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Orally administered *Lactobacillus plantarum* reduces pro-inflammatory interleukin secretion in sera from *Listeria monocytogenes* infected mice

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Lactic acid bacteria have traditionally been thought to have immunomodulating effects. To verify this property, *Lactobacillus plantarum* was orally administered to mice (5×10^7 colony forming units (c.f.u.)), prior to infection with *Listeria monocytogenes* in order to evaluate the host resistance against an infectious micro-organism and to better define the influence of *L. plantarum* on such responses. Balb/c mice were treated daily with *L. plantarum* or received PBS (sham-treated mice as controls) for 4 weeks. Subsequently, mice were intravenously infected with a clinical isolate of *L. monocytogenes*. Our study revealed that the administration of *L. plantarum* did not significantly increase the survival ($P=0{\cdot}13$) of mice (fifteen in each group) after *L. monocytogenes* infection (10^6 c.f.u./ml), whereas a sub-lethal dose of *L. monocytogenes* (10^5 c.f.u./ml) was eliminated from liver and spleen 5 d after the challenge in both *L. plantarum*- and sham-treated mice (*n* 5). Nevertheless, the levels of IL-1β and IL-6 from sera of orally administered *L. plantarum* were drastically reduced at 0, 4 ($P<0{\cdot}01$) and 6 d after *L. monocytogenes* infection, whereas TNF-α production was unaltered. In conclusion, administration of *L. plantarum* reduced pro-inflammatory IL production after challenge with *L. monocytogenes*, although it did not significantly impact the survival of mice. We speculate that *L. plantarum* could exert anti-inflammatory effects, which may represent an important model to reduce inflammatory disorders. Therefore, further studies in human subjects should determine the role of *L. plantarum* as an immunomodulatory micro-organism and its relationship in the host protection to pathogens.

Probiotics: *Lactobacillus plantarum*: *Listeria monocytogenes*: Pro-inflammatory cytokines

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Probiotic lactobacilli have been widely defined as living micro-organisms with low or no pathogenicity that exert beneficial effects on human health by improving the balance of gut indigenous microbiota when they are administered in adequate amounts[1,2]. These micro-organisms have been associated with the modulation of immune functions because they participate in the enhancement of both humoral and cell-mediated immune responses[3,4], conferring a protective effect against pathogens. For this reason, lactic acid bacteria (LAB) are considered as micro-organisms capable of preventing infection by pathogenic bacteria, offering an alternative to traditional therapies for the prevention or treatment of intestinal infections. The antimicrobial activities of probiotics have been evaluated against *Escherichia coli*, *Salmonella*, *Listeria* species, *Helicobacter pylori*[5,6] and *Candida albicans*[7]. Therefore, there is a large body of evidence showing the involvement of LAB in the modulation of the intestinal mechanisms of defence against pathogenic bacteria in *in vivo* models[8,9]. In human subjects, pathogen challenge investigation is obviously not possible, but clinical studies have demonstrated an increase of Ig secretion following exposure to attenuated or non-virulent strains of enteric pathogens[10]. The mechanisms by which probiotics exert a protective effect are not clearly elucidated; however, antimicrobial characteristics may be attributed to different factors such as organic acid production, NEFA, hydrogen peroxide, bacteriocin-like compounds[11] or modulation of immune response[12]. This last aspect acquires a clinical importance because LAB may prevent the attachment of pathogen micro-organisms by competitive inhibition for microbial adhesion sites favouring the elimination of these detrimental agents[13].

Lactobacilli isolated from the human and mouse gastrointestinal tracts are considered a part of commensal microbiota with beneficial effects on health, including enhanced lymphocyte proliferation[14], innate and acquired immunity[3] and anti-inflammatory cytokine production[15]. Therefore, there are numerous application areas for use of LAB both in industry and human health, including the preservation of foods and use as probiotics.

Lactobacillus plantarum constitutes the dominant species in fermented food products such as sour dough, green olives or natural wines, because this micro-organism tolerates a lower pH than other bacteria. This bacterium is more common in the commensal microbiota of vegetarian people than in the microbiota of omnivores[16] and it is known to produce important antimicrobial substances[17]. The present study was designed to determine whether the oral administration of

Abbreviations: c.f.u., colony forming unit; LAB, lactic acid bacteria; MRS, Mann Rogosa Sharpe.
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L. plantarum influences immune resistance in a murine model infected with *Listeria monocytogenes* by an intravenous route. *L. monocytogenes*, a food-borne pathogen of major importance for man, has been extensively used for some years as an experimental model to better define molecular pathogenesis and cellular immunity involved in the host defence against an intracellular pathogen[18], since protective immunity to *L. monocytogenes* requires a coordinated action between many cell types and the production of numerous cytokines[19].

