Experimental Parasitology 127 (2011) 107–112

Contents lists available at ScienceDirect

Experimental Parasitology

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

Toxoplasma gondii: The effects of infection at different stages of pregnancy on the offspring of mice

Tao Wang [a], Min Liu [b], Xiao-Jie Gao [a], Zhi-Jun Zhao [a,c], Xiao-Guang Chen [b], Zhao-Rong Lun [a,*]

[a] Center for Parasitic Organisms, State Key Laboratory of Biocontrol, School of Life Sciences, and Key Laboratory of Tropical Diseases Control (The Ministry of Education), Zhongshan Medical College, Sun Yat-Sen (Zhongshan) University, Guangzhou 510275, PR China
[b] Department of Parasitology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou 510515, PR China
[c] Affiliated Hospital of Ningxia Medical University, Yinchuan 750004, PR China

ARTICLE INFO

Article history:
Received 27 April 2010
Received in revised form 25 June 2010
Accepted 1 July 2010
Available online 7 July 2010

Keywords:
Toxoplasma gondii Prugniaud strain
Congenital transmission
Mouse
Offspring
Growth and development

ABSTRACT

Congenital toxoplasmosis can cause fetal damage in humans and domestic animals. This study was focused on the effects of *Toxoplasma gondii* (Prugniaud strain) infection at different stages of pregnancy on the offspring of mice. Results showed that newborn mice from all infected groups were significantly lower in weight than those from the control group but significant difference was not found among these groups at day 60 after birth. The survival rate of the offspring from the group of mice infected at the earlier stage of pregnancy was significantly lower than those of infected and control groups. The positive offspring (with cysts found in their brain tissues) born from the mice infected at the earlier and intermediate stages of pregnancy showed a shorter latency and greater number of errors in the step-through passive avoidance test than those born from the mice infected at the late stage of pregnancy, the control group and the negative offspring from the infected groups. The number of cysts in the brain tissue was significantly higher in the offspring born from the groups of mice infected at the earlier and intermediate stages of pregnancy than those from the group of mice infected at the late stage of pregnancy. In addition, our results indicated that a high congenital transmission rate (90%) occurred in this NIH mouse model. In conclusion, the earlier and intermediate maternal infection of *T. gondii* can result in severe congenital toxoplasmosis, exhibiting conditions such as stillbirth or non-viability, and learning or memory capability damage in this mouse model. These results not only provide useful data for better understanding the effects of *T. gondii* infection on the offspring of mice infected at different stages of pregnancy but also for better consideration of the effect of this infection on other mammalian hosts including humans.

1. Introduction

Toxoplasma gondii is an intracellular protozoan parasite that infects all warm-blooded animals including humans. It causes significant morbidity and mortality in congenitally infected and immunocompromised individuals such as AIDS and organ transplantation patients (Petersen et al., 2001; Kim and Weiss, 2008). This parasite is transmitted by the ingestion of tissue cysts which are found in the tissues of infected animals, by ingestion of food or water contaminated with oocysts and congenital transmission (Dubey, 2000).

Congenital toxoplasmosis can cause fetal damage not only in humans but also in sheep, goats, pigs, rabbits as well as other domestic animals (Gandahusada, 1991). The incidence of human congenital toxoplasmosis has been reported to be 1–6/1000 births in the world (Freyre et al., 2006b). The risk of congenital toxoplasmosis is dependent on a number of factors including the number of cysts or oocysts ingested, virulence of the parasite, the immunological status of the mother, and particularly the time of gestation at which infection occurs (Tenter et al., 2000). It is known that congenitally infected children usually show a wide spectrum of clinical diseases (Jones et al., 2001). The main clinical signs may consist of low intellectual quotient, which occurs in the majority of infected children, retinochoroiditis, hydrocephalus and intracerebral calcification (Gagne, 2001; Saxon et al., 1973; Wilson et al., 1980).

Congenital toxoplasmosis is not only a serious public health problem in humans, but also a significant economic importance for the livestock industry. It has been estimated that 1.2–2.2% of the 16 million ewes in the UK suffer from abortion due to *T. gondii* infection (Bennett et al., 1999). The severity of human congenital toxoplasmosis and its importance as a public health concern is well known, while congenital toxoplasmosis has also been considered an important economic problem in the livestock industry, where goats and sheep are affected (Bennett et al., 1999).

* Corresponding author.
E-mail address: lsslzr@mail.sysu.edu.cn (Z.-R. Lun).

0014-4894/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2010.07.003

Although the limitations on physiological, immunological, ethological and therapeutic knowledge of maternofetal transmission has been accepted, animal models with mouse, rat, guinea-pig and rabbit have been developed for investigating congenital toxoplasmosis (Darcy and Zenner, 1993; Wong and Remington, 1994). Among them, the mouse is the most frequently adopted model because the similarities in histological structures of placentas between human and rodents (Darcy and Zenner, 1993). Mouse congenital toxoplasmosis was described as early as in 1950 (Cowen and Wolf, 1950). Subsequently different strains of mouse have been extensively and frequently used as the model for studying the congenital toxoplasmosis. This murine model has been employed to analyze the role of parasite multiplication and the immune response on foetal death and transmission control by vaccination. Moreover, the mouse model has also been widely used to test the ability of chemotherapeutics to limit the vertical transmission of *T. gondii* (Fux et al., 2000). In addition, the relatively short period of gestation in the mouse facilitates effective experimentation. Furthermore, the availability of numerous immunological reagents and the construction of different kinds of transgenic knock out mice will ensure its continual utility and should provide valuable information for better understanding the pathogenesis of congenital toxoplasmosis (Mévélec et al., 2005). Thus, the murine model has proven a useful model for understanding the congenital toxoplasmosis.

