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TOXOPLASMA GONDII INFECTION IN RATS BY THE RH STRAIN: INOCULUM AND AGE EFFECTS

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Summary :

Toxoplasma gondii strains are classified according to their virulence in mice. Rats are considered to be resistant to the infection, depending on the age. Newborn rats are fully susceptible but weaned rats are resistant. However the effect of inoculum has not been examined. Using RH strain inocula of 10^2, 10^4, 5×10^7 and 10^8 tachyzoites intraperitoneally inoculated into Wistar and Fischer rats of 7, 11, 21, 24 and 46 days old, the authors show that inoculum and not the age of the host had a statistically significant effect ($p < 0.01$) on the survival curve.

KEY WORDS : *Toxoplasma gondii*, RH strain, virulence, rat, liver lesion.

Résumé : INFECTION DU RAT PAR LA SOUCHE RH DE *TOXOPLASMA GONDII* : ROLE DE L'EFFET INOCULUM

Les souches de Toxoplasma gondii *sont classées en fonction de leur virulence pour la souris. On considère que la résistance du rat à l'infection est habituellement liée à l'âge. Les rats nouveau-nés sont très sensibles mais après le sevrage les rats sont résistants. En utilisant la souche RH à des inoculums de 10^2, 10^4, 5×10^7 et 10^8 tachyzoïtes par voie intrapéritonéale à des rats Fischer et Wistar âgés de 7, 11, 21, 24 et 46 jours, les auteurs montrent que l'importance de l'inoculum a un effet statistiquement significatif sur la survie ($p < 0,01$) alors que celui de l'âge à l'inoculation n'en a pas.*

MOTS CLÉS : Toxoplasma gondii, *souche RH, virulence, rats, lésions hépatiques.*

INTRODUCTION

Toxoplasma gondii strains are classified as virulent or non-virulent based on infectivity in mice. The RH strain of *T. gondii* is the most virulent strain for mice. It was isolated from the brain of a six-year old child (Sabin, 1941). Genetically, it is one of the type I strains (Howe & Sibley, 1995) which are extremely virulent in mice; one infective tachyzoite is lethal to mice (Dubey, 1998). Virulence of types II and III strains of *T. gondii* is variable (Howe & Sibley, 1995). Rats are considered to be one of the most resistant hosts for RH strain *T. gondii* but opinions differ with respect to age of resistance acquisition, lethal dose, and formation of tissue cysts (Lainson, 1955; Lewis & Markell, 1958; Kulasiri, 1962; De Meuter, 1972; Chinchilla *et al.*, 1981; Dubey & Frenkel, 1998). There is general agreement, except for the conflicting report of Remington *et al.* (1958), that natural resistance starts at an early age and that inoculums $< 10^7$ do not kill the animal. The aim of this study was to determine the effect of dose on morbidity and mortality in rats inoculated intraperitoneally (i.p.) with RH strain.

MATERIAL AND METHODS

Tachyzoites of the RH strain of *T. gondii* were obtained from mouse peritoneal fluid three days after i.p. inoculation and washed twice in physiological saline (0.85 % NaCl). The strain was subinoculated twice weekly into Swiss OF1 mice (IFFA-CREDO, L'Arbresle, France) in our laboratory.

In total 30 rats were used in the present study (Table I). 24 male and female Wistar rats from three litters were assigned to 12 pairs. Three different pairs were i.p. inoculated at the age of 7, 11, 24 and 46 days with either 10^2,10^4 or 10^8 tachyzoites of the *T. gondii* RH strain (De Champs *et al.*, 1997). In each pair, one was killed two weeks and the other four weeks after inoculation by barbiturate overdose. They were weighed at the time of inoculation and at sacrifice.

A quarter of each rat brain was homogenized in 1 ml of phosphate buffer saline (PBS) and 500 µl inoculated into a Swiss mouse and another quarter was used for histological examination. The remaining half was

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Age (days)	No of dead rats/ No of rats injected with stated number of tachyzoites 10^2	10^4	5.10^7 *	10^8	No of dead rats /No of rats injected
7	0/2	1/2	0	2/2	3/6
11	0/2	0/2	0	2/2	2/6
21	0/0	0/0	1/2	0/0	1/2
24	0/2	0/2	0	2/2	2/6
46	0/2	0/2	1/4	0/2	1/10
No of dead rats/ No of rats injected	**0/8**	**1/8**	**2/6**	**6/8**	**9/30**

* The 6 rats inoculated with 5.10^7 tachyzoites were Fischer rats, all others were Wistar rats.

Table I. – Mortality in rats in relation to age and the number of tachyzoites of *T. gondii* inoculated.

homogenized in PBS in a Potter's tube, purified by centrifugation on a discontinuous density gradient of 30 % and 90 % Percoll (Blewett *et al.,* 1983) and examined for the presence of tissue cysts. For seven of the rats inoculated at 24 and 46 days, histological sections of the liver were also examined.

Six male Fischer rats were inoculated i.p. with 5×10^7 tachyzoites, two at the age of 21 days and four at 46 days. They were weighed during the first 10 days and killed seven months later. The brain was homogenized in 2 ml PBS in a Potter's tube and purified in Percoll gradient. All the rats were fed *ad libitum.*

Weights of the rats inoculated after 11 days were expressed in standard deviation (SD) according to the mean weight for the age, using the growth curve for the species given by IFFA CREDO. Weight losses were calculated by substracting from the weight (in SD) at inoculation, the weight (in SD) at the 10th, 15th or 30th days, according to the date of sacrifice. They were compared according to inoculum or age at inoculation by the Krushall-Wallis rank-test (Woolson, 1987).

