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Increasing of *Toxoplasma gondii* (Coccidia, Sarcocystidae) infections by *Trypanosoma lewisi* (Kinetoplastida, Trypanosomatidae) in white rats

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Abstract: To demonstrate that *T. lewisi* infection increases *T. gondii* multiplication in white rats, groups of five Wistar or Sprague Dawley rats were inoculated with 10^6 *T. lewisi* trypomastigotes and four or seven days later infected with *Toxoplasma* tachyzoites. Host survival time was monitored, and the presence of *T. gondii* was confirmed in all dead rats by studying peritoneal exudate smears and lung tissue sections stained with haematoxylin-eosin. The presence of *Toxoplasma* cysts or antibodies was checked in the brain of surviving rats. The increase is observed four days after trypanosome inoculation and is dependent on rat strain, but not on inoculum size or rat age. Humoral and cellular factors may have a role in the increase as has been reported for other experimental infections with African trypanosomes and *T. cruzi*.

Key words: *Toxoplasma gondii, Trypanosoma lewisi*, immunosuppression, rats.

Since 1951-1955 it has been known that white rats are remarkably resistant to *Toxoplasma gondii* infection (Ruchman and Fowler 1951, Lainson 1955).

We have been trying to explain the reasons for such resistance. In our first studies we demonstrated, with controlled inocula, that even 10^8 tachyzoites do not give rise to fatal infection in adult white rats (Chinchilla *et al.* 1981). We showed the importance of age resistance (Chinchilla *et al.* 1981) and macrophages activity (Chinchilla *et al.* 1982), for that natural resistance to *T. gondii* and studies in progress, including new information about other factors that could play an important role in this phenomenom, comfirm the low susceptibility of white rats to *T. gondii.*

Immunosuppression of protozoan infections with other parasites has been reported. For example *Trypanosoma cruzi* infection appears to be increased by some immunosuppressive effects due to malaria infections in host (Krettli 1977).

This effect has been also demonstrated in African tripanosomiasis (Olsson *et al.* 1991. Darji *et al.* 1992). Particularly in toxoplasmosis, reactivation due to virus infection was reported in mice (Pomeroy *et al.* 1989) and cats (Witt *et al.* 1989). However, we have not found other studies of *T. gondii* immunosuppression due to *Trypanosoma* infections.

We have found that *Trypanosoma lewisi* increases *T. gondii* infection of white rat, changing the susceptibility of this rodent to

the parasite. Studies of different factors effecting this phenomena are presented in this paper.

MATERIAL AND METHODS

Animals: Wistar or Sprague Dawley rats feed with a local chow and water **ad libitum** were used in these experiments. *Toxoplasma* tachyzoites for serology tests were obtained from NIH white mice peritoneal exudate.

Parasite strains: Tachyzoites of *Toxoplasma* RH strain and partially characterized TCR-2 and TCR-3 strains (Holst and Chinchilla 1990, Guerrero *et al.* 1991) were inoculated in different concentrations according to each experiment. These strains are mantained by regular passages (twice a week) in the acute phase.

T. lewisi strain was locally isolated from a *Rattus norvegicus,* gray rat, and mantained by weekly passage in white rats.

General experimental models: Groups of five Wistar or Sprague Dawley rats were inoculated i.p. with 10^6 *T. lewisi* trypomastigotes obtained from one week infected animals and four or seven days later, according to the experiments, these rats were infected with different number of *Toxoplasma* tachyzoites.

Simultaneously, other animals were inoculated only with *T. gondii* or *T. lewisi*, representing the corresponding controls. All infections were done intraperitoneally.

Survival time of rats was monitored, checking the presence of *Toxoplasma* in the peritoneal exudate or lungs in all the dead rats. Lung tissue sections were stained by hematoxylin-eosin technique to demonstrate lesions. Animal surviving for 30 days, the end point for all the experiments, were studied for presence of brain *Toxoplasma* cysts or for antibody presence. The Carbon Immuno-Assay (CIA) of known specificity (Chinchilla *et al.* 1992) and used for us in other studies (Arias *et al.* 1994) was performed for serologic analysis.

A "t" student test for small samples was used for statistical analysis.

