocyst shedding (Frenkel et al., 1991; Freyre et al., 1993; Choromanski et al., 1994, 1995), that affords a high rate of protection. Since it is a live vaccine, it needs a cold chain for distribution, which is not practical and/or of low benefit for the potential manufacturer. To overcome this difficulty, a second generation (subunit) cat vaccine is desirable.

Several problems would have to be solved for the design of such a vaccine. For instance, there is a need for more knowledge on cross-protection among toxoplasma strains against the enteric cycle in the cat. Concerning homologous challenge of cats against the enteric cycle (Table 1), Dubey and Frenkel (1974) found that of 28 cats younger than 13 weeks when first inoculated with strain M-7741, 17 re-shed oocysts after homologous challenge, whereas none of 15 cats older than 13 weeks did so on reinoculation. However, Kühn and Weiland (1969), found that one of five adult cats

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Table 1
Previous research on homologous and heterologous challenge of cats against the enteric cycle of *T. gondii*

T. gondii strain	Results[a]		Reference
Homologous challenge			
M-7741	17/28[b]		Dubey and Frenkel (1974)
M-7741	15/15[c]		Dubey and Frenkel (1974)
[f]	4/5[d]		Kühn and Weiland (1969)
[f]	5/5[e]		Kühn and Weiland (1969)
T-263	24/24		Freyre et al. (1993)
M-7741	12/13		Frenkel and Smith (1982)
T. gondii immunizing strain	*T. gondii* challenge strain	Results[a]	Reference
Heterologous challenge			
Aldrin, Gail	M-7741	0/3	Dubey and Frenkel (1974)
M-7741	Aldrin	1/1	Dubey and Frenkel (1974)
M-7741	Gail	11/13	Frenkel and Smith (1982)
Ts-2	ME-49	3/3	Dubey (1995)

[a] Number of cats protected/number of cats immunized.
[b] Cats were younger than 13 weeks when immunized.
[c] Cats were older than 13 weeks when immunized.
[d] Challenge performed 5 weeks after immunization.
[e] Challenge performed 12 weeks after immunization.
[f] Name of strain not available.

re-shed oocysts when challenged 5 weeks after primary infection, but none of five cats did so after a second challenge with the same strain 12 weeks after primary infection. All of 24 cats, 9–11 weeks of age, were protected against shedding of oocysts, when vaccinated with the T-263 mutant vaccine strain of *Toxoplasma*, and challenged with M-7741 strain, that gave origin to strain T-263 (Freyre et al., 1993). Frenkel and Smith (1982) found that 12 of 13 cats were immune when first inoculated with the M-7741 strain and challenged with the same strain 28–62 days later.

With regards to heterologous challenge in the cat, there is limited evidence that some strains could have a broader range of protection than others (Table 1). For instance, three adult cats inoculated with cysts of the Aldrin or Gail strains of *Toxoplasma* excreted oocysts, and then all of them reexcreted oocysts after challenge with the M-7741 strain 3–6 weeks later (Dubey and Frenkel, 1974). Conversely, one cat primary inoculated with the M-7741 strain, was immune when challenged with the Aldrin strain 6 weeks later (Dubey and Frenkel, 1974), but 11 of 13 cats were immune when immunized twice with the same strain and challenged 71–116 days later with the Gail strain (Frenkel and Smith, 1982). Three cats 4–6 months old, inoculated with the ts-2 strain, were all immune when challenged with strain ME-49, 40 days after primary inoculation (Dubey, 1995).

There is also a need for knowledge on whether asexual, sexual stages or both, are necessary for protection against the intestinal cycle. Frenkel and Smith (1982) determined that the enteric cycle can be terminated by the administration of sulfadiazine and pyrimethamine given to cats 2 days before feeding them with toxoplasma cysts to 1 day after inoculation. Oocysts were not shed; however, the animals became immune and did not shed oocysts when challenged with Toxoplasma cysts some weeks later. *Toxoplasma* stages responsible for immunity were not identified, however.

The objectives of the present work were: (1) to test cross-immunity against the enteric cycle of *Toxoplasma* and (2) to determine the toxoplasma stages responsible for gut immunity in the cat.

2. Materials and methods

2.1. Experimental designs

2.1.1. Experiment 1

In Experiment 1, cross-immunity against the enteric cycle was tested. Nine cats were fed cysts of toxoplasma strain ME-49. Oocyst shedding was tested 3–15 days later. After 6 weeks, oocyst shedding was tested for 3 consecutive days, and the next day, the cats were challenged with three strains of *Toxoplasma*. Oocyst shedding was tested again, as above. When feces were microscopically negative, they were processed by a method to concentrate toxoplasma oocysts, left for 4–5 days at room temperature for sporulation, and concentrates were bioassayed for *Toxoplasma* in mice. Another three cats were fed toxoplasma cysts of the challenge strains, to serve as controls of oocyst shedding. Their feces were screened for toxoplasma cysts, 3–15 days after inoculation (Table 2).