In the present study, a strain of *L. plantarum* was orally administered to Balb/c mice, prior to *L. monocytogenes* infection. Our purpose was to evaluate the protective effects of *L. plantarum* after oral administration of this probiotic. Therefore, in the current study we investigated the effects of the administration of *L. plantarum* on resistance to infection in an experimental model based on the exposure to *L. monocytogenes*, an intracellular pathogen. In addition, we examined the action of *L. plantarum* administration on the pro-inflammatory cytokine production during the course of infection with *L. monocytogenes*.

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Materials and methods

Animals and treatment with Lactobacillus plantarum

BALB/c mice, 8 to 10 weeks old, were purchased from the University of Jaen (breeding colony of Servicios Técnicos de Investigación, Universidad de Jaén). Mice were housed in cages in an environmentally controlled room at a temperature of 24°C with a 12 h light–dark cycle and divided into two groups: *L. plantarum*-treated mice or sham-treated mice (received PBS). Mice were randomly allocated to receive daily *L. plantarum* or sterile PBS by oral route for 4 weeks, respectively. Each group was allowed access *ad libitum* to water as well as to standard mouse chow (PanLab A04 mouse maintenance diets, Barcelona, Spain). The animal procedures complied with the national and European Union legislation on the care and use of animals and related codes of practice (86/609/EEC).

Bacteria preparation

L. plantarum isolated from kefir[20] (kindly provided by Dr. Ruiz-Bravo, Facultad de Farmacia, Universidad de Granada, Spain) was grown overnight at 37°C in Mann Rogosa Sharpe (MRS) broth medium (Scharlau Chemie, Barcelona, Spain). The number and viability of LAB were determined by aerobic culturing on MRS plates. A clinical isolate of *L. monocytogenes* was grown in blood tryptic soya agar medium (Scharlau Chemie) for 24 h at 37°C. At the end of the experimental feeding period, each mouse was intravenously infected with a virulent strain of *L. monocytogenes*, injected through the tail vein.

Analysis of acid and bile salt tolerance

To test acid tolerance, we used the assay described by Chou & Weimer with minor modifications[21]. *L. plantarum* was grown for 24 h at 37°C and, subsequently, cells were collected by centrifugation at 4300 ***g*** for 10 min at 4°C, washed three times in sterile saline solution and inoculated (1 %) onto MRS broth acidified with concentrated hydrochloric acid to pH 3·5 or non-acidified MRS broth to pH 6·8. Counts of viable cells were performed at 0 and 90 min, by pour plating onto MRS agar (pH 6·8). To confirm the resistance to acids of this strain, because variances in acid tolerance may occur, individual colonies were grown in acidified MRS broth (pH 3·5) and incubated at 37°C for 0, 24, 48 and 72 h. The growth of bacteria was quantified spectrophotometrically (BioRad, Hercules, CA, USA) at 650 nm. These cells were then removed to tubes for the determination of bile salt tolerance. Acidified MRS broth (pH 4·0) was inoculated with the cells and each tube contained a concentration of bovine bile (0, 0·5, 1·0, 2·0 and 4·0 %, w/v) (Sigma Chemical, St. Louis, MO, USA). Tubes were incubated at 37°C for 30 min and dilutions were plated onto MRS agar and incubated at 37°C for 24 h for colony forming unit (c.f.u.) counts. Results are expressed as $\log_{10}$ c.f.u./ml.

Oral feeding, Listeria monocytogenes *infection and blood collection*

Mice were fed a dose of 5×10^7 c.f.u. viable *L. plantarum* in 100 μl PBS by gastric intubation using a feeding needle (822-gauge stainless steel) with a spherical tip (Harvard Apparatus Ltd, Edenbridge Kent, UK), every day for 4 weeks (five in each time point). Similarly, sham-treated mice received the same amount (100 μl) of sterile PBS each day. Three independent control and treated groups (sham- or *L. plantarum*-treated mice, respectively) were challenged with a similar amount of *L. monocytogenes* 1 d after the last administration of lactobacilli, in order to examine host resistance to this infectious micro-organism. Two levels of infection have been used. To measure survival, mice were intravenously injected with a lethal dose of 10^6 c.f.u./ml. To recover viable bacteria from both liver and spleen and to determine pro-inflammatory cytokine production from the sera, mice were intravenously injected with a sub-lethal dose of 10^5 c.f.u./ml. At each time-point (from 1 to 8 d), mice were killed to determine the level of colonization by *L. monocytogenes*. Peripheral blood was isolated at 0 (before infection), 2, 4, 6 and 8 d after experimental infection with this pathogen for the determination of cytokine secretion (five in each time point). Mice were anaesthetized with diethyl ether and blood was drawn from the retro-orbital plexus into tubes containing heparin (20 U/ml blood). Serum was obtained after centrifugation of the tubes at 1500 ***g*** for 30 min. Finally, serum samples were stored at −80°C for subsequent analysis.

