American Journal of Immunology 1 (1): 1-5, 2005
ISSN 1553-619X

Materno-Fœtal Transmission of Murine Toxoplasmosis after Oral Infection

[1,2]Jamal Hafid, [1]Bahrie Bellete, [1]Pierre Flori, [2]Philibert Sawadogo, [1]Yves Boyer, [1]Hélène Raberin, and [1]Roger Tran Manh Sung[1]

[1] Groupe Immunité Des Muqueuses et Agents Pathogènes (GIMAP), Faculté de Médecine Jacques Lisfranc, 15 Rue Ambroise Paré, 42023 Saint Etienne cedex 02. France.

[2] Unité d' Immunologie et de physiologie, Département de Biologie, Faculté des Sciences et Techniques, Avenue A. El Khattabi, B.P 549, 40000 Marrakech, Maroc

Abstract: The effect of maternofoetal transmission of *T. gondii* as measured by the mortality rate in the mother, the fœtus and the neonate were studied in the mouse model OF1. Ninety six female mice divided into two groups of 48 were infected with the Prugniaud strain before or after mating. When the time of mating was near the day of infection (Day 1), no mouse survived the pregnancy in the two groups. When the infection preceded the mating, the percentage of neonates who died was 68%, 58%, 74% and 52% on day 5, 9, 13 and 18 respectively. In contrast, when the mating preceded the infection, these percentage were much more elevated with 96%, 94%, 74% and 92% on days 5, 9, 13 and 18. This shows a higher transmission rate in the latter case. The brains of the surviving neonates were reinoculated into healthy mice, but the results were all negative. This shows that these neonates were not infected although they originated from a pregnancy where the number of litters was greatly reduced by the parasite.

Keywords: mice, vertical transmission, toxoplasmosis, neonates.

INTRODUCTION

Toxoplasma gondii is an ubiquitous and obligate intra-cellular parasite of worm-blood animals and humans. It is able to cause severe damage in both immunocompromised adults and in new-borns (Wong and Remington, 1994).It is considered to be a major cause of abortions in certain species mainly in sheep and goats (Buxton, 1990 ; Krupa et al., 1990; Buxton and Innes, 1995). Of the only known species of Toxoplasma several strains with varying degrees of virulence were isolated and are at present being used in vivo and/or in vitro. In experimental toxoplasmosis, the mouse is often used as a model for immunologic (Eisenhauer et al., 1988; Chardes et al., 1990, Hafid et al., 1991)as well as parasitologic (Dubey, 1998), pathophysiologic (Dubey, 1997; Zenner et al., 1998) and pharmacologic studies (Isamida et al., 1998). As for vertical transmission studies of the parasite using animals, there are a few studies done using the oocysts, cysts and trophozoites of different *T. gondii* strains in order to understand the mode of transmission and the consequences in the mother as well as in the foetus and the neonate (Stahl and Kaneda, 1998; Stahl et al., 2002). Several vaccination studies have also been done in this animal using different formes of the parasite as well as protein and nucleic acid extracts administered by the intraperitoneal, intradermal or nasal routes (Ali et al., 2003; Bonenfant et al., 200; Letscher-Bru et al., 2003; Hafid et al., 2004).

This study represents one phase of a large study to evaluate the immunoprotective power of certain metabolic extracts of the parasite in congenital toxoplasmosis of mice. We have tried to better understand the consequences of transpacental transmission of *T. gondii* on the mothers and their neonates by infecting the animals with cysts of the avirulent Prugniaud strain via the oral route at different gestationel dates.

MATERIALS AND METHODS

Animals: OF1 female mice weighing 22-28 g were used to maintain the cysts of T. gondii and to study the effect of materno-fœtal transmission to the neonates after infection of the mother by the oral route.

Parasite: The cysts of brain PRU of T. gondii were maintained in the laboratory by monthly intraperitoneal passage from an infected mouse to healthy mice. In this study, the mouse inoculations were made by the oral route with cysts derived from brain homogenates of mice infected two months before.

