

Infection of white rat peritoneal macrophages with *Toxoplasma gondii*, (Coccidia: Sarcocystidae) after *Trypanosoma lewisi* (Kinetoplastida: Trypanosomatidae) infection

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Abstract: Peritoneal macrophages from Wistar rats, inoculated and non-inoculated with 10^6 *T. lewisi* trypomastigotes, were cultured and infected with 10^6 *T. gondii* tachyzoites. Multiplication rates of this parasite were studied after 1, 24 and 48 h of infection but there were not significant differences between the number of parasites found inside of macrophages coming, either from *T. lewisi* infected or non infected rats. On the other hand, in vivo studies of *Toxoplasma* multiplication inside peritoneal macrophages, showed that there is an increase of parasite number in cells from *T. lewisi* infected rats, as compared with those macrophages from non infected rats. This effect was statistically significant and was more evident after four days of infection. Therefore, it has been demonstrated that in vivo, but not in vitro *T. lewisi* infections, causes an important decrease of the natural resistance to *T. gondii* of the white rats, which is manifested by the major invasion and multiplication of the parasite inside of peritoneal macrophages.

Key words: Toxoplasmosis, *Toxoplasma gondii*, immunosuppression, Wistar white rat.

White rats may be considered as model for humans because of their natural resistance to *Toxoplasma* infections (Ruchmand & Fowler 1951, Lainson 1955). To demonstrate that resistance, several studies have been done. For example, 1, 2, 10 or 15 days old rats were inoculated with different number of tachyzoites (RH strain) and it was shown that even young animals (five days) are able to resist 10^4 organisms (Chinchilla et al. 1981). This natural resistance was reduced in Sprague Dowley rats by cortisone treatment, which indicates that cellular immunity is involved in such phenomenon (Chinchilla et al. 1985, 1992). In addition, Chinchilla et al. (1982),

demonstrated that *T. gondii* multiplication is lower inside rat peritoneal macrophages than in hamster, mouse or guinea pig phagocytic cells. Rat macrophage activity studied by electron microscopy showed vacuolization and lysis of the parasite after eight hours of infection. This effect was not observed in macrophages from mice and hamsters (Chinchilla et al. 1986).

Recently, Guerrero *et al.* (1997) reported for the first time a significant increase of *Toxoplasma* multiplication in white rats previously infected with *T. lewisi* trypomastigotes. This effect was present only if the animals were inoculated with the

trypanosome four to six days before the *T. gondii* infection, and it was rat strain dependent but not parasite or inoculum size dependent.

In order to further investigate this property of macrophages (Chinchilla et al. 1982, 1986), a study was performed using, as target cells, peritoneal macrophages from infected and non infected rats with *T. lewisi*, and then infected with *T. gondii*. The results of these experiments are reported in this paper.

MATERIALS AND METHODS

Animals: Wistar o Sprague Dowley rats (100g body weight) were used as source of macrophages and *T. lewisi* trypomastigotes and NIH mice (20-35g body weight) to obtain *T. gondii* parasites for experimental infections.

Parasites: Tachyzoites of the well known *Toxoplasma* RH strain were obtained from the peritoneal exudate taked out with 0.85 % saline solution. The organisms were counted in a Neubauer camera and then adjusted to 106 parasites per ml.

The TL-2 strain of *T. lewisi*, isolated and maintained in our laboratory, was used in these experiments. Blood trypomastigotes were suspended in 0.85 % saline solution, counted in a Neubauer chamber, and the inocula was adjusted to 106 organisms per ml.

Model for peritoneal macrophages in vitro infection: Peritoneal exudate was obtained using Minimal Essential Medium (MEM) supplemented with 20% fetal calf serum, and then the macrophages were concentrated, counted in a Neubauer chamber and cultured on clean-steril coverslips (Chinchilla et al. 1995). These cells were incubated at 37° C (5% CO₂, 95% O₂) and 90% relative humidity for 24 h. The macrophages were infected with *T. gondii* tachyzoites in a 1:1 relation. The cells were incubated again and studied after one, 24 and 48 h of infection coverslips were Giemsa stained and the number of intracellular parasites per macrophage or per 100 cells was determined.

