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


Summary:
The capacity of viable, dead Lactobacillus casei and supernatant 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 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 means 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., 1995a), 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 determine if viable or dead L. casei administered orally to mice would induce a similar protective response against T. spiralis infection.

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 x 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 1 (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 x 10⁶ 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 x 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 x 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).

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Group</th>
<th>Number of mice</th>
<th>Adult worm recovery</th>
<th>% Reduction compared with control</th>
<th><em>P</em>-valuea</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>6</td>
<td>40.5 ± 2.7</td>
<td>58</td>
<td>&lt; 0.01</td>
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<tr>
<td></td>
<td>Viable <em>L. casei</em></td>
<td>6</td>
<td>17.0 ± 3.1</td>
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<td>2</td>
<td>Control</td>
<td>6</td>
<td>47.0 ± 7.6</td>
<td>56</td>
<td>&lt; 0.01</td>
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<td>21.0 ± 2.2</td>
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<td>3</td>
<td>Control</td>
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<td>289.0 ± 14</td>
<td>53.1</td>
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<td>Viable <em>L. casei</em></td>
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<td>135.0 ± 62.3</td>
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<tr>
<td>4</td>
<td>Control</td>
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<td>272.5 ± 12</td>
<td>53.4</td>
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<td>Viable <em>L. casei</em></td>
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<td>127.0 ± 35</td>
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<tr>
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<td>Control</td>
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<td>252.0 ± 4.8</td>
<td>44</td>
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<tr>
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<td>Control</td>
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<td>241.0 ± 16.6</td>
<td>32.5</td>
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<tr>
<td></td>
<td>Supernatant <em>L. casei</em> culture</td>
<td>6</td>
<td>163.0 ± 38.4</td>
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</tr>
</tbody>
</table>

*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.

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).
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REFERENCES