Corresponding Author: Pr. J. Hafid, Laboratoire de Parasitologie, C.H.U. de Saint Etienne, Hôpital Nord, 42055 Saint Etienne cedex 2, France. Phone: (33) 4 77 82 83 08, fax: (33) 4 77 82 84 82, e-mal: hfjamal@yahoo.frEduardo A. Castro División Química Teórica, INIFTA, Suc.4, C.C. 16, La Plata 1900, ca.unlp.edu.ar

Experimental infection and mating: Ninety six female OF mice having the same age, weight and origin were divided into two groups of 48 :

The first group consisted of 48 mice all of which were given 5 cysts of strain PRU of *T. gondii* by the oral route. Eight mice were isolated on each of D1, D5, D8, D13 and D18 post-infection and were made to mate with two males by keeping them together for four days. The remaining eight were kept as controls of infection (without mating).

The 48 mice in the second group were made to mate with to males the same day. Eight of these were isolated on each of D1, D5, D9, D13 and D18 and were immediately infected with 5 cysts of the PRU strain of *T. gondii* by the oral route. The remaining eight served as controls of mating (non infected).

One week after mating each mouse of the two groups was isolated in a cage in order to follow the impact of infection on itself during pregnancy and on the neonates.

One week after infection, the search for anti-toxoplasma gondii antibodies of the class G was made on the sera using indirect immunofluorescence (IIF). Briefly, blood was collected from a tail vein and diluted at 1 :20 in PBS at a pH 7.2 and was deposited on the trophozoites of the RH strain of *T. gondii*. After 30 minutes of contact at 37°C, fluorescein iso-thiocyanate labelled anti-mouse Immunoglobulin M and G conjugate (Bio-Rad, Marnes-la-Coquette) diluted at 1:25 in Evans blue solution was added and kept for another 30 minutes at 37°C before reading under a fluorescent microscope.

The newborns which were alive were kept for tree weeks to measure their viability and then samples were taken from their brains to be inoculated into other healthy mice.

Mouse inoculation and anti-T. gondii antibody detection: The brain of each neonate was mixed with 250 ml of physiologic saline (0.9% NaCl) and homogenized by mortar and pestle. The final volume was administered into two healthy OF1 mice intraperitoneally. Seven weeks after inoculation, blood samples were taken from the tail of each mouse and the sera were tested by IIF as described above to detect anti-Toxoplasma IgG.

The experimental infections following inoculation were done two times and the results were the same.

RESULTS

In the first three days following infection, all the animals have shown a reduction in their movement with a change in their fur coat. Once this stage has passed, they became normal again. The quantity of parasites administered orally in this study is normally a non-lethal dose for healthy non gestational mice.

The anti-*T. gondii* specific IgG antibodies detected by the IIF were positive in all the mice proving that they have all received the cysts.

The mice which have survived the infection and in whom the pregnancy was brought to term gave birth three weeks after the contact with the male.

When the mating was near the date of infection (D1), no mouse survived in the course of the pregnancy in both experimental groups.

In the experimental protocol where the mating precedes infection, only ten mice had viable litters (2 at D5, D9 and D18 and 4 at D13) with a total number of viable neonates and the percent dead (deduced from the controls of mating) was found to be 4 (96%) at D5, 6 (94%) at D9, 26 (74%) at D13 and 8 (92%) at D18 (Table 2). Ten mice did not get pregnant, two aborted and six gave birth to non-viable litters. As for the number dead, in addition to the eight of D1, four other mice (2 infected at D18, 2 at D13) died during the course of the pregnancy (Table1).

In the case where inoculation has preceded mating, the number of viable litters was the same for the days between D5 and D18 (a total of 24) with a non- negligible difference in the total number of viable neonates : 32 on D5, 42 on D9, 26 on D13 and 48 on D18. Regarding the percentage dead among the neonates, it was 68%, 58%, 74% and 52% for D5, D9, D13 and D18 respectively. Six mice remained non-pregnant during the entire experimental period. There were two mice who died during pregnancy on D13 (Table 2).

The control group for mating had the expected results where all the females were pregnant and all the litters were viable with 12 to 13 neonates per pregnancy.

Concerning the control group for infection, no death was recorded during the entire experimental period.

The IIF test to detect anti-*T. gondii* antibodies in the sera of mice inoculated with brain homogenates of the surviving neonates were all negative on the seventh as well as on the tenth week.

