Natural and induced blood dissemination of *Toxoplasma gondii*: experimental model in white mice and hamsters

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Abstract: Blood inoculation in mice showed that *Toxoplasma* organisms circulate in blood after 1 h of oocyst infection. Parasites were detected up to 15 days later and then disappeared from the bloodstream concomitantly with cyst formation in the brain, probably due to antibody presence. Immunosuppression caused by cortisone acetate treatment induced *Toxoplasma* bloodstream invasion in chronically infected mice and hamsters, causing death in some. Natural dissemination is discussed in relation with congenital toxoplasmosis. Induced immunosuppressive effect is compared with that produced by natural diseases such as Hodgkin, lymphoma, AIDS and others.

Key words: *Toxoplasma gondii*, immunosuppression, corticosteroids.

Toxoplasmosis causes, by congenital transmission, abortus or important sequelae, as well as ocular pathology (Desmonts and Couvreur 1979, Remington 1980, Koppe et al. 1986 and Koskiniemi et al. 1989). Since oral infection is the normal way to get toxoplasmosis, either by meat (Frenkel 1973) or oocyst ingestion (Frenkel and Ruiz 1980), parasites need blood transport to invade organs and be congenitally transmitted. In fact, the evolutive stages penetrate the mucosa and then, by blood circulation, they can reach several organs. Therefore the presence of *Toxoplasma gondii* outside the intestine indicates blood dissemination.

In addition it is also known that a reduced immune response due to diseases such as AIDS (Luft 1988) Hodgkin (Frenkel et al. 1975) and probably others (Jehe et al. 1984), as well as treatment with immunosuppressive drugs (Derouin et al. 1986), exacerbate *Toxoplasma* infections. It has been demonstrated that chronic infections usually caused by low virulence strains can relapse to acute manifestation if immunosuppressive treatment is applied (Frenkel et al. 1975). Then it is important to determine the normal disseminative pathway of an avirulent strain of *T. gondii* and the effect of corticosteroid treatment on blood re-invasion from chronic infected mice and hamsters.

MATERIAL AND METHODS

**Animals:** NIH male and female white mice (20-25 g) and hamsters (150±20 g) from University of Costa Rica animal care unit, were used throughout these studies and all animals were caged and fed *ad libitum*.

*T. gondii* strains: We used an avirulent strain isolated from an owl (*Glaucidium brasillianum*), called TCR-2 and studied under several biological and immunological aspects (Holst and Chinchilla 1990); the known RH strain was used to test chronic infections.
Experimental model for detection of Toxoplasma dissemination

a. Acutely infected mice: One hundred animals were inoculated per os with $10^3$ oocysts of TCR-2 strain. After one, 2, 4, 6, 8, 12 and 24 hours and then daily until 10 days, following after 15, 20, 25 and 30 days of infection, groups of 5 mice were studied as follows.

Blood was collected by cardiac puncture and a part was mixed with heparin to prevent coagulation and then injected i.p. in mice to determine Toxoplasma presence; remaining blood was used for serological studies. After 3 days of infection, brains of these animals were studied by Toxoplasma cyst presence and in those positive animals number of cysts per gram of brain was determined. Mice inoculated with blood from acutely infected animals were studied according to survival time, brain cyst presence and serology. Moreover weekly body weight determination was performed.

b. Chronically infected mice: To study the effect of corticosteroids in Toxoplasma blood invasion and dissemination, animals with chronic infection were injected s.c. twice a week with 2.5 mg of cortisone acetate (Sigma). Treatment was maintained for 2 months and after different periods of time (See Table 2) 5 animals were sacrificed and studied as previously described for acute infections.

Hamsters with acute and chronic toxoplasmosis were studied similarly to mice, except that we did not determine the number of cyst in any of the animals.

Brain cyst count: Three small fractions from different sites of brain from infected animals were placed in previously weighed slides and coverslips. These slides plus brain pieces were weighed again to determine the actual brain weight. Number of cysts was determined in each fraction and then cysts per gram of brain was calculated for each animal. Final results for all the groups were the average of counts found in the survivor mice.

Sero logical tests: The well known Sabin-Feldman and the Carbon immunoassay (CIA) tests were used in these studies. This last technique has been described elsewhere (Pakes and Lai 1985). Briefly, formalin fixed Toxoplasma tachyzoites were placed in contact for 30 m at 370°C with sera from studied animals. After adequate washing with phosphate buffer (PBS), parasites were stained for 5 m. with black India ink (Water proof drawing ink, Higgins Faber Castell). Slides were washed again, air dried and observed at immersion oil objective; more than 50% stained parasites was considered as the final positive point.

RESULTS AND DISCUSSION

Blood circulation is important for dissemination of T. gondii from the intestinal mucosa after infection and to transfer the parasite from the mother to the newborn in congenital toxoplasmosis (Remington and Krahenbuhl 1982). To this respect it has been demonstrated that there is a higher probability of fetal Toxoplasma infection when women acquire the parasite during pregnancy (Desmonts and Couvreur 1974), this is explained by blood transport of tachyzoites released due to the acute onset produced after a primary infection.

Circulating antigen of T. gondii has been detected either in experimental acute infections (Hassl et al. 1987) or in patients with AIDS (Hassl et al. 1988). They consider these findings as "an expression of multiplication and subsequent destruction of parasites".

