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Toxoplasma gondii (Eucoccidia: Sarcoystidae) dissemination pattern in rats after oral infection with oocysts of an avirulent strain

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Abstract: Differences in *Toxoplasma gondii* dissemination in white rats (Sprague Dowley) and mice (Wistar) after oral oocyst inoculation are described. Groups of five animals (both hosts) were infected per os with oocysts of the TCR-2 avirulent strain and the dissemination pattern was compared in brain tissue or by serology. Early dissemination was similar in both species. One hr after infection the parasite was present in blood and peritoneal exudate as well as in heart, lung, liver, spleen, lymph nodes and brain. However, after five days there were important differences between both hosts and after 30 days, the parasite was detected only in rat heart and brain, while in mice it persisted in fluids and all organs.

Key words: *Toxoplasma gondii* pathology, natural resistance, rats, mice.

White rat resistance to *Toxoplasma gondii* hast not been clearly studied. In early studies, Ruchman and Fowler (1951), using a virulent strain, demonstrated that this parasite was present in the blood for 4, 24, 48 and 72 hr after infection. Four days later they were found in all organs and remained in the brain for two years. Comparing *T. gondii* dissemination in rats and rabbits, Remington *et al.* (1961) reported that it was impossible to find parasites in blood of chronicly infected rats, while in rabbits it was present in blood for up to six months.

All previous studies on rat resistance were done by intraperitoneal inoculation of tachyzoites and remarkable differences in susceptibility between mice and rats were found. There are many studies dealing with the presence of *T. gondii* in organs of mice infected with different strains, under several inoculation conditions (Remington *et al.* 1961, Dubey and Frenkel 1973, Araujo *et al.* 1976, McLeod *et al.* 1984, Sumyuen *et al.* 1995). However, there is no comparative analysis on natural dissemination of *T. gondii* in both animals when they are inoculated per os with oocysts of an avirulent strain. Since it is well known that this is the normal mode of infection in intermediate hosts (Miller *et al.* 1972), it was interesting to study the dissemination pattern under the conditions. Therefore in this work we report some important differences in the behaviour of *T. gondii* in a resistant and a susceptible host after oral inoculation oocyst.

MATERIALS AND METHODS

Animals: Two months old cats, Sprague Dowley rats (150-200 body weight) and Wistar mice (20-25 g) were used in these experiments. In addition, C3H mice (20-25 g) were sub- inoculated with fluids and organs to demonstrate the presence of parasites. All the animals were caged and fed with water and a local concentrate.

Animal infection: To obtain oocysts for the experiments, brain cysts from mice, infected with the partially characterized TCR-2 *T. gondii* strain (Holst and Chinchilla 1990, Guerrero *et al.* 1991), were inoculated per os in cats. Oocysts were collected and prepared as previously described (Dubey *et al.* 1970 1972). Thirty three rats or mice were infected per os with 10^5 oocysts per rat and 10^2 per mouse. These inocula were selected on a basis of previous reports (Chinchilla *et al.* 1981) and other unpublished studies.

Experimental model of Toxoplasma dissemination: During one, 4, 8 and 24 hr as well as 2, 3, 4, 5, 10, 15 and 30 d after infection, the blood and peritoneal exudate (pex) were studied in each animal. The first fluid obtained by cardiac puncture using heparin as anticoagulant, was inoculated in two C3H mice (0.5 ml from rat or 0.25 ml from white mice) to determine parasite circulation. Pex, taking out in supplemented 199 Medium, was stained by Giemsa method to determine cell type and presence of *T. gondii.* Part of this fluid was also inoculated in C3H mice to detect parasites.

Simultaneously the animals were dissected and parts of heart, brain, lung, lymph node, liver and spleen were fixed in 10% formalin (pH 7) and treated as usual for histological examination. The remaining portion of those organs were ground with sterile permutite, suspended in 2 ml saline solution and inoculated in C3H mice, 1.8 ml for one and 0.2 ml for other mouse (Chinchilla and Frenkel 1978).

Survival time of all the sub-inoculated animals was recorded, and the serum of all the survivors for 30 d was examined for *T. gondii* antibodies (Sabin and Feldman 1948) and for brain tissue cysts.

Statistical tests were not applied because of the qualitative evaluation of parasite occurrence.

RESULTS

Serologic analysis indicated that in both animals, rats and mice, the parasite was in blood and Pex after 1 hr inoculation.

T. gondii tissue cysts were found in animals sub-inoculated with blood after 1 d and then 5, 10, 15 and 30 d or with pex of 10 d infected mice. It was not the same for rat infections (Fig.1).

In rats these fluids were negative at 10,15 and 30 d after infection, while in mice,blood was always positive.

