

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 environmental 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 asymptomatic (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 non-virulent 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 *ad libitum*.

Inocula preparation. mature and viable oocysts from a *Toxoplasma* strain (TCR-2) isolated from an owl (*Glaucidium brasilianum*) and preserved in 2% H₂SO₄ were used throughout this study. This material was washed 5 times with distilled water by centrifugation at 2200 rpm and using a Thoma white cells pipette, oocysts 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 oesophagus. The syringe with the inoculum was shaken before infecting each animal in order to obtain the correct oocyst distribution in mice.

Animal body weight was weekly recorded for six weeks and after this period of time mice were weighed and bled by cardiac puncture 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% objective. 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 month, 10 of the above infected mice were sacrificed and studied as follows:

- Bleeded by cardiac puncture to obtain serum for serological analysis.
- Determination of *Toxoplasma* cysts in fresh brain preparation under microscope observation.
- 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 testing sera for 30 m at 37°C, 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

Quantification 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 insignificant differences.

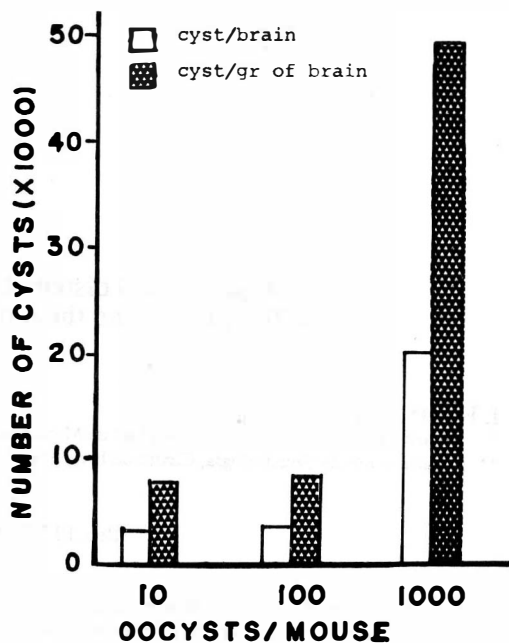


Fig. 1. Average number of *Toxoplasma* cysts per brain or gram of brain from mice infected with different inocula.

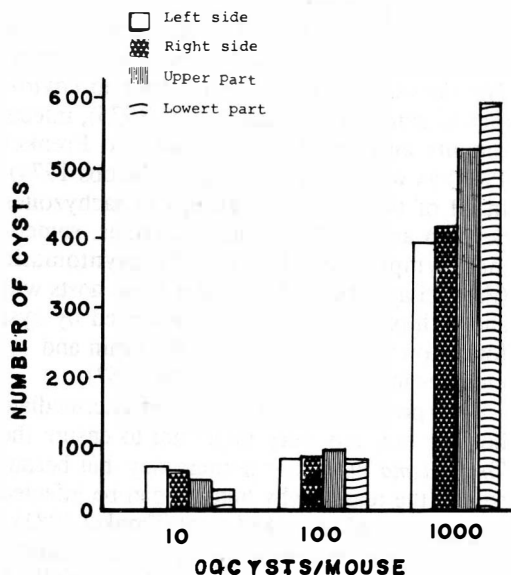


Fig. 2. Average number of *Toxoplasma* cysts in several brain sections (0,01 g) of mice infected with different inocula.

TABLE 1

Average (arithmetic mean) size of *Toxoplasma* cysts from mice after different infection time

| Infection time (days) | Cysts size ($\mu\text{m} \pm \text{Sd}$) |
|-----------------------|--|
| 15 | 5.85 \pm 1.40 |
| 18 | 10.12 \pm 1.60 |
| 22 | 9.50 \pm 0.94 |
| 25 | 11.78 \pm 0.68 |

TABLE 2

Toxoplasma antibodies in mice sera after seven weeks of infection

| Inoculum (oocysts/mouse) | Negative | Positive | | | Total |
|--------------------------|--------------------------------------|----------|-------|--------|-------|
| | | 1:64 | 1:256 | 1:1024 | |
| 10 | 22* | 22 | 45 | 11 | 78 |
| 100 | 10 | --- | 60 | 30 | 90 |
| 1000 | Only one survivor was positive 1:256 | | | | |

* (%)

Toxoplasma cyst development: Cysts were detected in the mice brain tissue 15 days after the oocyst infection. Cysts average sizes after 15, 18, 22 and 25 days of infection are shown in table 1. Size differences observed between animals with 15 and 18, 22 or 25 days of infection or between 22 and 25 days of infection were significant (t^{**}), but not those between animals with 18 days of infection and those studied 22 or 25 days later.