RESULTS

Determination of *T. lewisi* previous infection time: In a controlled experiment, rats were infected with *T. lewisi* and after different periods of time (0 to 21 days), inoculated with *T. gondii* tachyzoites. Data indicate (Table 1) that *T. lewisi* inoculated to rats, four to six days previously to *T. gondii* infection produces major increases of the multiplication of this last parasite (see underlined numbers).

In other experiment 30 or 60 days old rats were inoculated with 10^4 trypomastigotes and four or seven days later infected with 10^6 *T. gondii* tachyzoites. As it is shown in Table 2, both age animals infected with *T. lewisi* four days before to *Toxoplasma* infection, died earlier (see underlined data) than those infected seven days previously with trypanosomes. Rats inoculated only with *T. gondii* (controls) survived 30 days, end of the experiments in any case.

Rat strain differences (Table 3): Either 30 or 60 day old Wistar rats inoculated with *Toxoplasma,* previously infected with *T. lewisi,* survived no more than 12 days. On the other hand, Sprague Dawley rats survived more than 23 days. In both cases, animals inoculated with *T. gondii* alone, survived 30 days, the end of the experiment.

***Toxoplasma* strain differences**: The apparent immunosuppressive effect of *T. lewisi* was demonstrated for three strain studied (Table 4). However the survival time of the RH infected mice was lower.

Effect of *Toxoplasma* inoculum: The average survival time was shorter in animals previously infected with *T. lewisi*, as compared with the controls, independent of the inocula (10^5, 10^6, 10^7) of *Toxoplasma* tachyzoites (Table 5).

All the *Toxoplasma* infected rats surviving 30 days were positive, either by brain cyst presence or the CIA test and many tachyzoites were observed in peritoneal macrophages and lung lessions (Fig.1) of rats previously inoculated with *T. lewisi.*

TABLE 1.

Survival of rats with* T. gondii *infection and pre-infected with* T. lewisi.

Number of *T. lewisi* trypomast./inoculated animal	Number of *T. gondi* tachyz./inoculated animal	Time interval between *T. lewisi* and *Toxoplasma* infections (d)	Survival time (d)	% Surviving 30 d
0	0	0	30	100
10^6	0	0	30	100
0	10^6	0	30	100
10^6	10^6	3	30	100
10^6	10^6	**4**	**20.8**	**60**
10^6	10^6	**5**	**7**	**0**
10^6	10^6	**6**	**25.4**	**80**
10^6	10^6	10	30	100
10^6	10^6	14	30	100
10^6	10^6	17	30	100
10^6	10^6	21	30	100

*5 rats for each group.

TABLE 2

Effect of strain or age of rats on T.lewisi *"immunosuppression" against* T. gondii, *inoculated four d later*

Rat strain	Age (d)	*T. lewisi* infection			
		Infected		Non-infected	
		Survival time (d)	% surviving (30 d)	Survival time (d)	% surviving (30 d)
Wistar	30	**11.4**	**20**	30	100
	60	**12.7**	**25**	30	100
Sprague Dawley	30	23	60	30	100
	60	30	100	30	100

All the differences between *Toxoplasma* multiplication of *T. lewisi* infected and non-infected animals showed a $P<0.001$.

DISCUSSION

Although we have demonstrated a remarkable resistance of white rats to *Toxoplasma* infection (Chinchilla *et al.* 1981), it is possible to dicrease it by treatment with immunosuppresive agents (Chinchilla *et al.* 1992). In fact we found that cortisone injection induced a reversion of immunity during chronic infection, giving rise to a relapse. Likewise, the natural resistance of rats was inhibited by the same treatment (Chinchilla *et al.* 1992). However, this resistance was evident after several days, which is different to what we report in this study. The effect exerted by *T. lewisi* on *Toxoplasma* infection ocurred in only four to five days (Table 1 and 2) and it was not dependent on parasite strain or age (Table 3 and 4). This means that we are in presence of a strong effect against the acquired immune response. Since *T. gondii* is an intracellular parasite in which cellular immunity is more protective than humoral (Frenkel 1985), our model suggests a suppression of any cellular manifestations.

Immunosupression in experimental toxoplasmosis, probably due to cellular immunity impairment, has been observed in concomitant infections with Louping-ill Virus (Reid *et al.* 1980) murine leukemia virus, feline immunodeficiency virus (Watanabe *et al.* 1993) and Cytomegalovirus (Pomeroy *et al.* 1989).