2.1.2. Experiment 2

The aim of the first part of Experiment 2, was to determine the toxoplasma stages responsible for immunity against the enteric cycle in the cat. Eight cats were fed a large inoculum of toxoplasma cysts of strain ME-49 (day 0), and were medicated with sodium sulfamerazine (Sigma Co.) and pyrimethamine (Daraprim, GlaxoWellcome). Four medication timeschedules were used in relation to inoculation, each in two cats (Frenkel and Smith, 1982), (see Section 2.2.1). The cats were killed 6 days after inoculation with *Toxoplasma*, at which time sexual stages are

Table 2
Experiment 1: Cross-immunity in 9 cats fed 3000 cysts of strain ME-49 of *Toxoplasma* and challenged 6 weeks later with 1000 cysts of three strains of *Toxoplasma*, by mouth

Moment of screening for oocysts	Challenge strain M-7741			Control cat	Challenge strain Prugniaud			Control cat	Challenge strain M3			Control cat
Cat no.	1	2	3	4	5	6	7	8	9	10	11	12
Oocyst shedding[a]												
3–15 days after immunization	+	+	+	NA[b]	+	+	+	NA	+	+	+	NA
3 days pre-challenge	–	–	+[c]	NA	–	–	–	NA	–	–	–	NA
3–15 days after challenge	–	+[c]	–	+	–	–	–	+	–	–	–	+

[a] +, oocyst shedding; –, no oocyst shedding.
[b] Not applicable.
[c] Oocyst shedding only apparent at bioassay.

known to be present in the highest numbers (Dubey and Frenkel, 1972) and so, they would be readily detected by microscopy. One more (unmedicated) cat was fed a similar inoculum of toxoplasma cysts and was killed 6 days later. Fecal samples of the nine cats in this part of the Experiment 2 were screened for toxoplasma oocysts on days 3–6 after inoculation. *Toxoplasma* stages were screened for in H&E stained sections of the jejunum and ileum. Approximately 25 sections from different parts of the small intestine of each cat were screened for *toxoplasma* stages. After learning if any toxoplasma stages were present in the gut of the medicated cats, another four cats were inoculated with toxoplasma cysts of strain ME-49 and medicated for 20 days, starting 2 days after inoculation with *Toxoplasma*, in the second part of Experiment 2. Six weeks later, the four cats were orally challenged with 200 cysts of the same toxoplasma strain. Simultaneously, a unimmunized cat received a similar inoculum of toxoplasma, to serve as control of oocyst shedding. Fecal samples of the five cats of the second part of this experiment, were tested for toxoplasma oocysts starting 3 days after challenge until 15 days after challenge (immunized cats), or after inoculation (control cat), and bioassayed in mice if negative at microscopy.

2.1.3. Animals

Cats of the European breed were used, because it is the most common breed in most countries. They were weaned cats of both sexes, 6 weeks old, obtained from the breeding colony of the Laboratory for Toxoplasmosis, College for Veterinary Sciences of Montevideo.

The queens and their kittens were fed dried commercial cat food ("Gati," Cargill, Bs. As. Argentina), and were screened for *isospora* and *toxoplasma* oocysts three times during pregnancy and during the 2 weeks that preceeded the inoculation with *Toxoplasma*, respectively. A total of 24 cats were used. Twenty gram CF-1 mice were used. Cats and mice were free of toxoplasma infection, as ascertained with the direct agglutination (DA) reaction for toxoplasmosis of Desmonts and Remington (1980).

2.1.4. Toxoplasma strains

Strain ME-49 was used to immunize cats, because it is a world wide known complete strain of *Toxoplasma*. Strains Prugniaud, M-7741 and M3 were used as challenge strains. Strains ME-49 and Prugniaud belong to genotype II of *Toxoplasma*, and strain M-7741 to genotype III (Howe and Sibley, 1995). The strain M3 has a genotype II (M.L. Dardé, personal communication). These strains have been characterized by Freyre et al. (2001).

2.1.5. Toxoplasma cysts

Cysts were obtained from the brains of mice with chronic toxoplasma infections, that had been inoculated at least 1 month before. Thirty mg % sulfamerazine in drinking water was used preventively 3–15 days after inoculation of mice with the pathogenic toxoplasma strains M-7741 and M3. Brains of donor mice were homogeneized by passing a mouse brain and 1 cc of 0.9% NaCl solution 12 times through a 19 gauge needle inserted in a 3 cc hypodermic syringe. Cysts were counted in 25 µl aliquots placed on a slide with a coverslip, at 100×.