Survival analysis

Mice fed orally with *L. plantarum* or sterile PBS were infected with a virulent strain of *L. monocytogenes* (fifteen in each group). For the determination of mice survival, *L. monocytogenes* was suspended in PBS at a concentration of 10^6 c.f.u./ml. Then, this bacterial suspension (100 μl) was injected into each mouse through the tail vein. The survival was monitored every 8 h for 8 d after the *L. monocytogenes* challenge. Mice found to be moribund were killed by cervical dislocation. The mice were closely monitored during the next

12 h as this is the predicted life expectancy for this lethal dose of bacteria. Results were expressed as percentage of survival.

Determination of numbers of viable bacteria in spleens and livers

To this assay, a *L. monocytogenes* cell suspension at 10^5 c.f.u./ml (100 μl) was inoculated through the tail vein (five in each time point). The spleens and livers of infected animals were aseptically isolated in sterile PBS and weighed under sterile conditions. Then, spleen and liver cells were prepared by homogenizing these organs between frosted-glass slides in distilled water under sterile conditions. To evaluate the extent of systemic listerial infection, the number of viable bacteria within spleens and livers were determined at various time points from 1 to 8 d after *L. monocytogenes* challenge. Thus, cells were disrupted by treatment with distilled water in order to lyse host cells and release intracellular bacteria. Then, serial 10-fold dilutions of each sample were made and an aliquot (10 μl) of each dilution was transferred onto blood tryptic soya agar medium to determine the number of live *L. monocytogenes* in the spleens and livers. Plates were incubated at 37°C for 24 h. Finally, the number of c.f.u. was counted and the values were expressed as $\log_{10}$ viable bacteria. The limit of detection was fixed approximately at 10^2 c.f.u. bacteria/organ.

Cytokine production

ELISA kits were used for determination of pro-inflammatory cytokine concentrations such as IL-1β (R&D Systems, Minneapolis, MN, USA), IL-6 (R&D Systems) and TNF-α (BioSource, Camarillo, CA, USA) in the sera samples. Results were calculated against standard curves generated using known amounts of recombinant cytokines in accordance with the manufacturer's instructions in a microplate reader (BioRad) at a wavelength of 450 nm. Limits of detection for these assays were <3 pg/ml (IL-1β), 1·6 pg/ml (IL-6) and <3 pg/ml (TNF-α). Samples were assayed in duplicate.

Statistical analysis

Results are expressed as means with their standard errors of the mean. The main effects of treatment and time of infection were compared using two-way ANOVA. Significant differences between treatments were identified by the least significant difference test at each time point. The effects of acids and bile salts in *in vitro* assays were evaluated with the Student's *t* test. Survival curves of *L. monocytogenes*-infected mice were compared using the Kaplan–Meier log rank test. The survival data from three independent experiments were pooled for statistical analysis. Viable bacteria counts from spleens or livers were $\log_{10}$ transformed prior to analysis. Data were analysed using SPSS Version 12.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences were considered as statistically significant at a value of $P<0·05$.

Results

Acid and bile salt tolerance

The *L. plantarum* strain tested in the present study was acid-tolerant to pH 3·5 for 90 min at 37°C (Table 1). In addition, the transference of this strain from MRS agar plates to acidified MRS broth for 0 to 72 h showed that the cell viability was not modified in acid medium. Fig. 1 illustrates the resistance of *L. plantarum* to the action of different concentrations of bovine bile, when this strain was grown on MRS broth at pH 4·0 for 30 min. Counts of viable *L. plantarum* after the treatment with different concentrations of bile salts has demonstrated a significant reduction of cell viability ($P<0·05$, treated *v.* untreated cultures), but there was not a dose dependency. In fact, the cell viability was maintained to approximately 10^5 c.f.u. after the treatment with bile salt from 0·5 to 4·0 % (w/v). As a result, this strain may be considered as acid- and bile-tolerant.

Table 1. Survival of *Lactobacillus plantarum* on acidified Mann Rogosa Sharpe (MRS) agar (pH 3·5) and non-acidified MRS agar (pH 6·8)*

(Values are means with their standard errors of the mean of two independent determinations in triplicate)

	Counts ($\log_{10}$ c.f.u./ml)			
	Control (pH 6·8)		Treatment (pH 3·5)	
Time (min)	Mean	SEM	Mean	SEM
0	4·43	0·07	4·64	0·05
90	4·50	0·10	4·65	0·09

c.f.u., colony forming unit.
* For details of animals and procedures, see Materials and methods.

