Experimental Parasitology 120 (2008) 142–146

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Toxoplasma gondii: An improved rat model of congenital infection

A. Freyre [a,*], J. Falcón [a], J. Méndez [b], M. González [b]

[a] *Laboratory for Toxoplasmosis, Department of Parasitology, College of Veterinary Sciences, Montevideo, Uruguay*
[b] *Laboratory for Parasitology, College for Chemistry, Montevideo, Uruguay*

ARTICLE INFO

Article history:
Received 14 December 2007
Received in revised form 7 May 2008
Accepted 19 June 2008
Available online 25 June 2008

Keywords:
Toxoplasma gondii
Congenital toxoplasmosis
Rat model
Intrauterine infection

ABSTRACT

The objective of this study was to refine the rat model of congenital toxoplasmosis. In Fischer rats we found that visualization of spermatozoa in vaginal exudates and the detection of at least 6 g body weight increase between days 9 and 12 of pregnancy, allowed the diagnosis and timing of pregnancy with 60% specificity and 84% sensitivity. A dose of 10^4 *Toxoplasma gondii* bradyzoites or 10^2 *T. gondii* oocysts of the Prugniaud strain resulted in more than 50% of congenital infection of the rat litters. Transmission of *T. gondii* via lactation was not detected in rats inoculated with either bradyzoites or oocysts. Bioassays of 51 neonates born from mothers inoculated with bradyzoites (in tissue cysts) and 29 neonates from mothers inoculated with oocysts demonstrated that both liver and lungs can be used for the diagnosis of congenital transmission in this model.

1. Introduction

Toxoplasmosis can cause fetal damage in humans and abortion in sheep, goats, pigs, and rabbits if seronegative (i.e. naïve) hosts are infected during pregnancy (Dubey and Beattie, 1988; Remington and Desmonts, 1990). The incidence of congenital human toxoplasmosis has been demonstrated to be from 1 to 6 per 1000 live births. Although most infected newborns are asymptomatic at birth, adverse sequelae, e.g. cognitive difficulties and chorioretinitis, may develop later in life in a large proportion of the infected neonates (Remington and Desmonts, 1990). In immunocompetent hosts primary infection with *Toxoplasma gondii* results in a lifelong protective immune response that prevents transmission during pregnancy. This suggests that control of the disease by vaccination is a feasible goal.

A considerable experimental effort has been put into developing a vaccine to prevent congenital toxoplasmosis in animals (Nielsen et al., 2000). While several animal laboratory species have been used to investigate immunity against congenital toxoplasmosis, the rat has been the animal of choice for the purpose (Dubey and Shen, 1991; Dubey et al., 1997; Freyre et al., 1998, 2001, 2003, 2004; Paulino and Vitor, 1999; Thiermann, 1957; Zenner et al., 1993; Zenner et al., 1999). The usual rat congenital *T. gondii* model includes immunization of females 1–2 months before conception, followed by challenge during pregnancy, and then bioassay of newborn tissue samples in mice. Non-immunized (control) rats that receive similar challenge inocula are also used. While this model has been widely used, a number of improvements are needed which were the objectives of the present study. We identified the following as the major problems that needed to be addressed in this rat model:

1. It is necessary to have a method to ascertain the date of copulation of each rat, and to ascertain before challenge, that the rats are pregnant. This would permit challenge of rats with *T. gondii* between 12 and 15 days of gestation. Copulation does not necessarily follow a few days after caging of both sexes together, and pregnancy does not necessarily follow copulation.
2. The challenge dose with *T. gondii* should be proportional to the body weight of rats. This would allow some comparison to the infective doses ingested by people, which are the referent of the model. For instance, there might be approximately one tissue cyst of *T. gondii* for every 50 g pork consumed (Dubey and Thayer, 1994). In our previous work (Freyre et al., 2006a) partial protection was obtained in the rat model using a high challenge dose and this was the principal hypothesis to explain this partial protection. In the present work, the congenital transmission of *T. gondii* from an infection initiated by low doses of bradyzoites and oocysts, will be tested. In this way, the use of lower stringency challenges in future research on *T. gondii* will allow the detection of protection conferred also by antigens of limited immunogenicity. In addition, the use of precise number of bradyzoites, instead of cysts, containing variable number of bradyzoites, will improve the reproducibility of these experiments as it

* Corresponding author. Address: Universidad de la Republica, Facultad de Veterinaria, Departamento de Parasitologia, Alberto Lasplaces 1550, Montevideo 11600, Uruguay. Fax: +598 2 6280130.
E-mail address: freyrealvaro@hotmail.com (A. Freyre).

0014-4894/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2008.06.007

is known that cysts contain variable numbers of bradyzoites (Motomoura and Jo, 1970). At the same time, transmission rates of several *T. gondii* strains will be determined in the present study to determine the best strain for this congenital model.

3. It is also necessary to ascertain whether or not lactogenic transmission is important in this rat model. It has been shown that *T. gondii* is present in milk of rats infected per os during pregnancy (Sepúlveda, 2003). Lactogenic transmission of a *T. gondii* infection initiated during the rat pregnancy, might interfere with the interpretation of the results, if it was not coincident with the congenital transmission.
4. Another objective of the present work is to refine the detection of the congenital transmission. The underlining hypothesis is that during congenital transmission, *T. gondii* may be unevenly distributed in newborn tissues and it is useful to ascertain the most sensitive tissues for bioassay of infection in newborns.