DISCUSSION

The positive IIF tests obtained signify that all the mice of the two groups have received the cysts of *T. gondii* by the oral route and that they produced specific antibodies. *T.gondii* specific antibodies, despite their presence and persistence in the first few days following inoculation have a weak protective effect against virulent *T. gondii* (Hafid et al., 1991)

At first sight, when comparing the two experimental protocols, it appears that whatever the time of infection in relation to mating the consequences are severe on the mother, the fœtus and the neonates. They are much more accentuated when infection occurs after mating.

Table 1 : Number of pregnant females and percentage of deaths in the neonates of mice made to mate followed by infection with 5 cysts of *T. gondii* PRU strain

Date of infection (post-mating)	Number of pregnant females	Number of viable neonates	% dead
D1	8 died during pregnancy	0	100
D5	4 non-pregnant 2 with non-viable litters 2 with viable litters	2x2 (4)	96
D9	2 died during pregnancy 4 with non-viable litters 2 with viable litters	2x3 (6)	94
D13	2 died during pregnancy 2 non-pregnant 4 with viable litters	2x8 et 2x5 (26)	74
D18	4 non-pregnant 2 pregnant but abortion 2 with viable litters	2x4 (8)	92
Mating control	8 with viable litters	4x12 et 4x13 (100)	0

Table 2 : Number of pregnant females and percentage of deaths in the neonates of mice infected with 5 cysts of *T. gondii* PRU strain followed by mating.

Date of mating	Number of pregnant females	Number of viable neonates	% dead
D1	8 died during pregnancy	0	100
D5	2 non-pregnant 6 with viable litters	2x4, 2x5 et 2x7 (32)	68
D9	2 non-pregnant 6 with viable litters	2x7, 2x9 et 2x10 (42)	58
D13	2 died during pregnancy 6 with viable litters	2x3 et 4x5 (26)	74
D18	2 non-pregnant 6 with viable litters	6x8 (48)	52

In each experimental protocol, the date of infection is determinant in the follow up of the animals but also in their capacity to become pregnant and in the number as well as the viability of the litters.

In the two experimental protocols, the death of the 8 mice during pregnancy when the date of infection and mating were closer (D1), could be explained by the fact that the beginning of pregnancy is a physiological stage that weakens the host and favours multiplication of bradyzoites with a parasitemia acute enough to cause the death of the females in the days that follow infection. These results are supported by those of Thouvenin et al., (1997) who showed that in mice infected by 20 cysts of the strain PRU on the 11^{th} day of gestation, the cerebral and pulmonary parasite loads were much greater than those of mice who were non pregnant. Likewise, the intravascular transfer of the parasite into the brain during a congenital transmission following infection of mice with *T. gondii* on the 7^{th} day of pregnancy, entails a number of inflammatory lesions in the fœtus (Stahl and Kaneda, 1998).

When infection preceded mating except for the two mice who died during pregnancy, the rest of the females

were either not pregnant (6 mice) or they gave birth to viables litters (24 mice). The number of litters per pregnancy was variable between 3 and 10 with a total of 26 on D13 and 48 on D18. In these cases, the number of mice which died was low and that of the viable litters was high. This is probably due to the fact that the beginning of gestation is around D5 which is after the parasitemic stage which lasts three to four days resulting in consequences which are much less pronounced as compared to those observed when mating precedes infection. Therefore, the number of non-pregnant females was much higher (10 mice), that of the non-viable litters was 6 (against none) and that of the pregnant who aborted was 8 (against none). The number of viable litters thus remains low (10) with 2 to 8 litters per pregnancy. These findings show that during pregnancy, infection has severe repercussions on the fœtus whatever the date of infection.

The present results suffer from lack of other studies with which to compare them. The majority of the studies were done do not address the follow up of vertical transmission of *T. gondii* in the mouse which for several years is considered to be the biological model the most studied for toxoplasmosis. On the other hand, some stages in certain of these studies are devoted to this aspect and allow us to compare our results. Thus by inoculating cysts of *T. gondii* intraperitoneally into NYLAR mice on the 7^{th} day of gestation, Stahl et al., (2002) have shown a clear reduction in the number and survival of the neonates per pregnancy. Likewise, oral infection of non-immunized BALB/c mice (control group) with 4 cysts of the strain P between the 10^{th} and 14th day of pregnancy has permitted to obtain a high percentage of infected litters by congenital transmission of the parasite (Elsaid et al., 2001). Using the ELISA technique to determine the incidence of congenital toxoplasmosis in the litters of BALB/c mice, Roberts and Alexander, (1992) have noted that 5/6 mice infected on the 12^{th} day of gestation gave birth to approximately 50% of infected litters ; whereas the litters of mice infected eight weeks before mating were healthy. Our results of D13 agree with those of Abou-bacar et al., (2004) who by infecting BALB/c mice on the 11^{th} day of gestation by the oral route with 20 cysts of strain PRU found only 63.9% of the foetus as infected whereas all the placenta were infected.

