EFFECT OF VIABLE OR DEAD *LACTOBACILLUS CASEI* ORGANISMS ADMINISTERED ORALLY TO MICE ON RESISTANCE AGAINST *TRICHINELLA SPIRALIS* INFECTION

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Summary :

The capacity of viable, dead *Lactobacillus casei* and supernantant from *L. casei* culture, administered by oral route, to induce resistance in mice against *Trichinella spiralis* infection was evaluated. The percentage of adult worm reduction in the intestine five days after *T. spiralis* infection as compared with the worm burden in the control group fluctuated between between 53.1 and 58 % in mice treated with viable *L. casei*, while reductions in animals treated with dead lactobacilli or supernatant from *L. casei* culture were of 44 and 32.5 % respectively. The percentage of larvae per gram of muscle tissue reductions, as compared with controls, obtained 30 days after infection ranged from 48.4 to 70.7 % in rodents which ingested viable *L. casei* compared with the percentage reductions of 65.9 and 24 % obtained respectively in mice treated with dead lactobacilli or with *L. casei* supernatant. The protective response observed in the present study may be explained on the basis of 1) lactobacilli colonization of the intestine, 2) macrophage processing of dead lactobacilli in local immune tissues and presentation of *L. casei* antigens to Th1 cells which, in turn, produced IL-2 to activate B cells and other T cells.

KEY WORDS : oral *Lactobacillus casei*, mice, resistance, *Trichinella spiralis*.

The growing evidence of drug-resistance in parasites and the difficulty to produce vaccines based on defined antigens in the short term have emphasized the need for new alternatives for the control of parasitic diseases; the use of immunostimulants is one of such alternatives. In this context, it has been shown that by mean of the intraperitoneal inoculation of immunostimulants in different animals before infection it is possible to induce non specific resistance against parasites such as *Fasciola hepatica* (Bautista-Garfias *et al.*, 1992), *Haemonchus contortus* (Bautista-Garfias *et al.*, 1991) *Toxoplasma gondii* (Bautista-Garfias *et al.*, 1995b), *Eimeria tenella* (Bautista-Garfias, *et al.*, 1996), and *Trichinella spiralis* (Bautista-Garfias *et al.*, 1995a). In further experiments we also demonstrated that *L. casei* administered by intraperitoneal route in mice generated a significative resistance against *Trichinella spiralis* infection (Bautista-Garfias *et al.*, 1999). The aim of the present study was to determinate if viable or dead *L. casei* administered orally to mice would induce a similar protective response against *T. spiralis* infection.

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MATERIALS AND METHODS

TRICHINELLA SPIRALIS

The strain of *T. spiralis* used (ISS3) has been maintained by passage in NIH mice and Wistar rats. Muscle larvae utilized for infection were isolated by pepsin digestion (Slayton Blair, 1983).

LACTOBACILLUS CASEI

The strain ATCC7469 cultured in Ellefer broth was used. The microorganisms were harvested by centrifugation at 5,000 × g for 10 min, and washed several times with sterile saline solution.

MICE

Parasite-free NIH female mice, aged six-eight weeks, with an average weight of 25 g were obtained from the Hygiene Institute (Health Secretariat) in Mexico City.

EXPERIMENTAL DESIGN

Four independent experiments were carried out. In the first experiment, mice were allocated at random into six groups of 12 animals each: Viable *L. casei*-treated (VLc), VLc-control l (VLc-C), Dead *L. casei*-treated (DLc), DLc-control (DLc-C), Supernatant of *L. casei* culture-treated (SLc) and SLc-control (SLc-C). In each of the other three experiments only two groups were involved: VLc and VLc-C (12 mice/group). In groups VLc each mouse received orally 1.8×10^9 viable *L. casei*, in groups VLc-C, DLc-C and SLc-C each animal received

orally 0.1 ml of sterile MRS broth (MRSB). In group DLc each mouse was inoculated orally with 1.8×10^9 dead *L. casei* (organisms were killed in boiling water during 30 min) and in group SLc each rodent received orally 0.1 ml of cell-free supernatant of *L. casei* cultured for 24 hours (centrifuged at 5,000 × g for 10 min).

WORM BURDEN

Five days after challenge (a.c.) half of the mice from each group (six animals) were sacrificed to determine the number of adult worms recovered from the intestine (AWI) by a modified Baermann technique (Manson- Smith *et al.*, 1979). At day 30 a.c. the remaining mice from each group, were processed to determine the number of larvae per gram of muscle tissue (LPGMT) by the standard digestion procedure, using 1 % pepsin and 1 % HCl (Peña-Piña *et al.*, 1988). Additionally, from each mouse small intestine a sample was taken and cultured in MRSB at 37° C for 24 hours to attempt *L. casei* isolation.

