Experimental Infection of *Calomys callosus* (Rodentia, Cricetidae) by *Toxoplasma gondii*

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Calomys callosus, Rengger 1830 (Rodentia, Cricetidae), a wild rodent found in Central Brazil, was studied to investigate its susceptibility to *Toxoplasma gondii* experimental infection and its humoral immune response against this parasite. The electrophoretic profile of the serum proteins of *C. callosus* showed that IgG, which shows no affinity to Protein A, has higher cross reactivity with rat IgG than with IgG from other rodents. The susceptibility assay was performed by inoculation groups of animals with various suspensions of *T. gondii* tachyzoites from $10^3$ to $10^6$ parasites. All animals died between 3 and 9 days after infection and the kinetics of antibody synthesis was determined. Basically, they recognized predominantly the immunodominant antigen SAG-1 (P30). The immunohistochemistry assays revealed that the liver was the most heavily infected organ, followed by the spleen, lungs, intestine, brain and kidneys. It can be concluded that *C. callosus* is an excellent experimental model for acute phase of *Toxoplasma* infection.

Key words: *Toxoplasma gondii* - *Calomys callosus* - experimental infection

*Toxoplasma gondii*, an intracellular coccidian, infects a wide range of eukaryotic cells and it is an important opportunistic pathogen for humans and others animals (Jackson & Hutchison 1989). The infection is frequently assymptomatic but there are two groups of high-risk individuals: the human foetuses and the immunosuppressed patients, particularly those with acquired immunodeficiency syndrome (AIDS), that develop fatal toxoplasma meningoencephalitis.

Several aspects of the mechanisms responsible for the pathological lesions leading to the clinical toxoplasmosis have been elucidated by using experimental models (Hutchison et al. 1982, Graham et al. 1984, Sims et al. 1988) and mouse has been the most employed animal in these studies (Gazzinelli et al. 1992). However, the experimental infection of wild rodents is poorly explored. On the other hand, it has been demonstrated that wild animal species present unique aspects when they are studied in investigation focusing host-parasite interactions (Deane et al. 1984, Jansen et al. 1985, Borges et al. 1992).

*Calomys callosus* (Rodentia, Cricetidae) has been described as a reservoir for various infectious agents (Petter et al. 1967, Justines & Johnson 1970, Ribeiro 1973, Borges et al. 1992, Vaz-de-Lima et al. 1992). This paper presents the results of a study involving the experimental infection of *C. callosus* by *T. gondii* in order to evaluate the degree of its susceptibility to the parasite and its applicability as a model for experimental toxoplasmosis.

**MATERIALS AND METHODS**

Parasites - *T. gondii* parasites of the RH strain were maintained by serial passage in Swiss mice by standard procedure as described previously (Camargo et al. 1978, Mineo et al. 1980).

*Calomys callosus* specimens - The specimens of *C. callosus* of the Canabrava strain used in this study belong to a resident colony housed at the Laboratory of Histology from Universidade Federal de Uberlândia and were kindly provided by Dr Judith Kloetzel. The animals were kept on a 12 hr light:12 hr dark cycle in a temperature controlled room (25°C) with food and water *ad libitum*. All animals were approximately 60 days old males, weighing about 25 g on inoculation. Serum samples were obtained from 20 animals and the immunoglobulins were isolated by precipitation on 40% ammonium sulphate solution. Both total serum proteins and immunoglobulins were analyzed by SDS-PAGE and submitted to western-blotting.

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analysis after being transferred to nitrocellulose membranes (Burnette 1981). Experiments were carried out with protein-A and antibodies against rat, mouse, or rabbit in order to detect cross-reactivity to C. callosus immunoglobulins.

Experimental infection - Six groups of five animals were used in these experiments. The animals of each group were inoculated intraperitoneally with 0.2 ml of various suspensions calculated to contain 10^2, 10^3, 10^4, 10^5 and 10^6 tachyzoites of T. gondii. Animals from the control group were inoculated with 0.2 ml media only. Parasites were obtained from mice peritoneal exsudate, washed twice in 1640 RPMI (Sigma Co., St. Louis, USA) containing 50 mg/ml of gentamycin. The viable parasites were counted in Neubauer hemocytometer chamber in suspensions containing trypan-blue. Blood samples were collected from day 1 to day 9 after infection to determine the kinetics of antibody synthesis. Mortality was also observed up to nine days. As soon as the animals died, peritoneal exsudate was collected for parasite search. The next step was the collection of organs for histopathology and immunohistochemistry analysis. Liver, spleen, intestine, lung, testis, kidney and brain were examined.

