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Evaluation of *Toxoplasma gondii* placental transmission in BALB/c mice model

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ABSTRACT

Toxoplasma gondii infection is common worldwide and highly important to pregnant women as it can be transmitted to the fetus via the placenta. This study aimed at evaluating the prevention of placental transmission in two different strains after chronic infection with each one of the strains. A BALB/c mice model was inoculated 30 days before breeding (immunization) and re-infected 12 and 15 days after pregnancy (challenge). Seven experimental groups were assayed: G1: ME49-immunization (type II), M7741-challenge (type III); G2: M7741-immunization, ME49-challenge; G3, ME49-immunization; G4: M7741-immunization; G5: ME49-challenge; G6: M7741-challenge; G7: saline solution inoculation. Serology, mouse bioassay, PCR and RLFP of the uterus, placenta and fetus were performed to determine the congenital transmission of the strains challenged after chronic infection. IgG *T. gondii* antibodies were detected in G1, G2, G3 and G4, but not in G5, G6 and G7. All animals of G5 and G6 were IgM-positive. Congenital infection was not detected by bioassay and PCR. Nonetheless, placentas from G3 and G4 resulted positive but no corresponding fetal infection was detected. G1 and G2 did not show the genotype of the strain challenged during pregnancy, only those of chronic infection. Thus, the chronically infected BALB/c mice showed no re-infection after inoculation with another strain during pregnancy. Further studies with different parasite loads and different mice lineages are needed.

1. Introduction

Toxoplasmosis is a disease that affects warm-blooded animals, including humans. It is caused by the intracellular obligatory parasite protozoan, *Toxoplasma gondii* (Tenter, 1998). It can cause abortion in many animal species of economic interest (Tenter, 1998), and is a disease of relevance to public health for being an opportunistic zoonosis in immunosuppressed or HIV-positive individuals. It is equally an important cause of abortions or congenital disease resultant from maternal primary infection during pregnancy (Derouin et al., 1995).

The population structure of *T. gondii* is dominated by three lineages, designated strain types I–III. The type I strain, RH, is uniformly lethal in mice prior to establishment of cyst-associated persistence, causing death of BALB/c mice in 4–6 days, whereas types II (ME49) and III (M7741) are less virulent and can establish chronic infection. M7741 is more virulent than ME49 (Roberts and Alexander, 1992; Howe and Sibley, 1995; Fux et al., 2000).

The histology of human and rodent placentas is very similar and the mouse is the most frequently studied experimental model of congenital toxoplasmosis (Darcy and Zenner, 1993). Vertical transmission has been reported to occur through successive generations in mice. However, Roberts and Alexander (1992) demonstrated that female BALB/c mice infected many weeks before breeding developed immunity capable of totally protecting their fetuses, even when re-infected during pregnancy. Dao et al. (2001), studying re-infection in chronically infected mice, demonstrated that a primary infection with a type II strain does not protect the host against the production of tissue cysts secondary to re-infection with a type III strain. Thus, whether a given *T. gondii* genotype can evade the host immune response after a previous infection with another variant due to antigenic differences (Lehmann et al., 2000) is debatable. Araujo et al. (1997) suggested that the immunity induced by a previous infection with a strain of *T. gondii* in mice is not sufficient to prevent acute toxoplasmosis and death after host re-infection with parasites of another strain.

The present study aimed at developing a chronic infection in a mouse model using a type II strain to prevent placental transmission and/or re-infection with a more virulent type III strain or vice versa. Placental transmission was evaluated in various mouse tissues and sera by PCR and IFAT.

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2. Materials and methods

2.1. Animals and parasites

Swiss albino mice were inoculated, s.c., with the strains ME49 (type II) and M7741 (type III) (Howe and Sibley, 1995). The brains of the infected mice were isolated and digested in pepsin for the liberation of bradyzoites from cysts, according to Dubey (1998a,b). Released bradyzoites were counted in a Neubauer chamber and their viability was determined by Trypan blue dye exclusion following pepsin release.

Isogenic BALB/c female mice, 30 days old, provided by and kept at the Vivarium of Botucatu Medical School Experimental Laboratories, UNESP, were used for inoculation. Swiss albino mice, 30 days old, from Botucatu Central Vivarium-UNESP, were used to obtain tissue cysts of *T. gondii* ME49 and M7741 strains, as well as for the bioassay of fetal samples.

2.2. Experimental plan

This experiment was approved by the Research Ethics Committee of Botucatu Medical School (#16/20020-CEEA). Seven experimental animal groups were studied. Two main groups were used to evaluate the cross protection between types II and III strains and the remaining five groups were used to control the efficiency of the experiment (Table 1).