Our study was focused on the effects of congenital *T. gondii* infection on the development and behaviour of mice. In particular, we investigated the effect on the weight, survival rate, learning and memory capability, the cyst number in the brain tissue and the congenital transmission rate of the offspring from pregnant mice infected at different gestational periods. This information will provide a better understanding of congenital toxoplasmosis.

2. Materials and methods

2.1. Animals and parasites

NIH mice weighing from 35 to 40 g used for congenital transmission and BALB/c mice used for the maintenance of *T. gondii* were purchased from the Experimental Animal Centre of Guangdong province. NIH mice were bred by National Institutes of Health, USA and belonged to an outbred stock. The protocol was conducted according to international regulations for animal experimentation and approved by the Institutional Review Board of Sun Yat-Sen (Zhongshan) University, School of Life Sciences (973 project, #2010CB530000).

The Prugniaud (Pru) strain of *T. gondii* was used throughout the experiment. It was maintained by oral inoculation of cysts from brain tissue of infected BALB/c mice every 3 months. Cysts for the infection of NIH mice were isolated from the brain tissue of infected BALB/c mice 2 months after infection through oral inoculation. Briefly, under sterile conditions, brain tissues obtained from the infected mice were homogenized in a 1-ml homogenizer and the number of cysts was counted under a microscopy with a 10x objective. They were diluted if necessary.

2.2. Mating and infection

Female and male NIH mice were caged together at 2:1. Copulation was assessed every 12 h by vaginal suppository. The procedure was repeated until all NIH mice were found to have copulated.

Four groups each with 5 confirmed pregnant mice were used in this study. In the control group, each mouse was inoculated by the oral route (OR) with 0.2 ml saline solution (0.85%), while in the other three groups, each was infected with 5 cysts of *T. gondii* by the OR on the 5, 10 and 15th day after gestation respectively. Each of the offspring from the groups was weighed at day 1 and day 60 after birth and their survival rate was recorded.

2.3. Step-through passive avoidance test

The learning ability of each mouse was evaluated by the Single Trial Passive Avoidance Test (STPAT). The step-through test was performed as described previously (Zarrindast et al., 2002; Wang et al., 2007, 2009). Briefly, the apparatus for the step-through passive avoidance test was an automated shuttle-box divided into an illuminated safe compartment and a dark shock compartment of the same size, separated by a wall with a guillotine door.

The experiment was divided into learning and memory trials. A mouse was put into the illuminated compartment, facing away from the dark compartment. After 180 s, the door between these two boxes was opened and the mouse was allowed to move into the dark compartment freely. When the mouse stepped into the dark compartment, an inescapable foot-shock (36 V) was delivered through the grid floor and the number of errors within 5 min were recorded. The number of errors was used to assess the learning ability of each mouse. The retention of passive avoidance response, named the memory trial, was measured at 24 h after the learning trial. During the memory trial, each mouse was put into the illuminated compartment and the latency of the first time to enter the dark compartment and the error number within 5 min was recorded. The maximum cut-off time for the latency was 300 s. The latency and number of errors were used to assess the mouse memory. All training and testing were performed between 10:00 am and 3:00 pm.

2.4. Isolation and calculation of cysts from the mouse brain

The calculation of numbers of cysts in the mouse brain was performed as described previously (Goodwin et al., 2008). Briefly, mouse was anaesthetized by CO_2 and the whole brain was removed and homogenized in 1 ml PBS (pH 7.2). The number of cysts in 10 μl sample was calculated by microscopy with a 10× objective and repeated five times. The total number of cysts in the brain was determined by the total number of cysts in the 50 μl sample examined multiplied the total volume of the homogenate.

2.5. DNA extraction and PCR detection

About 50 mg of brain tissue were used for DNA extraction. Total genomic DNA was extracted by using the Genomic DNA extraction kit (Axygen, Hangzhou, China). Forward primer: 5′-CGCTGCAG GGAGGAAGACGAAAGTTG-3′ and reverse primer: 5′-CGCTGCAG ACACAGTGCATCTGGATT-3′ were used for generating a specific fragment at 529 bp (Homan et al., 2000).

2.6. Statistical analysis

All data were shown as the mean ± S.D. Statistical analysis of the data for multiple comparisons was performed by one-way ANOVA. $P < 0.05$ was considered statistically significant and $P < 0.01$ was considered highly significant.

3. Results

3.1. The mean weight of the offspring

All surviving offspring were weighed on day 1 and day 60 after birth respectively and the results are shown in Table 1. The offspring from the non-infected control group showed a higher body