Spleen, liver, heart, lungs and brain were processed for histological examination from three of the rats that died of acute toxoplasmosis. Paraffin-embedded sections were cut at 5 µm and sections were stained with Giemsa, periodic acid Schiff (PAS), hematoxylin-eosin-saffron (HES) and indirect immunofluorescent assay (IFA) (Derouin *et al.*, 1989).

After paraffin removal and rehydration, the sections were incubated for 30 min at 37 °C with a rabbit polyclonal anti-*T. gondii* hyperimmune serum diluted 1/300 in PBS. After washes in PBS they were treated with anti-rabbit IgG conjugate (Sanofi Diagnostic Pasteur, Marnes la Coquette, France) at 1/100 dilution in PBS. The specimens were observed by epifluorescence microscopy. The survival curves were compared using the Mantel-Haenzel test (Woolson, 1987).

RESULTS

The mortality rate according to age and inoculum are summarized in Table I. All deaths occurred within the first eight days after inoculation. No rat died after inoculation with 10^2 tachyzoites. The rat that died after an inoculation with 10^4 tachyzoites was seven days old when inoculated. The statistical analysis of the survival curve showed a significant difference for the inoculum ($p < 0.01$) but not for age.

Among the surviving rats, one rat inoculated with 10^2 tachyzoites at seven days lost its hair. With the inoculum $\geq 10^4$ a delay in growth was observed, and with 5.10^7 and 10^8 the rats lost almost three standard deviations in weight (Table II).

On histological examination, the rats that died during the acute infection had no lesions in the lungs or spleen. In the liver, the Glisson capsule was thicker and infiltrated by lymphocyte cells and numerous tachyzoites were seen. Tachyzoites were observed in the brain of two rats, and inflammatory lesions in their heart were associated with tachyzoites-shaped organisms. A total amount of 10^2 to 10^6 tachyzoites was harvested from the ascites.

Tissue cysts were observed in the homogenate brain of nine killed rats (Table III) and none at the histological examination of the brain or the liver (De Champs *et al.*, 1997). Eleven of the 17 mice inoculated i.p. with the brain of these rats died.

Inoculum	10^2 (n = 4)**	10^4 (n = 4)	$5 . 10^7$ (n = 4)	10^8 (n = 2)
Standard deviation (mean)	+ 0.1	– 1.9	– 2.8	– 2.8

* Difference between the standard deviation at inoculation and 15 or 30 days after inoculation, according to the growth curves of the species given by IFFA CREDO.
** Number of surviving rats inoculated after 11 days.

Table II. – Weight growth delay *.

Day rat was killed	No of rats killed	No of rats with tissue cyst in brain homogenate	No of *T. gondii* infected rats*
15	9	3	6
30	8	6	5
207-230	4	0	2

* By bioassay in mice inoculated i.p. with rat brains.

Table III. – Persistence of RH strain in brains of rats according to the date of kill.

No tissue cyst was observed in the homogenate brain of the four rats inoculated with 5.10^7 tachyzoites and killed seven months later. The mice subinoculated with the brain of two rats survived, and those inoculated with the brain of two other rats died 87 and 130 days later. *T. gondii* were observed in ascites from one of the mice. The proportion of mice that died after i.p. subinoculation with the brain of rats decreased when the rats were killed later and when the rats were older at inoculation (Table IV). The association between the age of rats at inoculation and the number of subinoculated mice that died was statistically significant ($p < 0.01$).

Age at injection (days)	No of rats killed	No of rats with tissue cyst in brain homogenate	No of infected rats*
7	3	1	2
11	4	3	4
21	1	0	1
24	4	2	4
46	9	3	2

* By bioassay in mice inoculated i.p. with rat brains.

Table IV. – Persistence of RH strain *T. gonidii* in brains of rats according to the date at inoculation.

DISCUSSION

The results of this study show that the resistance of rats to infection by the RH strain is only partially age-dependent and that large inocula can be lethal for adult animals. These results agree with those obtained with RH strain (Remington *et al.*, 1958) and with VEG strain oocysts (Dubey, 1996) in Sprague-Dawley rats. Our findings do not support the hypothesis that weaned rats are resistant and that resistance is acquired at an early age (Lainson, 1955; De Meuter, 1972; Chinchilla *et al.*, 1981).

The lethal dose of *T. gondii* for rats varies with age of the rat. De Meuter (1972) observed 88 % (22/25) mortality in one day old rats inoculated with 10^5 tachyzoites and Remington *et al.* (1958) found 33 to 50 % mortality in Sprague-Dawley rats weighing 120 g (then probably about five week old) inoculated with 10^8 tachyzoites. From these studies it is concluded that three rat species, Wistar, Fischer and Sprague-Dawley, have similar resistance to RH strain and that lethal dose of tachyzoites is $> 10^7$ for adult rats and 10^4 for younger rats.

There is little documented evidence on clinical disease in rats. Toxoplasmosis in rats had been considered asymptomatic (Frenkel, 1956; Lewis & Markell, 1958). In our studies infection with inoculum $\leq 10^7$ tachyzoites was followed by growth delay.

Dubey (1996) showed that the number of tissue cysts observed in tissue sections was significantly lower than in brain homogenates. This could explain why no tissue cyst was found in the histological sections of our study because very few tissue cysts were obtained in the brain homogenates. However, subinoculation of rat brains into mice indicated that rats were infected with *T. gondii*. The persistence of the RH strain in rats is variable and depends on the infecting dose (Pettersen, 1988). In our study, the survival of the i.p. subinoculated mice was more closely related to the age of the rats at infection than to the date of the kill. But the absence of early fatal toxoplasmosis among the mice subinoculated with the brain of rats killed after 200 days post-infection suggests that the stage or the virulence of the RH strain in these rats is not the same as during the first month post-infection.

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