STATISTICAL ANALYSIS

Comparison of data obtained between groups was made using the Mann-Whitney U-test. A value greater than $p = 0.05$ was considered non-significant.

RESULTS

The percentage of adult worm reduction oscillated between 53.1 and 58 % in mice treated with viable *L. casei*, while reductions in animals treated with dead lactobacilli or supernatant from *L. casei* culture were of 44 and 32.5 % respectively (Table I).

The values of larvae per gram of muscle tissue ranged from 48.4 to 70.7 % in rodents which ingested viable *L. casei* compared with the percentage reductions of 65.9 and 24 % obtained respectively in mice treated with dead lactobacilli or with supernatant (Table II).

DISCUSSION

The results indicate that viable or dead *L. casei*, administered orally to mice seven days before *T. spiralis* challenge, promote a protective response against this nematode. However, the adult worm reductions observed were not as good as those obtained previously with viable *L. casei* administered by intraperitoneal route (Bautista-Garfias *et al.*, 1999). We speculate that protective response observed in the present study may be explained on the basis of the following facts: 1) lactobacilli colonization of the intestine, 2) macrophage processing of dead lactobacilli in local immune tissues and presentation of *L. casei* antigens to Th1 cells which, in turn, produced IL-2 to activate B cells and other T cells and also gamma interferon (IFN-γ). It is probable that IFN-γ activated macrophages in such a way that these cells rapidly processed *Trichinella spiralis* antigens improving the acquired immune response against the parasite. These macrophages also produced nitric oxide (NO) and probably promoted an inflammatory response in the intestine. In this respect, we have found that sera from *L. casei* treated mice recognize, by Western blot, at least four components from the lactobacilli showing apparent molecular weights of 24, 54, 62 and 128 kilodaltons (C.R. Bautista-Garfias *et al.*, unpublished data)

Experiment number	Group	Number of mice	Adult worm recovery Mean ± SEM	% Reduction compared with control	*P*-value[a]
1	Control	6	40.5 ± 2.7		
	Viable *L. casei*	6	17.0 ± 3.1	58	< 0.01
2	Control	6	47.0 ± 7.6		
	Viable *L. casei*	6	21.0 ± 2.2	56	< 0.01
3	Control	6	289.0 ± 14		
	Viable *L. casei*	6	135.0 ± 62.3	53.1	< 0.01
4	Control	6	272.5 ± 12		
	Viable *L. casei*	6	127.0 ± 35	53.4	< 0.01
1	Control	6	252.0 ± 4.8		
	Dead *L. casei*	6	105.0 ± 12.1	44	< 0.01
1	Control	6	241.0 ± 16.6		
	Supernatant *L. casei* culture	6	163.0 ± 38.4	32.5	< 0.05

[a] Mann-Whitney *U*-test

Table I. – Effect of *Lactobacillus casei* oral treatment on the number of adult worms recovered from NIH mice, five days after the *Trichinella spiralis* challenge.

Experiment number	Group	Number of mice	Larvae per gram of muscle tissue Mean ± SEM	% Reduction compared with control[a]	*P*-value[b]
1	Control	6	535.8 ± 5.4		
	Viable *L. casei*	6	157.2 ± 5.8	70.7	< 0.01
2	Control	6	1594.5 ± 31.6		
	Viable *L. casei*	6	738.3 ± 5.4	53.7	< 0.01
3	Control	6	2165.0 ± 45.8		
	Viable *L. casei*	6	1117.3 ± 53.6	48.4	< 0.01
4	Control	6	581.0 ± 3.8		
	Viable *L. casei*	6	231.3 ± 8.9	60.1	< 0.01
1	Control	6	1515.0 ± 12.6		
	Dead *L. casei*	6	516.2 ± 10.5	65.9	< 0.01
1	Control	6	1532.0 ± 53.4		
	Supernatant *L. casei* culture	6	1166.0 ± 37.9	24	< 0.05

[a] Lactobacilli were isolated only from the intestines of mice treated with viable *L. casei*.
[b] Mann-Whitney *U*-test.

Table II. – Effect of *Lactobacillus casei* oral treatment on the number of larvae per gram of muscle tissue recovered from NIH mice, 30 days after the *Trichinella spiralis* challenge.

and that peritoneal macrophages from these mice induced a significantly increase in the production of IFN-γ (Bautista-Garfias *et al.*, 1999) and produced high levels of NO as compared with non-treated controls (R. Hernández *et al.*, unpublished data). Other researchers, have also demonstrated that *L. casei* administered orally in mice induces the production of IL-12 and IFN-γ by splenocytes (Kato *et al.*, 1999).

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