Pex from 5, 10 and 15 d infected mice stimulated *T. gondii* antibodies in sub-inoculated animals (Fig. 2). On the other hand, studies of stained Pex smears did not show extra or intracellular parasites. The only interesting observation was the higher number of eosinophils found in rat exudate as compared with mice, specially 8 hr after infection.

T. gondii migration through internal organs was demonstrated by direct and indirect finding of parasites in sub-inoculated mice. According to antibody presence we found that the parasite was already present after 1 hr infection in the liver and the lung of the mice (Fig. 3) and the organisms remained in these organs for 30 d. Although the parasite was also present in those organs of rats after 24 hr infection, the organisms dissapeared as long as the infection time increased and 30 d later there was no *T. gondii* antibodies in the subinoculated mice.

According to serological analysis of sub-inoculated mice, lymph node and spleen were also positive in both animals after 24 hr infection. However, while in 5, 10 and 15 d infected rats a decrease in organ positivity was observed, in mice the organs remained positive, independent of the infection day (Fig. 4).

The positivity of mice sub-inoculated with brain and heart of both animals indicated a constant presence of the parasite during the 30 d (Fig. 5). In rats there was a decrease of heart positivity, specially after 15 d and then remained low until 30 d of infection.

Histopathological analysis of lung, liver, spleen and lymph node showed the presence of *T. gondii* and some lesions in rats with less than 5 d of infection. After that time, those organs were negative and normal. On the other hand, organs from mice demonstrated the usual and known pathology.

Rat intestinal tissues were always normal and without parasites, which was different in mice whose organs presented *T. gondii* tachyzoites after 4 and 10 d of infection, disappearing later on. Despite of the absence of parasites, eosinophilic cells were observed in the small intestine and in the lung of the infected rats.

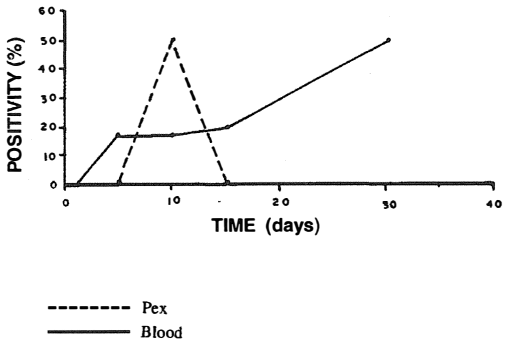

Fig. 1. Presence of brain cysts in mice inoculated with blood or peritoneal (Pex) from mice infected with 10^2 *T. gondii* oocysts.

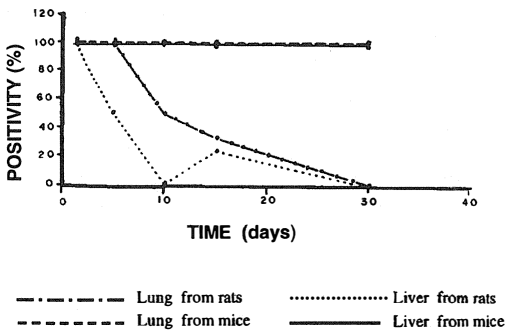

Fig. 3. Positivity (%) by antibody presence in mice inoculated with lung or liver from rats or mice infected with *T. gondii* oocysts (10^5 / rat, 10^2 / mouse).

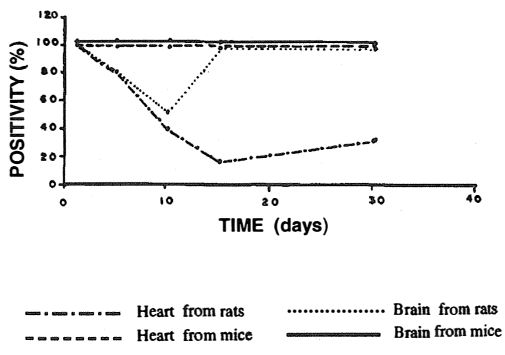

Fig. 5. Positivity (%) by antibody presence in mice inoculated with heart or brain from rats or mice infected with *T. gondii* oocysts (10^5 / rat, 10^2 / mouse).

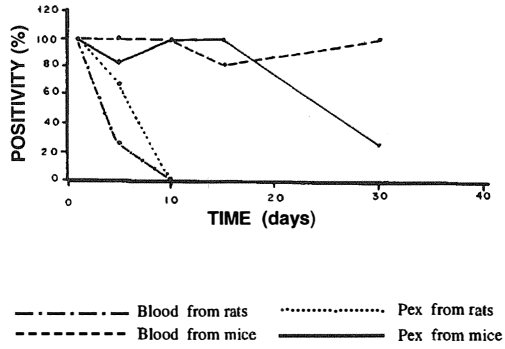

Fig. 2. Positivity (%) by antibody presence in mice inoculated with fluid from rats or mice previously infected with *T. gondii* oocysts (10^5/rat, 10^2/mouse).