Antibody detection by CIA test: after 12 days of infections antibody levels (1:16 to 1:64) were detected by the CIA test in 20% of the infected mice. Eighteen days later 67% of the animals were positive and after 22 days all animals presented *Toxoplasma* antibodies with titles up to 1:256.

Serological study of animals infected with different number of oocysts is shown in Table 2. 78% of mice inoculated with 10 oocysts presented titles from 1:64 to 1:1024 and 90% of the animals infected with 100 oocysts were seropositive with higher titles (1:256 to 1:1024).

Only one mouse survived to 1000 oocysts infection and presented a title of 1:256.

Body weight variation: Animals inoculated with 10 or 100 *Toxoplasma* oocysts, at the end

of the experiment, had a total weight increase of 4 g (22%) approximately. On the opposite side, mice who received 1000 oocysts had a body weight decrease of 1 gr which represents about 4.8% of the initial weight.

Fig. 3 shows the sequence of the body weight control during the 7 weeks of infection period.

After the first week of infection there was a weight increase in the three groups but in the second week a considerable body weight loss (5.4 g) was observed for those mice infected with 1000 *Toxoplasma* oocysts. Then there was a slow increase and in the last week a small weight loss was found. There were no significant variations for groups of mice inoculated with 10 or 100 oocysts.

DISCUSSION

Toxoplasma oocysts of this strain produced an important amount of cysts in all the infected mice (Fig. 1). Although significant differences in the number of cysts were observed only between animals infected with 1000 oocysts and those inoculated with 10 oocysts, there was a clear correlation of the number of cyst and inocula. This means that this strain can induce good infection in mice, producing cysts in their brains even if the oocyst ingestion is low. Since mice are the principal source of *Toxoplasma* infection for cats (Chinchilla 1978) this TCR-2 strain or other similar keeps the natural life cycle of this parasite. Moreover, some animals infected with the higher inoculum (1000 oocysts) survived 30 days, which indicates that this strain is avirulent: virulent strains kill mice in a very short time (Chinchilla and Frenkel 1978). This is also interesting from the epidemiological point of view because strains like these are responsible for the rodent natural infection already reported in Costa Rica (Chinchilla 1978).

As has been described (Fujita 1960, Dubey & Frenkel 1976) *Toxoplasma* cysts were observed in the brain two weeks after the infection. In addition, significant differences were observed in the size of cysts found in the brains of mice after 15 or 18 days of infection. This appears to be caused by the rapid bradizoite multiplication at the beginning of the brain invasion when the immune response is just starting. However, as soon as immunity develops, parasite multiplication slows down, explaining

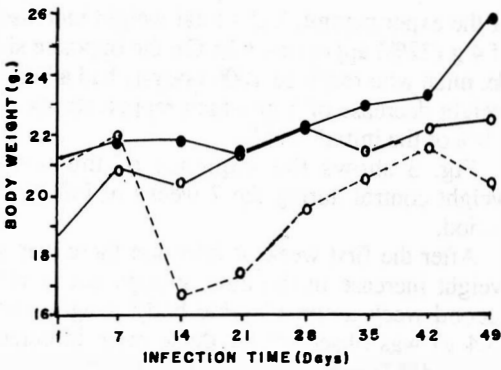


Fig. 3. Body weight variation on mice infected with different *Toxoplasma* oocyst concentrations.

●—● 10 oocysts/mouse
 0—0 100 oocysts
 0.....0 1000 oocysts

why there were no significant differences in the size of cysts from 18, 22 and 25 days-infected mice (Table 1).

We did not observe any differences in the number of cysts in each brain section (Fig.2). These results indicate the need of studying samples of different parts of the brain. Pathological effects of this strain were determined by recording the body weight variation (Frenkel and Havenhill 1963) throughout the infection as well as the survival time of the groups inoculated with different *Toxoplasma* oocysts concentrations. Infections with 10 or 100 oocysts did not produce a considerable body weight diminishing which was evident for mice inoculated with 1000 oocysts (Fig. 3). In addition, only two out of ten mice survived for more than 15 days. Thus, the strain TCR-2 produces some pathogenic effect in higher but not in lower infections which, as discussed before, is very important in the epidemiology of *T. gondii* in Costa Rica.

Analysis of humoral immunity response for this strain (Table 2) indicates that antibody presence was detected by CIA technic after 12 days of infection, 3 days before the cyst finding. This results correlate with other reports (Remington 1974). We expected an earlier immune response but it is known that increased immunity and formation of brain cysts do not seem to be parallel (Akao *et al.* 1989). Furthermore, CIA sensitivity is lower than other tests (Chalupsky 1981). However this

technic is very easy, specific and useful in laboratory studies where we are working with the TCR-2 strain.