2.2. Feeding of cats with Toxoplasma cysts

Cats were each fed approximately 1000 brain cysts of *Toxoplasma* in Experiment 1 and in challenge inocula of the second part of Experiment 2. Greater immunizing inocula (3000 brain cysts) were chosen for inoculation in the first part of Experiment 2, to be able to easily detect toxoplasma stages in gut sections.

2.2.1. Medication of cats

In Experiment 2, cats were medicated with 60 mg/kg sodium sulfamerazine and 1 mg/kg pyrimethamine. Due to the low palatability of the drugs used, they were mixed with sardine. The medication was started on days −2, 0, 1, and 2 in relation to inoculation with *Toxoplasma*, and sustained until the cats were killed, 6 days after inoculation, in the first part of this experiment. Two cats were used for each timeschedule. In the second part of Experiment 2, four cats were medicated 2 days after inoculation with *Toxoplasma*, for 20 days.

2.2.2. Search for oocysts of Toxoplasma

Fecal samples were concentrated by the method of Frenkel (1977) and screened under the microscope at 200× for toxoplasma oocysts. Concentrates that were negative at

microscopy, were mixed 1:5 with 2% sulphuric acid in tap water and agitated in an orbital shaker at room temperature (20 °C) for 4–5 days. Resulting fecal emulsions were centrifuged 10 min at 2000 rpm. Pools were made with the feces of each cat that were shed on three consecutive days, neutralized with 3.3% sodium hydroxide and fed to mice. Four mice per pool were inoculated. Mice were screened with the DA reaction for toxoplasma antibodies 25 days later.

3. Results

Results of Experiment 1 are shown in Table 2. All of nine kittens fed cysts of strain ME-49 for the first time, shed toxoplasma oocysts. Only cat no. 3 shed oocysts during the 3 days previous to challenge, judging from a positive bioassay result. After challenge with three different strains of *Toxoplasma*, one of the nine cats shed toxoplasma oocysts, as ascertained with bioassay. The other eight cats were immune to *Toxoplasma* shedding. The three control cats shed oocysts after an inoculation with cysts of the challenge strains. Regarding the first part of Experiment 2, toxoplasma sexual and asexual stages were seen in gut sections of the unmedicated control cat inoculated with toxoplasma cysts, which shed *toxoplasma* oocysts. Conversely, only asexual stages were seen in the sections of the gut of the cats medicated 2 days after inoculation with *Toxoplasma*. None of the four cats inoculated with *Toxoplasma*, medicated with the same drugs and doses starting 2 days after inoculation for 20 days and then challenged in the second part of this experiment, shed oocysts. Oocysts were shed by the control cat inoculated simultaneously with the challenge inoculation of the immunized cats.

4. Discussion

Cross-protection among the immunizing strain ME-49 and three challenge strains used in the present work, was not complete in terms of the proportion of cats that were protected. One of three cats was not immune when challenged with strain M-7741 of *Toxoplasma*, although oocysts shedding was only apparent after bioassay of the feces shed on days 4–6 after challenge. The remaining eight cats became immune, however. The results obtained are in agreement with previous research, as discussed in Section1, in that absolute protection among different toxoplasma strains against the enteric cycle in the cat cannot always be expected. Although more cats could be tested for immunity, and more strains could be used for challenge, it seems reasonable to consider that an acceptable degree of cross-immunity was achieved with strain ME-49, and therefore, it can be used in future assays as a candidate strain from which antigens may be tested for protection against the enteric cycle of *Toxoplasma*.

Sexual stages were not seen in the gut sections of cats inoculated with *Toxoplasma* and treated with sulfamerazine and pyrimethamine. Sexual stages are readily observed by microscopy however, due to their size, larger than that of asexual stages. Cats treated similarly were nevertheless immune, since they did not shed toxoplasma oocysts when challenged. If it could be proved that sexual *T. gondii* stages were totally absent in the inoculated cats, these stages could be considered irrelevant to achieve immunity against the enteric cycle in the cat. If it was so, subunit antigens to test for protection against the enteric cycle of *Toxoplasma* in the cat, should be derived from those contained in asexual stages.

Future goals will be: (1) in vitro cultivation of cat enterocytes, to obtain large amounts of toxoplasma asexual stages. Figures for quantities of asexual and sexual toxoplasma enteric stages obtained from the gut of cats experimentally inoculated (Omata et al., 1997), show that this source of parasites is insufficient to perform antigen studies; (2) separation of asexual stages from the enterocytes in tissue culture. In this respect, separation of toxoplasma stages from cat gut has already been achieved (Omata et al., 1997) and toxoplasma stages were infective for kittens, which shed oocysts 2–8 days posinoculation (Omata et al., 1999); (3) identification of the protective antigens of asexual stages of *Toxoplasma*. There is work in progress on antigens of the enteric stages of *Toxoplasma* (Koyama et al., 2000, 2001).

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