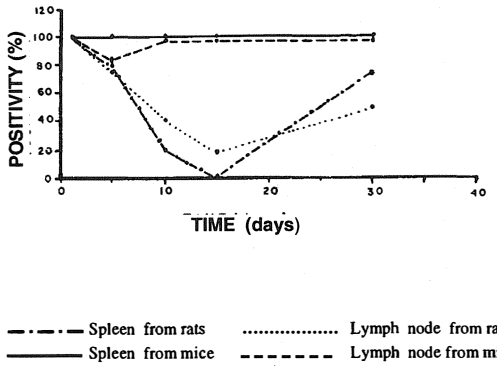

Fig. 4. Positivity (%) by antibody presence in mice inoculated with spleen or lymph node from rats and mice infected with *T. gondii* oocysts (10^5 / rat, 10^2 / mouse).

Non-inoculated animals, used as controls, did not present any pathological lesions.

DISCUSSION

Our results show that there is a rapid parasite dissemination in both hosts and in the first days, parasitaemia fluctuates but after 5 d, the pattern is different for mice and for rats. In fact, the parasite presence in blood and pex from rats was decreasing and quickly disappeared. On the contrary, mice pex was clearly positive until 15 d, decreasing later, while organisms were detected in the blood throughout all the experiment (Fig.2). The results observed for us in mice infection, specially

TABLE 1

Survival time (days) of mice inoculated with fluids from rats and mice infected with 102 oocysts of T. gondii

Source	Animal fluid	Infection time: Hours				Days						
		1	4	8	24	2	3	4	5	10	15	30
Rats	Pex	30	30	30	30	30	30	30	25	30	28	30
Mice	Pex	30	27	27	30	30	30	30	30	30	30	30
Rats	Blood	30	30	30	30	30	30	27	26	30	30	27
Mice	Blood	27	23	30	30	30	30	30	30	24	30	30

regarding to early presence of parasites, are different of those reported by Sumyuen *et al.* (1995), since they did not find organisms in blood or some organs in the first days. However they infected the animals with tissue cysts, and monitored the parasite presence by an "in vitro" method. We used oocysts as inoculum and *T. gondii* infection was followed by animal subinoculation.

The results observed for *T. gondii* rat infections correlate with those reported by Ruchman and Fowler (1951), who found that RH strain infected animals showed the parasite in blood after 4 hr infection finding the major number of organisms 4 or 5 d later. After that time they were found sporadically.

On the other hand, comparing our studies of an avirulent strain with those reported by Remington *et al.* (1961), there is certain concordance. In fact these authors did not find parasites, in the blood of rats after 2, 3, 5, 8, 9 and 11 months of infection with avirulent strains, while in mice, *T. gondii* was present in blood, as expected, for 8 months which demonstrates the differences between these 2 rodents.

Our data confirm the natural resistance of rats, resistance that could be due to several factors such as macrophage activity (Chinchilla *et al.* 1981, 1982) or a lytic hostil factor described by Petersen (1988).

According to the serological analysis of subinoculated mice, it was posible to observe that while the rats showed a diminishing of the *T. gondii* presence after two weeks of infection, mice hold the parasite throughout the 30 d infection (Figs. 2, 3, 4). Data obtained for mice correlates with those previously reported (Sumyuen *et al.* 1995). In addition there was evidence of important lesions in the organs of rats only after the first week of infection. In mice it was observed in almost all the infection periods. These data relate with those reported by Ruchman and Fowler (1951) who worked with the virulent RH strain as well as with other reports (Mc Leod *et al.* 1984).

T. gondii tissue cysts presence in the brain of both host after 30 d of infection (Fig.5), indicates that the parasite encysts in this organ independent of the host. Recently this fact has been demonstrated for mice by Sumyuen *et al.* (1995).

With these results it has been shown again that despite of mice strain susceptibility (Araujo *et al.* 1976), mouse organs present a major *Toxoplasma* presence.

In epidemiological studies it has been found that *T. gondii* is more present in rats that in mice (Chinchilla 1978, Burridge *et al.* 1979). This low prevalence is maybe due to the higher susceptibility of mice that easily die before the infection can be detected (Jacobs 1956).

On the other hand, as we have demonstrated in this work, rat present a clear parasite elimination capacity from most of the organs, leaving tissue cysts only in heart and brain which makes this animal an excelent *T. gondii* reservoir (Remington *et al.* 1961). However, we can not discard mice since they still are very important in the epidemiology of this organism; in fact, some animals survive low infections with avirulent strains (Chinchilla 1978, Guerrero *et al.* 1991), that eventually can infect domestic cats. Furthermore most strains of *T. gondii* are avirulent and do not kill mice.

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