Darji *et al.* (1992), have shown in several studies a trypanosome-elicited immunosuppression due to either suppression of TL-2 by generations of prostaglandin-producing macrophages, or a prostaglandin-independent suppressive mechanism that inhibit the expression of TL-2 receptors. Furthermore, they establish that TNF a and IFN-gamma play an important role in the pathway of T-cell (probably $CD4^+$) immunosuppression. Araujo (1992) has demonstrated, in very detailed experiments, the importance of $CD4^+$T cells

TABLE 3

Confirmation that previous T.lewisi *infection gives rise to increasing of* T.gondii *multiplication in rats**

Age (d)	Time interval between *T. lewisi* and *Toxoplasma* infections (d)	*T. lewisi* infection			
		Infected		Non-infected	
		Survival time (d)	% surviving (30 d)	Survival time (d)	% surviving (30 d)
30	0	30	100	30	100
	4	**15**	**35.7**	30	100
	7	23.3	70	30	100
60	0	30	100	30	100
	4	**15.6**	**37.5**	30	100
	7	27.7	90	30	100

* 5 rats for each group.

TABLE 4

Relation of Toxoplasma *strains with the effectof* T.lewisi *parasite sinoculated four days earlier in Wistar rats.*

T. gondii strain	*T. lewisi* infection (4 d before)			
	Infected		Non-infected	
	Survival time (d)	% surviving (30 d)	Survival time (d)	% surviving (30 d)
RH	16	40	30	100
TCR-2	20.4	60	30	100
TCR-3	24	75	30	100

TABLE 5

Effect of Toxoplasma *inoculum on"immunosuppression" of Wistar rats, induced by* T.lewisi, *infected four days earlier*

T gondii inocula (Tachyz./animal)	*T. lewisi* infection (4 d before)			
	Non-infected		Infected	
	Survival time (d)	% surviving (30 d)	Survival time (d)	% surviving (30 d)
10^5	25	80	6.4	0
10^6	30	100	11.4	20
10^7	7	80	25.3	10

and IFN-a in the immune response against *T. gondii.* Some of these studies and others with *T. brucei* (Bakhiet *et al.* 1990) as well as with *T. congolense* (Flyn and Sileghen 1991) explain some immunosuppressive mechanisms that can be suspected for our *Toxoplasma - T. lewisi* model. Sztein and Kierszenbaum (1992) suggest that alterations of the expression of TcR, CD_4, CD_8 and IL-2R due to *T. cruzi* infections, could be responsable of lymphocyte interference, giving rise to immunosuppression. This is another interesting aspect to take into account for our experiments.

Albright and Albright (1991) have suggested that in infections with *Trypanosoma musculi*, a mouse parasite biologically similar to *T. lewisi,* there are several factors blocking of antibody activity, interference that affect humoral immunity suppression. This could be another explanation for the apparent immunosuppressive effect that we have observed in our model.

This phenomenon appears to be related with the animal genetic origen as is shown in Table 3. Actually the effect was more evident in Wistar than in Sprague Dawley rats. Differences in animal immune response to intracellular parasites depending on animal,

specially rodent strains, have been reporte (Handman 1979). Specifically, for rat strains, in a study to determine *Pneumocystis carinii* karyotypes obtained by corticosteroid treatment, Hong *et al.* (1992) needed only five to eight weeks for immunosuppression of Wistar rats. On the contrary a nine to ten weeks treatment was neccesary to obtain the same effect for Sprague Dawley rats.

In conclusion, these preliminary studies demonstrate that *T. lewisi* infections can increase *Toxoplasma* multiplication in rats due to an apparent immunosuppressive effect. The presence of many tachyzoites in lung lesions (Fig. 1 a, b, c) as well as in peritoneal macrophages (Fig. 1d) of these animals, compared with the controls, support the interpretation that *T. gondii* was the cause of death.

Furthermore survival of rats infected with *T. lewisi* or *T. gondii* alone let us think that the effect is not produced by other disease, as has been mentioned for bartonelosis infections (Perla and Marmorston 1941) which can modified the natural resistance.

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