ELISA for determination of anti-T. gondii antibodies in serum samples - An indirect immunoenzymatic test was employed as described (Camargo et al. 1978), with some modifications. Protein binding ELISA microtiter plates (Interlab, Brazil) were coated with 50 ml of a suspension of 1 x 10^6 tachyzoites of T. gondii/ml diluted in PBS. The plates were dried at 37°C and kept at -20°C until the serum samples were tested. The plates were then washed three times with phosphate-buffered saline containing 0.05% Tween 20 (PBS-T) and blocked with 5% low fat milk solution for 30 min at room temperature and incubated with the samples. Twofold dilutions of serum samples, diluted in PBS-T from 1:16 to 1:64, were added in duplicates to the plates and incubated for 45 min at 37°C. The third incubation step was carried out with the conjugate consisting of the Protein A-affinity purified rabbit IgG against C. callosus immunoglobulins labelled with peroxidase (type VI, Sigma, USA). After incubation with this conjugate for 45 min at 37°C, the plates were incubated with substrate solution consisting of H2O2 (Merck, Germany) and o-phenylenediamine (Merck, Germany) in 0.1M citrate-Na2 HPO4 buffer (pH 5.5) for 15 min at room temperature. The reaction was stopped with 2N H2SO4 and the absorbance was read at 492 nm in a microwell reader system (Titertek Multiskan-Plus spectrophotometer, Flow Laboratories, USA). As controls, the serum samples and the conjugate were also incubated with uncoated plates in the same conditions. In addition, serum samples obtained from uninfected animals were also included.

The parasitological examination revealed presence of tachyzoites in the peritoneal fluids of the animals as soon as they died. The immunohistochemistry assays demonstrated that parasites were present in all examined organs, except testis. The liver was the most intensively infected organ. Fig. 3 shows that the parasitophorous vacuoles are related with the number of inoculated parasites. The parasitological examination revealed pres- ence of tachyzoites in the peritoneal fluids of the animals as soon as they died. The immunohistochemistry assays demonstrated that parasites were present in all examined organs, except testis. The liver was the most intensively infected organ. Fig. 3 shows that the parasitophorous vacuoles are related with the number of inoculated parasites.
The immune system components of *C. callosus* have been studied. Structural and functional similarities and differences with other organisms have been described. Homology between human and *C. callosus* complement components, particularly C3, C4, B and B, has already been demonstrated [Vaz de Lima et al. 1992]. The profile of hydrogen peroxide release from peritoneal macrophages from *Trypanosoma cruzi* infected mice and *C. callosus* shows some noteworthy differences, even though a parallel between macrophage activation and histopathological findings was observed in this work [Borges et al. 1992].

The present investigation showed cross-reactivity between IgG molecules from *C. callosus* and rat, demonstrating the existence of conserved epitopes which were not found among other studied rodents. Interestingly, *C. callosus* IgG shows no affinity to protein A which is one particular feature among other rodents.

The data presented in this investigation demonstrated that *C. callosus* is highly susceptible to infection by RH strain of *T. gondii*. High mortality and massive presence of parasites in peritoneal cavity of the infected animals were found. Different susceptibilities to *T. gondii* infection were shown among various inbred and outbred laboratory rodents [Fujii et al. 1983, Zemmour et al. 1993, Benedetto et al. 1996]. On the basis of several criteria, the animals can be divided into three groups: (i) highly sensitive (S) as mice, Syrian hamsters and Chinese hamsters; (ii) moderately sensitive (M) as Mongolian gerbils and mastomys; and (iii) resistant (R) as rats and Korean striped field mice. Indeed, we are now studying *C. callosus* as a model for congenital toxoplasmosis by looking on the invasion capacity of trophoblastic cells in *in vivo* experiments.

**REFERENCES**


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