In our work we demonstrate that in mice there is evidence of circulating live Toxoplasma as early as 1 h (Table 1). It is evident that after 7 days and then until 15 days there is a higher number of Toxoplasma organisms since all the tested animals were infected (Table 1). Similar results were found for acute infected hamsters (not shown). Later the parasitemia diminishes and after 25 days of infection Toxoplasma diagnosis is only possible by serology. The antibody presence plays an important role in this effect since we have shown that in experimental infections antibody detection by the CIA test starts after 12 days (Holst and Chinchilla 1990). Furthermore in our experiments higher titers (1:64 or more) were found in the acute infected mice after 10 days of infection and before this time, titers were lower (<1:64). These results are related with cyst development which represents initial chronic infections (Frenkel 1988). Our findings confirm this statement as is shown in Fig. 1;
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infected animals. Cell mediated immuni ty

plays the major role in protection against

acute toxoplasmosis (Brinkmann et al. 1987)

and this immunity is inhibited by cortisone

treatment, producing a reactivation of chronic

infections of intracellular parasites, especially
demonstrated in toxoplasmosis (Frenkel et al. 1975, Remington and Krahenbuhl 1982,
Chinchilla 1985) or leishmaniasi (Chinchilla et al. 1980).

Using models described by Frenkel et al.
(1975) we demonstrate Toxoplasma dissemi-
nation induced by corticosteroids treatment in
chronic infected animals (Table 2 y 3). In fact,
from mice that presented Toxoplasma
cyst in the brain (Fig. 2), circulating parasites
were detected (Table 2, Fig. 3). In some cases
the parasite was detected by serology and by
cyst presence in the sub-inoculated mice. This
indicates that even when Ferguson and
Hutchison (1987) propose that cyst located
within intact brain cells are protected against
immune attack, cortisone immunosuppression
can break that barrier and induce Toxoplasma
dissimulation.

Disintegration of cysts has been suggested by
Frenkel and Escajadillo (1987) as cause of
clinical encephalitis and probably explains
other pathologies of the central nervous system
observed in immunosuppressed patients with
AIDS (Snyder 1989). These relapsing diseases
resembles an acute initial infection, as has been
shown in human cases or in experimental
studies (Frenkel et al. 1975).

### Table 1

**Detection of Toxoplasma in animals inoculated with**

**blood from acutely infected mice.**

<table>
<thead>
<tr>
<th>Infection time</th>
<th>Cysts in brain</th>
<th>Serology (CIA)</th>
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</thead>
<tbody>
<tr>
<td>1h</td>
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<td>n.d.</td>
</tr>
<tr>
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<td>0/5</td>
<td>n.d.</td>
</tr>
<tr>
<td>4h</td>
<td>4/5</td>
<td>4/5*</td>
</tr>
<tr>
<td>6h</td>
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<tr>
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<td>7d</td>
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<tr>
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</tr>
<tr>
<td>30d</td>
<td>0/5</td>
<td>2/5</td>
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</tbody>
</table>

* Infected/total
n.d. Not done

*Infected/total
n.d. Not done

### Fig. 1
Fig. 1. Determination of *T. gondii* circulation by finding of brain cyst in blood inoculated mice. Cyst found in mice sub-inoculated with blood from infected mice: (Continuous line). Cyst found in infected mice (See text); (Discontinuous line).
Detection of Toxoplasma in animals inoculated with blood from chronically infected mice treated with cortisone

<table>
<thead>
<tr>
<th>Treatment time (days)</th>
<th>Cysts in brain</th>
<th>Serology (CIA)</th>
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<tbody>
<tr>
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</tr>
<tr>
<td>50</td>
<td>0/2</td>
<td>0/2</td>
</tr>
</tbody>
</table>

* Infected/total

In our work it was confirmed that lung invasion observed in chronic infected hamsters after 40 days of cortisone treatment was the cause of death in these animals. In addition, the weight loss pattern, as measure of pathogenicity in acute toxoplasmosis (Elwell and Frenkel 1984), was similar in acute infected mice and cortisone treated animals (Fig. 4).

The results of this study demonstrated that circulation of Toxoplasma tachyzoites starts as early as 1 hour. Then, they remain there until antibody arise and cyst formation takes place. On the other hand cortisone treatment induces release of Toxoplasma organisms from brain cysts and the parasites are transported by blood circulation to lung and other organs causing relapsing pathology. This pattern is probably...
similar to that observed in patients with Hodgkin’s disease (Frenkel et al. 1978) or AIDS (Parkash et al. 1988).

ACKNOWLEDGMENTS

This work was supported by the Vicerrectoría de Investigación, Universidad de Costa Rica (Grant 803-87-040). We thank Eddy Camacho for assistance.

REFERENCES


Fig. 4. Body weight sequence of Toxoplasma infected mice. Acute infected animals: (Continuous line). Chronic infected animals treated with cortisone (discontinuous line).

CHINCHA: Blood dissemination of Toxoplasma gondii in mice and hamsters

RESUMEN

Hemos demostrado que Toxoplasma gondii circula en la sangre de ratones después de una hora de infección con oquistes. Los parásitos estuvieron circulando a nivel sanguíneo hasta por 15 días, desapareciendo luego, al mismo tiempo que se observaba la formación de quistes en el cerebro estimulada posiblemente por la presencia de anticuerpos. La inmunosupresión inducida por medio del tratamiento con acetato de cortisona, produjo la re-invasión de parásitos en sangre de ratones y hamster crónicamente infectados, causando la muerte de algunos animales. La diseminación natural de T. gondii se discute relacionándola con la toxoplasmosis congénita y el efecto inmunosupresor inducido, el cual se compara con el producido naturalmente por enfermedades debilitantes tales como Hodgkin, SIDA y otras.


