Rev. Biol. Trop., 41 (2): 197-202, 1993

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

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(Rec. 21-V-1992. Acep. 19-I-1993)

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 dissapeared 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, lymphome, 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 (Jehn *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 brasilianum),* 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.

Serology tests: The well known Sabin-Feldman and the Carbon immunoassay (CIA) tests were used in these studies. This last tecnique has been described elsewhere (Pakes and Lai 1985). Briefly, formalin fixed *Toxoplasma* tachyzoites were placed in contact for 30 m at 37°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 objetive; 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;

TABLE 1

Detection of Toxoplasma *in animals inoculated with blood from acutely infected mice*

Infection time	Diagnosis by	
	Cysts in brain No	Serology (CIA) No
1h	2/5*	n.d.
2h	0/5	n.d.
4h	4/5	4/5*
6h	1/5	1/5
8h	0/5	2/5
12h	2/5	2/5
1h	2/5	4/5
2d	1/5	1/5
3d	0/5	1/5
4d	0/5	0/5
5d	1/5	1/5
6d	2/5	n.d.
7d	5/5	5/5
8d	5/5	5/5
9d	5/5	5/5
10d	5/5	5/5
15d	5/5	5/5
20d	1/5	3/5
25d	0/5	2/5
30d	0/5	2/5

* Infected/total
n.d. Not done

presence of cysts in acutely infected mice starts at about 20 days but might be earlier, between 15 and 20 days, as described elsewhere (Holst and Chinchilla 1990). In summary, as antibody titers rise, circulating parasite diminishes and cyst development starts in the original infected mice. Even when Jacobs (1967), described this phenomenom, in our work we follow the complete course of the infection, detecting important variations that could be related with the effect of cortisone treatment in *Toxoplasma* chronic infections. Moreover, our results confirm the suggestions of Brinkmann *et al.* (1987) who established that a high humoral response induces cyst formation.

We wanted to relate the results already discussed with artificial induced toxoplasmosis by immunosuppression of chronic infected animals. Cell mediated immunity plays the major role in protection against acute toxoplasmosis (Brinkmann *et al.* 1987) and this immunity is inhibited by cortisone

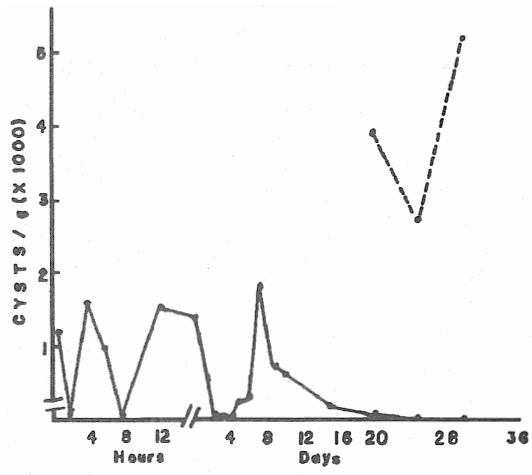

Fig. 1. Determination of *T. gondii* circulation by finding of brain cyst in blood inoculated mice. Cyst found in mice sub-inoculated with blood from infected mice: (Continuos line). Cyst found in infected mice (See text): (Discontinuos line).

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 leishmaniasis (Chinchilla *et al.* 1980).

Using models described by Frenkel *et al.* (1975) we demonstrate *Toxoplasma* dissemination 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* dissemination.

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 2

Detection of Toxoplasma *in animals inoculated with blood from chronically infected mice treated with cortisone*

Treatment time (days)	Diagnosis by: Cysts in brain No	Serology (CIA) No
4	0/5 *	2/5 *
6	2/5	2/5
8	1/5	2/5
11	0/5	2/5
14	3/4	3/4
18	1/5	2/5
22	2/5	4/5
25	0/5	3/5
27	0/5	1/5
28	0/5	4/5
32	0/5	0/5
34	0/5	1/5
35	0/5	1/5
39	0/5	3/5
41	1/4	1/4
43	1/4	1/4
46	0/5	2/5
48	1/4	1/5
50	0/2	0/2

* Infected/total

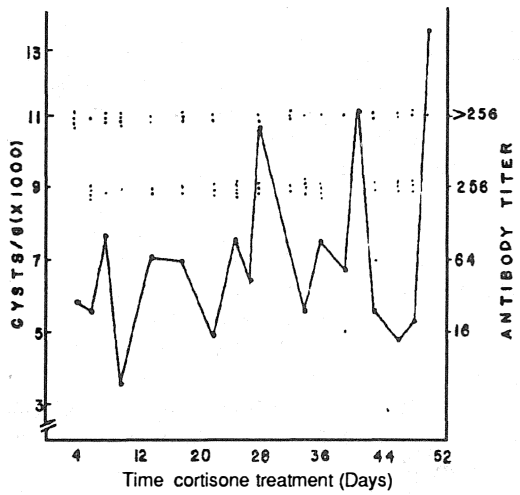

Fig. 2. Determination of *Toxoplasma* circulation in chronic infected mice treated with cortisone by brain cyst counts in sub-inoculated mice. Points indicate CIA test results.

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

TABLE 3

Toxoplasma *detection in chronically infected hamsters treated with cortisone acetate and in subinoculated mice*

Treatment time (days)	*Toxoplasma* presence: Hamster In brain	Hamster In lung	Mice In brain
2	+	-	-
5	+	-	-
9	+	-	-
12	+	-	-
16	+	-	-
19	+	-	-
22	+	-	-
26	+	-	-
30	+	-	-
36	+	-	-
40	+	+	+
43	+	+	+
47	+	+	+
50	+	+	+

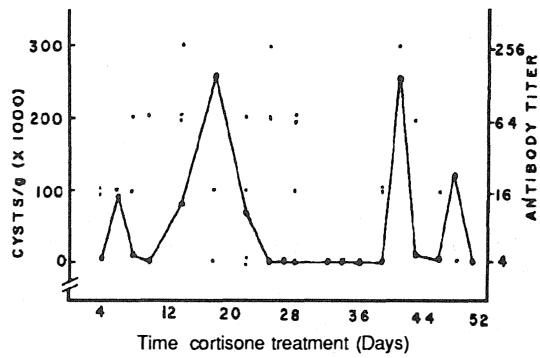

Fig. 3. Brain cyst determination in chronic infected mice treated with cortisone. Points indicate CIA test results.

weight loss pattern, as measure of pathogenecity 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

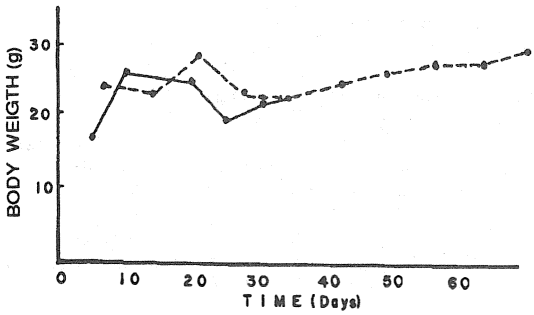

Fig. 4. Body weight sequence of *Toxoplasma* infected mice. Acute infected animals: (Continuos line). Chronic infected animals treated with cortisone (discontinuos line).

similar to that observed in patients with Hodgkin`s disease (Frenkel *et al.* 1978) or AIDS (Farkash *et al.* 1988).

ACKNOWLEGMENTS

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

RESUMEN

Hemos demostrado que *Toxoplasma gondii* circula en la sangre de ratones después de una hora de infección con ooquistes. 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.

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