With all these results it has been determined that the *Toxoplasma* strain isolated from a bird is able to produce cysts in intermediate hosts, it is not very pathogenic and therefore could play an important role in dissemination of the parasite. Strains like this are present in our country; in previous papers, rodents (Chinchilla 1978) and birds (Ruiz and Frenkel 1980) have been reported positive for *T. gondii*. Brain cyst distribution is another interesting study that will help in future experiments. Actually there is only one similar work in this sense (Akao *et al.* 1989) but our results differ, maybe our strain shows a different behaviour. Regarding the cyst formation and its effect in the host, Akao *et al.* (1989) have demonstrated a correlation between *Toxoplasma* cyst formation and the decrease in aminopeptidase activity of brains from chronic infected mice.

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RESUMEN

En el proceso de caracterización de una cepa avirulenta de *T. gondii* (TCR-2) aislada de un buho (*Glaucidium brasilianum*) se halló que produce quistes distribuidos aleatoriamente, en el cerebro de ratones, los cuales pueden sobrevivir a inóculos hasta de 1000 oquistes por ratón. Su presencia se determinó por primera vez a los 15 días de infección. En cuanto al desarrollo de la inmunidad humoral, determinada por la prueba del carbono inmunoensayo, anticuerpos contra *Toxoplasma* comenzaron a aparecer después de 12 días de infección.

REFERENCES

- Akao, S., K. Yamakami, M. Nishiyama & M. Tanaka. 1989. Aminopeptidase activity in the brains of mice with chronic *Toxoplasma gondii* infections. *Parasitol. Res.* 75:186-188.

- Bergquist, N.R. & T. Waller. 1983. A novel simple immunoassay for rapid detection of human IgG antibodies to *Toxoplasma gondii*. *J. Immunol. Methods* 61: 339-344.
- Chalupsky, J. 1981. Application of the India Ink immunoreaction for the diagnosis of toxoplasmosis. *Folia Parasitologica (Praha)* 28:131-136.
- Chinchilla, M. & J.K. Frenkel. 1978. Mediation of immunity to intracellular infection *Toxoplasma* and *Besnoitia*) within somatic cells. *Infect. Immun.* 19:999-1012.
- Chinchilla, M. 1978. Epidemiología de la toxoplasmosis en Costa Rica: importancia de los roedores domésticos. *Rev. Biol. Trop.* 26:113-124.
- Dubey, J.P. & J.K. Frenkel. 1972. Cyst-induced toxoplasmosis in cats. *J. Protozool.* 19:155-177.
- Dubey, J.P., N.L. Miller & J.K. Frenkel. 1970. Characteristics of a new fecal form of *Toxoplasma gondii*. *J. Parasitol.* 56:447-456.
- Dubey, J.P. & J.K. Frenkel. 1976. Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. *J. Protozool.* 23:537-546.
- Frenkel, J.K. & M.A. Havenhill. 1963. The corticoid sensitivity of golden hamsters, rats and mice. effects of dose, time and route of administration. *Lab. Invest.* 12:1024-1220.
- Frenkel, J.K. 1982. Common questions on toxoplasmosis: veterinary, medical and public health considerations. Annual Meeting of Am. Vet. Med. Ass., p. 1188-1196 *In General Practice.*
- Frenkel, J.K. & A. Ruiz. 1973. Toxoplasmosis humana. Una revisión. *Acta Médica Costarricense* 16:5-73.
- Frenkel J.K., A. Ruiz & M. Chinchilla. 1975. Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. *Am. J. Trop. Med. Hyg.* 24:439-443.
- Fujita, K. 1960. Some biological characteristics of *Toxoplasma* pseudocyst. *Keio Igaku* 27:961-975.
- Jacobs, L. 1974. *Toxoplasma gondii*: parasitology and transmission. *Bull. N. Y. Acad. Med.* 50:128-145.
- Jacobs, L. 1967. *Toxoplasma* and toxoplasmosis. *Adv. Parasitol.* 5:1-44.
- Remington, J.S. 1974. Toxoplasmosis in the adult. *Bull. N.Y. Acad. Med.* 50:211-227.
- Ruiz, A. & J.K. Frenkel. 1980. Intermediate and transport hosts of *Toxoplasma gondii* in Costa Rica. *Am. J. Trop. Med. Hyg.* 29:1161-1166.