The degree of persistence and transmission via the placenta or breast-milk is associated to the virulence of the strain injected, as was the case of the avirulent and mutant strain TS-4 which was not transmitted to the neonates of mice inoculated on the 5^{th} , 10^{th} and 15^{th} days of gestation and on the 2^{nd} day postpartum (Pinkey et al., 1995). The authors have then noted that this strain can cause death of the neonates but was avirulent to adult mice pregnant or otherwise.

The vertical transmission of *T. gondii* was studied by other authors (Freyre et al., 1999; Flori et al., 2002; Couper et al., 2003) in other animal species with totally different experimental protocols and any comparison with our results is difficult or even impossible.

The fact that the results of the detection of anti-*T.gondii* antibodies in mice inoculated with brain homogenates were negative makes us conclude that the neonates from whom the brain homogenates originated were spared from infection and those which were infected in utero died either before or just after birth. Another important element to support our hypothesis is the fact that the neonates which escaped infection remained completely normal during the three weeks preceding their sacrifice.

The transmission of *T. gondii* has also been studied after chronic infection of two species of mice (Mus musculus and Apodemus sylvaticus). Thus, these animals received 50 oocysts by the oral route and were mated six weeks after infection. Their pups were examined 3 weeks after weaning at 6 weeks of age. The vertical transmission was demonstrated by PCR in 82.7% and 85% of all pups respectively (Owen and Trees, 1998).

Our study has shown once more that the chronology of infection in relation to gestation is a predominant element in the vertical transmission of toxoplasmosis and that in mice the viable neonates are spared.

REFERENCES

1. Abou-Bacar, A., Pfaff, A.W., Georges, S., Letscher-Bru, V., Filisetti, D., Villard, O., Antoni, E., Klein, J.P., Candolfi, E., 2004. Role of NK cells and gamma interferon in transplacental passage of *Toxoplasma gondii* in a mouse model of primary infection. Infect. Immun. 72, 1397-1401
2. Ali, S.M., Allam, S.R., Negm, A.Y., El Zawawy, L.A., 2003. Vaccination against congenital toxoplasmosis.J. Egypt. Soc. Parasitol. 33, 863-874
3. Bonenfant, C., Dimier-Poisson, I., Velge-Roussel, F., Buzoni-Gatel, D., Del Giudice, G., Rappuoli, R., Bout, D., 2001. Intranasal immunization with SAG1 and nontoxic mutant heat-labile enterotoxins protects mice against *Toxoplasma gondii*. Infect. Immun. 69, 1605-1612
4. Buxton, D., 1990. Ovine Toxoplasmosis. J. R. Soc. Med. 83, 509-511
5. Buxton, D., Innes E.A., 1995. A commercial vaccine for ovine Toxoplasmosis. Parasitol. 110, 11-16
6. Chardes, T., Bourguin, I., Mevelec, M.N., Dubremetz, J.F, Bout, D., 1990. Antibody responses to *Toxoplasma gondii* in sera, intestinal secretions, and milk from orally infected mice and characterization of target antigens. Infect. Immun. 58, 1240-1246

