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Development and distribution of cysts of an avirulent strain of *Toxoplasma* and the humoral immune response in mice

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Abstract: An avirulent *T. gondii* strain isolated from a owl *(Glaucidium brasilianum)* produces randomly distributed cysts in the brain of mice which can survive to inocula as high as 1000 oocysts. Cysts appeared for the first time after 15 days of infection. Regarding to humoral immunity development due to our TCR-2 strain, detectable antibodies were found after 12 days of infection.

Key words: Toxoplasma infection, brain cysts, owl.

The life cycle of *Toxoplasma gondii,* an isosporoid parasite, has been correctly characterized (Dubey *et al.* 1970) and the presence of a fecal cystic form is very well known today. The oocyst, which is very resistant to enviromental conditions (Frenkel *et al.* 1975), infects rodents and small birds (Ruiz and Frenkel 1980) as well as cattle and pigs (Jacobs 1974). In all of these animals, groups of tachyzoites develop and produce acute infections, sometimes symptomatic but usually asyntomatic (Frenkel and Ruiz 1973). Later these hosts will show chronic infections characterized by cyst formation more frequently in the brain and sometimes in muscle tissue (Jacobs 1967).

The presence of these cysts in intermediate hosts is not only very important to ensure the *Toxoplasma* infections permanency, but because it is the reason why humans can be infected by ingestion of uncooked meat (Frenkel 1981).

Since avirulent strains of *T. gondii* usually produce the cystic form and the TCR-2 Toxoplasma strain, isolated from a bird *(Glaucidium brasilianum),* behaves as a nonvirulent one, it was convenient to study the cyst formation as well as the immune response to it in an experimental model as part of the characterization of this strain, which is used in our laboratory.

MATERIAL AND METHODS

Animals: male and female NIH mice (15-20 grs) were placed in plastic cages and concentrated food and water was giving *adlibitum.*

Inocula preparation. mature and viable oocysts from a Toxoplasma strain (TCR-2) isolated from an owl *(Glaucidium brasilianum)* and preserved in 2% H_2SO_4 were used throughout this study. This material was washed 5 times with destilled water by centrifugation at 2200 rpm and using a Thoma white cells pipette, occysts were counted and inocula of 10, 100 or 1000 mature oocysts per 0.1 ml was prepared to use according to each experiment.

Experimental model to study oocyst development and serological response: Part A: Groups of 10 mice were inoculated per os, with 10, 100 or 1000 oocysts per 0.1 ml using a 1 ml syringe with a special needle that was introduced in the aesophagus. The syringe with the inoculum was shaked before infecting each animal in order to obtain the correct occyst distribution in mice.

Animal body weight was weekly recorded for six weeks and after this period of time mice were weighed and bleeded by cardiac punction to collect serum for serological studies. Once the animals were killed in ether camera, their brains were taken out and weighed. Portions from

right or left side as well as the upper or lower area of the brains of each mouse were weighed and the number of cysts in all portions were counted in fresh preparations and observed under microscope using 45% objetive. With these data, number of cysts per brain or per gram of brain were determined.

Part B. Eighty mice were inoculated with 100 mature Toxoplasma oocysts following the methods described in part A. Ten non-infected animals were kept in the same conditions as normal controls.

Every 3 days and for 1 months, 10 of the above infected mice were sacrificed and studied as follows:

a) Bleeded by cardiac punction to obtain serum for serological analysis.
b) Determination of Toxoplasma cysts in fresh brain preparation under microscope observation.
c) Measurement of Toxoplasma cysts to determine their growth rate.

Control animals were killed and studied also, using the same procedures at the end of the experiment.

T. gondii infection in mice which died before the 30 days experimental period was confirmed by lung smears.

Serological studies: antibody presence was determined using the carbon immunoassay test (CIA). This technic has been described elsewhere (Bergquist and Waller 1983). Briefly, formalin fixed tachyzoites were in contact with texting sera for 30 m at 37ºC, washed and stained with Indian Ink for 5 m, washed again, air dried and observed in the microscope. Presence of more than 50% stained parasites was considered positive.

Statistical analysis: The "t" student test was used for all comparisons, significance is indicated by "t**".

RESULTS

Cuantification of cyst formation: Fig. 1. shows the average number of cysts per brain or gram of brain found in mice infected with different Toxoplasma concentrations. There was a proportional direct correlation between inocula and number of cysts in the infected animals.

A difference (t**) was found between the number of cysts present in mice inoculated with 1000 oocysts and those infected with 100 or 10 oocysts. There was no difference between animals inoculated with lower oocyst concentrations.

Cyst distribution in the brain tissue: The number of cysts was similar in each part of the brain of the mice inoculated with 10 or 100 oocysts (Fig.2). In animals infected with higher inoculum we saw some unsignificant differences.

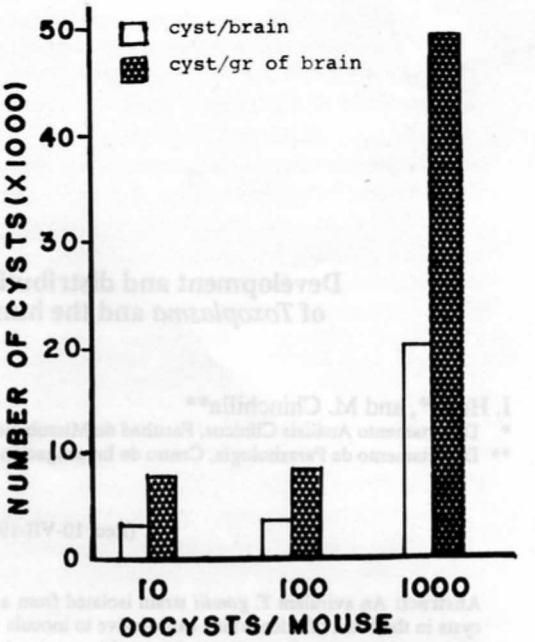

Fig. 1. Average number of *Toxoplasma* cysts per brain or gram of brain from mice infected with different inocula.

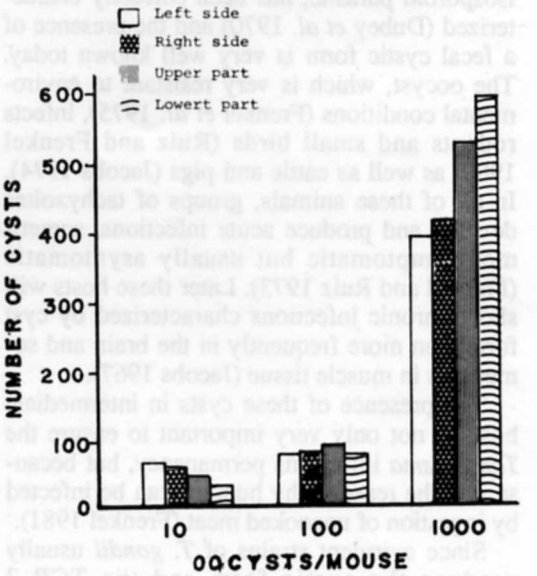

Fig. 2. Average number of *Toxoplasma* cysts in several brain sections (0,01 g) of mice infected with different inocula.