2. Materials and methods

2.1. Animals

Fischer rats were used. The Fischer strain is an endogamic rat strain that theoretically would give more homogeneous results among individuals, than an exogamic rat strain. Rats were obtained from in house breeding. The parents were purchased from the Laboratory Animal Facility of the Faculty of Veterinary Sciences of La Plata, Argentina (origin: National Institutes of Health, US). Twenty grams CF-1 mice were used as donors of tissue cysts of *T. gondii*, and for bioassays. Weaned cats of the European breed were used to obtain *T. gondii* oocysts. They were obtained from the breeding colony of the Laboratory for Toxoplasmosis, College for Veterinary Sciences, Montevideo. Rats, mice and cats were free of *T. gondii* infection, as ascertained with a home-made direct agglutination (DA) assay (Desmonts and Remington, 1980). A 12/12 h light cycle was used.

2.2. T. gondii strains

The following strains of *T. gondii* were used: M7741, M3, and Prugniaud, (Freyre et al., 2001, 2003). Strains M3 and Prugniaud belong to genotype II, and strain M7741 to genotype III (Howe and Sibley, 1995). Both genotype II and III have been isolated from cases of congenital human toxoplasmosis (Ajzenberg et al., 2002), although it should be noticed that we are mentioning genotypes and not immunotypes, a more appropriate classification for the present purpose, of which almost nothing is known at present (Smith and Frenkel, 2003). The geographic origins of the strains used, are considerably divergent: USA (M7741), Scotland (M3) and France (Prugniaud) (Freyre et al., 2003, 2004).

2.2.1. Bradyzoites

Brain cysts were obtained as described (Freyre et al., 2001). Brains were homogenized in PBS pH 7.2 in a hypodermic syringe by passing the emulsion 12 times through a 19 gauge needle. Equal parts of the emulsion and of digestive fluid (Freyre, 1995) were incubated for 5 min at 37 °C, then the mixture was neutralized with 1% Na_2CO_3, centrifuged at 500*g* 15 min, and the supernatant replaced by PBS pH 7.2 with 0.6% bovine albumin. A 1/10 dilution of this mixture was made in PBS pH 7.2 with 0.6% bovine albumin and the bradyzoites were counted in a hemocytometer.

2.2.2. Oocysts

Oocysts were obtained by feeding the brain and the carcass of a mouse with a persistent *T. gondii* infection to a weaned cat. Fecal specimens were collected 4–7 days after inoculation and oocysts were separated by sugar flotation (Frenkel, 1977). Oocysts were incubated in 2% sulfuric acid at room temperature with agitation for 4–7 days. The oocysts were then counted in a hemocytometer. After neutralization with 3.3% NaOH, they were titrated in mice, to assure that the dose administered to rats was calculated on a basis of live oocysts.

2.3. Experimental design

2.3.1. Diagnosis and timing of pregnancy

Female and male rats were caged together 4:1. Copulation was assessed every morning by microscopic visualization at 100× of sperm in vaginal exudates obtained with the help of a cotton plug soaked in 0.9% NaCl in water. The procedure was repeated until all rats were found to have copulated. The method was feasible because rats copulate only in the dark. Should rats copulate during the light period, sperm ejaculated in the vagina 24 h before might have not been noticed. Nine and 12 days after copulation, the rats were weighed. Rats with 6 and more grams were considered pregnant. Pregnancy was assessed by parturition.

2.3.2. Assessment of congenital transmission of T. gondii infections initiated by defined numbers of bradyzoites or oocysts

Groups of 8–12 female rats were inoculated by gavage per os each with 10^3 bradyzoites, 10^4 bradyzoites, 10^5 bradyzoites, 10^2 oocysts or 10^3 oocysts of Prugniaud strain *T. gondii* at 12 days of pregnancy. Newborns were bioassayed in mice at birth. Twenty five days later, the mice were bled and their sera were investigated with the direct agglutination (DA) assay of Desmonts and Remington (1980), using 1:64 as the threshold titer indicative of *T. gondii* infection.

2.3.3. Lactogenic transmission

Toxoplasma gondii infected mothers (as described above) were provided with three newborns from non-infected mothers, born the same day of parturition of the stepmother, or close to it. Three days later, these newborns were bioassayed in mice. Twenty five days later the mice were bled, and their sera were investigated with the DA reaction for toxoplasmosis.

2.3.4. Organs of choice to assess congenital transmission

The livers, lungs and brains of newborns from five rats inoculated with strain Prugniaud were each separately inoculated in one mouse. Twenty five days later, the mice were bled and their sera examined with the DA reaction for *T. gondii*.

2.3.5. Congenital infection bioassay

Newborns were rinsed with distilled water and killed by cervical dislocation. For the routine assay newborns from the same litter were blended altogether in a laboratory blender with 0.85% NaCl in distilled water with 1000 IU penicillin and 0.1 mg streptomycin/ml and the resulting homogenate was intraperitoneally (i.p.) inoculated in mice. Mice were bled 30 days later and their sera examined for specific antibodies with the DA reaction. To examine tissue specificity in the congenital model the liver, lungs and brain of each newborn were separately homogenized in PBS pH 7.2 in a hypodermic glass tuberculin syringe by passing the emulsion 12 times through a 19 gauge needle. The lungs were previously minced with scissors, in a tube before homogenization. Each homogenate was i.p. inoculated into one mouse. Mice were bled 30 days later and their sera examined for specific antibodies with the DA reaction.

2.3.6. Statistical analysis

To detect any association between congenital transmission rates in rats and the doses and *T. gondii* strains used, the exact Fisher test was used, at an $\alpha = 0.05$.