7. Couper, K.N., Nielsen, H.V., Petersen, E., Roberts, F., Roberts, C.W., Alexander, J., 2003. DNA vaccination with the immunodominant tachyzoite surface antigen (SAG-1) protects against adult acquired *Toxoplasma gondii* infection but does not prevent maternofoetal transmission. Vaccine 20, 2813-2820
8. Dubey, J.P., 1997. Bradyzoites-induced murine toxoplasmosis : stage conversion, pathogenesis, and tissue cyst forma bradyzoites of différent strains of *Toxoplasma gondii*. J. Eukaryot. Microbiol. 44, 592-602
9. Dubey, J.P., 1998. Comparative infectivity of *Toxoplasma gondii* bradyzoites in rats and mice. J. Parasitol. 84,1279-1282
10. Eisenhauer, P., Mack, D.G., McLeod, R., 1988. Prevention of peroral and congenital acquisition of *Toxoplasma gondii* by antibody and activated macrophages. Infect. Immun. 56 : 83-87
11. Elsaid, M.M., Martins, M.S., Frezard, F., Braga, E.M., Vitor, R.W., 2001. Related Articles, Links Vertical toxoplasmosis in a murine model. Protection after immunization with antigens of *Toxoplasma gondii* incorporated into liposomes. Mem. Inst. Oswaldo. Cruz. 96, 99-104
12. Flori, P., Hafid, J., Bourlet, T., Raberin, H., Genin, C.,Tran Manh Sung, R., 2002. Experimental model of congenital toxoplasmosis in guinea-pigs: use of quantitative and qualitative PCR for the study of maternofetal transmission. J. Med. Microbiol. 51:871-878
13. Freyre, A., Falcon, J., Correa, O., El Elhou, S., Mendez, J., Gedda, C., 1999. Congenital transmission of experimental chronic toxoplasmosis in rats. J Parasitol. 85, 746-748
14. Hafid, J., Tran Manh Sung, R., Pozzetto, B., Jaubert, J., Akono, Z.Y., Raberin, H., Jana, M., 1991. Kinetic of circulating antigens by capture-ELISA and Immunoblotting in murine toxoplasmosis. Europ. J. Protistol. 27, 40-45
15. Hafid, J., Vincent, N., Flori, P., Bellete, B., Raberin, H., Tran Manh Sung, R., in press. Production of antibodies in murine mucosal immunization with *Toxoplasma gondii* excreted/secreted antigens. Vet. Parasitol
16. Isamida, T., Tanaka, T., Omata, Y., Yamauchi, K., Shimazaki, K., Saito, A., 1998. Protective effect of lactoferricin against *Toxoplasma gondii* infection in mice. J. Vet. Med. Sci. 60, 241-244
17. Krupa, K., Bartoszcze, M., 1990. Rezerwuary toksoplazmozy. Przegl. Epid. 44, 317-321
18. Letscher-Bru, V., Pfaff, A.W., Abou-Bacar, A., Filisetti, D., Antoni, E., Villard, O., Klein, J.P., Candolfi, E., 2003. Vaccination with *Toxoplasma gondii* SAG-1 protein is protective against congenital toxoplasmosis in BALB/c mice but not in CBA/J mice. Infect. Immun. 71, 6615-6619
19. Owen, M.R., Trees, A.J., 1998. Vertical transmission of *Toxoplasma gondii* from chronically infected house (Mus musculus) and field (Apodemus sylvaticus) mice determined by polymerase chain reaction. Parasitol. 116, 299-304
20. Pinckney, R.D., Lindsay, D.S., Blagburn, B.L, 1995. Further characterization of the TS-4 temperature-sensitive mutant of *Toxoplasma gondii* in mice. J Parasitol. 81, 118-121
21. Roberts, C.W., Alexander, J., 1992. Studies on a murine model of congenital toxoplasmosis: vertical disease transmission only occurs in BALB/c mice infected for the first time during pregnancy. Parasitol. 104, 19-23
22. Stahl, W., Kaneda, Y., 1998. Cerebral anomalies in congenital murine toxoplasmosis: a preliminary report. Tokai J. Exp. Clin. Med. 23, 261-365
23. Stahl, W., Sekiguchi, M., Kaneda, Y., 2002. Cerebellar anomalies in congenital murine toxoplasmosis. Parasitol Res. 88 , 507-512
24. Thouvenin, M., Candolfi, E., Villard, O., Klein, J.P., Kien, T., 1997. Immune response in a murine model of congenital toxoplasmosis: increased susceptibility of pregnant mice and transplacental passage of *Toxoplasma gondii* are type 2-dependent. Parassitologia 39, 279-283
25. Wong, S.Y., Remington, J.S. 1994. Toxplasmosis in prenancy. Clin. Infect. Dis. 18, 853-862
26. Zenner. L., Darcy. F., Capron. A., Cesbron-Delauw, M.F., 1998. *Toxoplasma gondii*: kinetics of the dissemination in the host tissues during the acute phase of infection of mice and rats. Exp. Parasitol. 90, 86-94