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The Effect of *Plasmodium berghei* Infection on Mice Infected with Low-virulent Strains of *Trypanosoma cruzi* or *Leishmania donovani*

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Abstract: Malaria is considered to induce immunodepression in host-animals and to increase susceptibility to concurrent infection. Using this phenomenon, we attempted to produce apparently severer infection of *Trypanosoma cruzi* or *Leishmania donovani* by low-virulent strains which had been maintained in culture. *Plasmodium berghei* was inoculated into mice before or after *T. cruzi* or *L. donovani* infection. In general, *P. berghei* infection was shown not to enhance *T. cruzi* and *L. donovani* infection with low-virulent strains except for the prolongation of infection by a specific strain of *T.* cruzi.

Key words: Plasmodium falciparum, Immunosuppression, Concurrent infection, *Trypanosoma cruzi, Leishmania donovani*

Although the interaction between malaria parasites and host-immune system is exceeedingly complex, malaria is generally acknowledged to induce immunosuppression in antibody formation as well as in cell-mediated immune responses (Weidanz, 1982). Thus concurrent infection with various mibrobial agents is enhanced, for example, in protozoan diseases experimental animals with malaria have been shown to increase susceptibility to *Leishmania mexicana* (Coleman *et al.*, 1988), *Trypanosoma cruzi* (Kretti, 1977) and *Toxoplasma gondii* (Strickland *et al.*, 1972).

In the present work, we attempted to induce apparently severer infection with *L. donovani* and *T. cruzi* in mice infected with *Plasmodium berghei* using the strains which had decreased infectivity to mice by prolonged maintenance in culture. For *Plasmodium berghei* infection, the strain NK 65 was kindly provided from Prof. M. Suzuki, Department of Parasitology, Gumma University School of Medicine. The *L. donovani* strain, NLB−65 was obtained from Nairobi *Leishmania* Bank, Kenya Medical Research Institute in 1986 and has been maintained in modified NNN media. For *T. cruzi* infection the strain G1 was obtained from Prof. I. Tada, Kumanoto University School of Medicine, Japan as an isolate from a patient in Guatemala, 1986 and the strain CL and Colombia were obtained from Dr. M.N.L. Meirelles, Instituto Oswaldo Cruz, Brazil in 1988. They had been maintained in slightly modified LIT media, and transferred into fibroblast cultures derived from the skins of ICR newborn mice before the experimental use. Trypomastigotes developed in the overlaid medium were used for the infectious experiment. The two kinde of experiments were conducted.

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The schedule and result of the first experiment was shown in Table 1. On day 0, one drop of tail blood from a heavily infected mouse with *P. berghei* was diluted in 1 ml of saline and injected into each of 5 BALB/c mice (5 weeks old, female). On day 3, around 1% parasitaemia in all mice was confirmed and then 2.5×10^6 trypomastigotes from the G1 strain of *T. cruzi* or 10^7 culture forms of *L. donovani* were inoculated into each of *P. berghei* infected mice or 5 control mice. From day 4, 10 mg/kg/day of chloroquine was given for 3 successive days to cure malaria. On day 11, 18 and 30, *T. cruzi* parasitaemia was examined, and on day 30 and 60, respectively two and three infected mice with *L. donovani* alone or with *P. berghei* were sacrificed to examine parasites in spleens. The same chloroquine treatment was done on day 12 and 25 for a malaria relapse. Neither exacerbation of *T. cruzi* infection by concurrent *P. berghei* infection was observed nor apparent infection of *L. donovani* by the long-maintained strain in culture was induced.

The schedule and the result of the second experiment were shown on Table 2. On day 0, 2.5×10^6 trypomastigotes from the CL strain or the Colombia strain were inoculated into each of 10 ddY mice (5 weeks old, female). On day 7, low parasitaemia which meant at least one parasite in some fields (200 x) of a microscpoe, was confirmed in all mice and then *P. berghei* infection was done to 5 mice out of 10 infected mice with each strain. On

Table 1. The schedule and the result of the first experiment

	P. b. ↓ *T. c.* *L. d.* ↓		T ↓	T ↓	T ↓		T ↓	T ↓	T ↓		T ↓	T ↓	T ↓		
day	0	3	4	5	6	11	12	13	14	18	25	26	27	30	60
P. b.		~1%					>10%				>10%				
T. c.(G1)	alone					5+				4+, 1−				5−	
	with *P. b.*					5+				3+, 2−				5−	
L. d.	alone													2−	3−
	with *P. b.*													2−	3−

+: at least one trypomastigote in some fields.
−: no trypomastigote in a drop of tail blood or no amastigote in spleen stamps.
T: intramuscular injection of 10 mg/kg/day chloroquine.

Table 2. The schedule and the result of the second experiment

	T. c. ↓		*P. b.* ↓		T ↓	T ↓	T ↓	T ↓		T ↓	T ↓	
day	0		7	9	10	11	17	18	21	25	26	30
Colombia	alone		10+						3+, 2−			5−
	with *P. b.*								5+			5+
CL	alone		10+						5−			5−
	with *P. b.*								5−			4−, 1+
P. b.				~1%			>10%			>10%		

+: at least one trypomastigotes in some fields.
−: no trypomastigote in a drop of tail blood.
T: intramuscular injection of 10 mg/kg/day chloroquine.

day 9, *P. berghei* infection was confirmed and on day 10 and 11, 10 mg/kg/day of chloroquine was given to a mice. From day 17 and 25, the same treatment was done for two successive days to cure a malaria relapse. On day 21 and 30, all mice infected with the Colombia strain and *P. berghei* showed low parasitaemia, but three out of, and none out of five mice infected with the Colombia strain alone showed low parasitaemia respectively. None of mice infected with the CL strain showed parasitaemia on day 21 whether with concurrent infection of *P. berghei* or without, and only one mouse with concurrent *P. berghei* infection showed low parasitaemea on day 30. These two experimets indicated that *P. berghei* infection which showed two severe relapses enhanced the concomitant *T. curzi* infection only on limited conditions, because the infection only by the Colombia strain was prolonged. The reason of prolongation is not known because the characteristic difference between this strain and others in not determined. These results suggested that generally the mouse condition which was induced by *P. berghei* infection could not cause apparently severer infection for the infection with low-virulent strains of *T. curzi* or *L. donovani.* Furthermore, the mice which had survived the acute infection by the virulent strain (Tulahuen) of *T. cruzi,* did not show a relapse when they were infected with *P. berghei* 6 months after the *T. cruzi* inoculation (data not shown).

We concluded that *P. berghei* infection would enhance the concurrent *T. curzi* or *L. donovani* infection when it occurred timely on the mice in the limited conditions or on the mice infected with the limited strains of *T. curzi* or *L. donovani.* The factors limiting the conditions of hosts or parasites should be the next subject of investigation.

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