Female BALB/c mice infected with the ME49 or M7741 strains received, by the oral route (gavage), 100 μL of infected brain + saline suspension with 10^4 bradyzoites (adapted to Freyre et al., 2006b; Freyre et al., 2008). When appropriate, 0.85% saline solution in a volume of 100 μL animals was orally administered, by gavage. All groups were reinoculated according to different protocols 12 to 15 days after breeding (Roberts and Alexander, 1992) (Table 1). The female BALB/c mice infected with the M7741 strain 30 days before breeding were treated with sulfadiazine (400 mg sulfadiazine + 10 g of sodium bicarbonate/1 L water) in drinking water, from day 3 to day 20 post-inoculation as described by Villard et al. (1997). Chronic infection was thus induced because the M7741 strain is more virulent than ME49 and caused the death of the untreated animals starting at day 8 post-inoculation, as observed during the previous pilot experiment. Female BALB/c mice from each experimental group were placed with males (3:1 ratio) in boxes, and checked for the presence of mucous vaginal plug. The day on which there was evidence of mating was recorded as "day 0" of pregnancy (Roberts and Alexander, 1992), as the presence of vaginal plug indicates that mating occurred within the previous 24 h (Harkness and Wagner, 1993).

Caesarian was performed 19 days after breeding (and 4 days after the second inoculation) using isofluorane a vapor saturated chamber, as determined in a pilot experiment. Normally, the time for a BALB/c mouse to give birth is 19–21 days after breeding. All mice were sacrificed after the operation.

2.3. Infection detection by indirect fluorescent antibody test (IFAT)

IFAT was performed using commercial goat anti-mouse IgG conjugate (A90-131F, Bethyl Laboratories Inc., Montgomery, TX, USA) diluted at 1:180 for the detection of anti-*T. gondii* IgG antibodies in all groups. Additional research for IgM antibodies was performed in G5 and G6 using goat anti-mouse IgM conjugate (A90-101F, Bethyl Laboratories Inc., Montgomery, TX, USA). IgM and IgG IFAT were performed in the same serum samples. In all groups, blood samples were collected by retro-orbital sinus puncture 28 days after the first inoculation. Sera were diluted in phosphate buffered solution (PBS), pH 7.2, 0.01 M and endpoint titers were determined by serial dilution from 1:16 to 1:16,000 using a Zeiss SG250 fluorescence microscope. Positive and negative control sera obtained by previous inoculations in laboratory mice were provided by NUPEZO.

2.4. Preparation of tissue samples

Uterine samples obtained from female BALB/c mice, and fragments of fetal tissue and placenta obtained during caesarian section were crushed in a vertical homogenizer and a porcelain vessel, respectively. Homogenized tissue samples were diluted in 0.85% sterile saline solution (20% w/v) and kept frozen at −80 °C for PCR. Fragments of fetal tissue samples from all female BALB/c mice were homogenized and kept at 4 °C for bioassay. Brain samples from all female BALB/c mice were also homogenized as described above. PCR results were compared with those obtained from uterine, fetal and placental samples. Contamination was avoided by washing instruments with 1 N HCl solution between sample preparations.

2.5. Bioassay

Four albino Swiss mice were intraperitoneally inoculated with 1 mL from brain samples of a pool from all fetuses of each female BALB/c mouse, and observed for up to 60 days, to evaluate viability and to re-isolate the parasite strains used in order to control the efficiency of the experiment. Mice were sacrificed on day 60 in an isofluorane vapor saturated chamber. Blood samples were collected by orbital sinus puncture, and the sera obtained underwent IFAT.

2.6. Polymerase chain reaction (PCR)

T. gondii DNA was extracted from brain, uterus, placenta and fetal tissues according to the protocol described by Janssen (1994). Samples were kept at −20 °C.

PCR was performed using the primers described by Homan et al. (2000), which amplify a 529 bp fragment, AF146527 [GenBank], that is repeated 200- to 300-fold in the genome of *T. gondii*. Thus, primers TOX4 (5′CGCTGCAGGGAGGAAGACGAAAGTTG3′) and TOX5 (5′CGCTGCAGACACAGTGCATCTGGATT3′) were used. PCR was performed in a 25 μL reaction mixture containing 10 μM of each primer (Invitrogen, São Paulo, SP, Brazil), 10X PCR buffer (50 mM KCl, 10 mM Tris–HCl, Invitrogen, São Paulo, SP, Brazil), 1.5 mM $MgCl_2$ (Invitrogen, São Paulo, SP, Brazil), 1.25 mM dNTP (Invitrogen, São Paulo, SP, Brazil), 0.15 U of Platinum *Taq* polymerase (Invitrogen, São Paulo, SP, Brazil), and ultrapure water q.s.p. Amplification was performed in a MJ Research thermocycler (MJ Research Inc., Waltham, MA, USA). Initial denaturation for 7 min at 94 °C, was followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C, and a final extension for 10 min at

Table 1
Experimental groups (six BALB/c mice each) inoculated with either ME49 strain, or M771 strain, or 0.85% saline solution 30 days before breeding and between 12 and 15 days of pregnancy.

Inoculation dates	G1	G2	G3	G4	G5	G6	G7
30 days before breeding	ME49	M7741	ME49	M7741	Saline	Saline	Saline
12 and 15 days of pregnancy	M7741	ME49	Saline	Saline	ME49	M7741	Saline