Toxoplasma multiplication rate was calculated dividing the number of parasites found after 24 h infection by the number of organisms observed after 1 h infection (Chinchilla et al. 1995). All this procedure was repeated in macrophages from rats infected with *T. lewisi* for 1, 2, 3 or 4 d.

Model for peritoneal macrophages in vivo infection: Thirty Wistar rats were separated in 3 groups (10 rats each) according to the next distribution.

Group 1: *Toxoplasma* (106 tachyzoites) inoculated 4 d after the *T. lewisi* (10⁶ trypomastigotes) infection.

Group 2: Only *T. gondii* (infection control).

Group 3: Not inoculated with any parasite (General control)

Starting from the day of the *Toxoplasma* infection and daily thereafter until 15 d, samples of rat peritoneal exudate were air dried, fixed with methyl alcohol and Giemsa stained. The number of infected macrophages, as well as the number of parasites per macrophage was determined calculating the same parameters used for the in vitro infection. *T. gondii* multiplication rate was calculated dividing the number of parasites found after 24, 48, 72 and 96 h of infection by the number of parasites observed at the previous infection time (Chinchilla et al. 1995). The Systat program for microcomputer (Wilkinson 1990) was used for statistical analysis.

RESULTS

Statistical analysis showed that there were not differences between the in vitro *Toxoplasma* multiplication inside of macrophages from rats, previously infected with *T. lewisi* and those cells coming from non infected animals. Data are shown in Table 1.

In vivo studies, counting 100 peritoneal macrophages are presented in Table 2. Statistical analysis showed that there are significant differences between cells from rats infected with both parasites and those cells obtained from animals inoculated only with *Toxoplasma*. The following parameters

TABLE 1

Mutiplication rate of *T. gondii* in white rat peritoneal macrophage cultures

Group	Rat infection time (d)	Macrophage infection time (h)	Number of tachyzoites		Multiplication rate	
			per 100 macrop.	per 100 infect. mac. mac.	per 100 mac.	per 100 infect. mac.
1*	0	1	33	141	2.4	1.3
		24	78	186		
		48	30	115		
	1	1	16	190	0.9	1.0
		24	15	189		
		48	11	157		
	2	1	160	267	0.4	0.9
		24	68	227		
		48	14	200		
	3	1	23	164	0.6	0.7
		24	14	117		
		48	4	200		
	4	1	31	182	0.9	1.3
		24	28	233		
		48	17	250		
2**	0	1	9	138	7.1	1.5
		24	64	203		
		48	48	268		
	1	1	5	600	3.8	0.6
		24	19	380		
		48	6	67		
	2	1	26	130	1.3	1.7
		24	34	226		
		48				
	3	1	143	325	0.1	0.5
		24	14	175		
		48	50	274		
	4	1	20	105	1.4	2.4
		24	28	255		
		48	2	100		

* Macrophages from animals infected with *T. lewisi* 1, 2, 3 or 4 days before *Toxoplasma* infection.
** Macrophages from animals without *T. lewisi* infection.

TABLE 2
Multiplication in vivo of T. gondii in rat peritoneal macrophage
(100 cells studied)

Infection time (d)	Group*	Infected macrophages	Tachyzoite number		Multiplication rate	
			per 100 macrop.	per 100 infect. macrophages	Per 100 macrophages	Per 100 infect. macrophages
1	1	0.5	0.5	100.0	5.6	2.5
	2	0.0	0.0	0.0	4.4	0.6
	3	0.0	0.0	0.0	0.5	0.5
2	1	1.4	2.8	250.0	2.3	1.5
	2	0.2	0.4	200.0	0.04	1.1
	3	0.0	0.0	0.0		
3	1	<u>6.8</u>	<u>12.4</u>	<u>172.9</u>		
	2	0.2	0.2	100.0		
	3	0.0	0.0	0.0		
4	1	<u>10.8</u>	<u>29.6</u>	<u>262.0</u>		
	2	0.0	0.0	0.0		
	3	0.0	0.0	0.0		
5	1	0.4	1.2	300.0		
	2	0.0	0.0	0.0		
	3	0.0	0.0	0.0		
6	1	0.0	0.0	0.0		
	2	0.0	0.0	0.0		
	3	0.0	0.0	0.0		

* Group 1: *T. lewisi* y *T. gondii*; group 2: *T. gondii* only; group 3: whitout infection. Underlined data indicate statistically significant differences for the corresponding period.

showed statistically significant differences: number of infected macrophages, and number of tachyzoites per 100 macrophages.

In animals inoculated with both parasites, infected macrophages were found starting 24 h after infection following an ascendent multiplication curve, reaching a maximun average (9 organisms) at the fourth day. Then the number of intracellular parasites decreases, disappearing by the sixth day (Table 2). On the other hand, macrophages from rats inoculated only with *Toxoplasma* presented, at the second and third day but not later, a 0.2% of infection. Significant statistical differences were demonstrated for groups 1,2,3 for all days

(P=0.0), and for the third (P=0.04) or fourth day (P=0.05) of infection.

Tachyzoite multiplication as well as multiplication rates are shown in Table 2. In group 1 intracellular organisms are present already after the first day of infection, but higher numbers are found at the fourth day decreasing at the fifth day. Significant statistical differences (P=0.04 to P=0.05) were shown for the third and fourth day respectively. Cells from group 2 (*Toxoplasma* infected rats), presented very low number of parasites at the second and third day only.

Number of parasites per infected macrophages, a parameter that indicates the real capacity of intracellular multiplication,

showed a similar tendency since a higher number of organisms were present in those cells coming from rats infected with both parasites ($P=0.00$) specially for 3 or 4 d after infection.

DISCUSSION

Some studies in animals and humans infected with African trypanosomes have shown immunosuppression phenomenon, but the mechanisms for that are unknown (Bakhiet *et al.*, 1990, Darji *et al.* 1992, Olsson *et al.* 1991, Greenwood 1974). Trypanosome infections in rodents are specially useful models to study host-parasite relations. Some examples are the works of Albright & Albright (1980) with *Trypanosoma musculi*.

In recent studies, Guerrero *et al.* (1997) report the increasing of *T. gondii* multiplication in white rats because of *T. lewisi* previous infection, suggesting an immunosuppression effect.

Since *Toxoplasma* organisms multiply inside of the macrophages, any immune alteration caused by trypanosome infections could affect the parasite multiplication in these phagocytic cells. This supposition was demonstrated for the *in vivo* model as it is shown in Table 2, because a higher parasite multiplication rate was found for peritoneal macrophages obtained from rats previously inoculated with *T. lewisi*, as compared with those cells from non infected animals. *In vitro* studies (Table 1), on the contrary, showed no differences.

Different results between the *in vivo* and the *in vitro* models can be explained on the basis of macrophages activity. These cells are activated *in vivo* by several factors (Hiroashi & Morrison 1996, Briend *et al.* 1995, Zheng *et al.* 1995, Kuby 1994), and phagocytosis is increased by lymphokines and other factors such as gamma interferon (Borges *et al.* 1975, Anderson *et al.* 1996). In addition, activated macrophages are more efficient in microorganisms killing, express higher levels

of the Histocompatibility Major Complex (class II) and establish a two ways collaborative effect (Kuby 1994).

In our model *in vitro*, macrophages are cultured in an artificial environment without any humoral or cellular influence, which reduces phagocytosis and effective intracellular multiplication. (Tables 1 and 2). Destruction and multiplication were not separately evaluated.

In summary, after the analysis of the results, it is clear that *T. lewisi* infections induce a remarkable decrease in natural resistance to *T. gondii* in the white rats, increasing invasion and multiplication of this parasite inside of the macrophages. However, according to the *in vitro* studies and previous research (Borges *et al.* 1975, Anderson *et al.* 1996), it can be suspected that this is a complex phenomenon where many factors play a role. Identification and characterization of these factors and their relationship with rats peritoneal and alveolar macrophages are in progress.

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