Body, spleen and liver weights

The oral administration of *L. plantarum* did not affect the body weight of Balb/c mice that were intravenously infected with 10^5 c.f.u./ml *L. monocytogenes*. Initially, the spleen weights of mice treated with *L. plantarum* were significantly reduced from 0 to 4 d after experimental infection with *L. monocytogenes* ($P<0·05$). On the other hand, no differences in the liver weights were observed (Table 2).

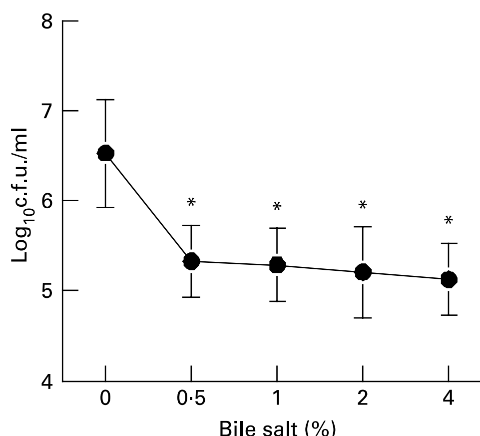

Fig. 1. Determination of bile salt tolerance. Acidified Mann Rogosa Sharpe (MRS) broth (pH 4·0) was inoculated with the cells and determination of bile salt tolerance was carried out at different concentrations from 0 to 4 %. Cells were incubated at 37°C for 30 min and dilutions were plated onto MRS agar plates and incubated at 37°C for 24 h for the counts of colony forming units (c.f.u.). Results are means with their standard errors of the mean of two independent determinations in triplicate. Values were significantly different from untreated bacteria (0 %) as calculated with the Student's *t* test: *$P<0·05$. For details of animals and procedures, see Materials and methods.

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Table 2. Effect of oral administration of *Lactobacillus plantarum* for 4 weeks on the body, spleen and liver weights of mice experimentally infected with *Listeria monocytogenes* for 8 d*

(Values are means with their standard errors of the mean of three independent experiments (five in each time point) and were analysed by two-way ANOVA)

	Body weight (g)				Spleen weight (mg)				Liver weight (g)			
	Sham		*L. plantarum*		Sham		*L. plantarum*		Sham		*L. plantarum*	
Days after challenge	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	23·67	1·42	24·23	1·84	198·3^{b}	8·9	98·2^{c}	3·3	1·05^{c}	0·36	1·12^{b,c}	0·19
1	25·77	0·21	24·26	4·71	200·1^{b}	20·1	90·1^{c}	2·2	1·08^{c}	0·22	1·17^{b}	0·06
2	23·54	5·42	22·43	2·74	270·3^{a}	15·0	120·5^{c}	1·5	1·36^{b}	0·24	1·39^{b}	0·13
3	26·93	3·28	23·53	3·05	280·2^{a}	11·1	120·5^{c}	2·5	1·58^{a}	0·12	1·60^{a}	0·26
4	21·20	5·57	21·98	3·13	280·1^{a}	14·2	130·5^{c}	4·5	1·80^{a}	0·30	1·88^{a}	0·36
5	21·97	1·81	23·35	1·48	260·3^{a}	7·3	240·1^{a}	8·2	1·25^{b}	0·19	1·66^{a}	0·12
6	22·99	1·22	22·12	4·24	190·2^{b}	6·2	190·3^{a,b}	6·0	1·22^{b}	0·03	0·96^{c}	0·22
7	21·01	1·13	24·49	2·55	200·3^{b}	3·1	170·0^{b}	3·1	1·30^{b}	0·32	1·22^{b}	0·23
8	23·85	1·48	23·40	2·36	200·2^{b}	8·3	190·2^{a,b}	4·0	1·45^{a,b}	0·19	1·37^{b}	0·24

^{a,b,c} Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of animals and procedures, see Materials and methods.

Survival of mice

The protective effects of *L. plantarum* were evaluated by measuring the survival time of Balb/c mice that received a daily dose of *L. plantarum* or PBS (sham-treated mice as controls) (Fig. 2). At 24 h of feeding treatment with either PBS or *L. plantarum*, animals were infected with a lethal dose of *L. monocytogenes*. After challenge with *L. monocytogenes*, most deaths occurred between 4 and 7 d post-infection. At day 8 after the challenge, 60 % of mice treated with *L. plantarum* survived to infection, whereas 40 % of control mice survived. Nevertheless, we did not find any significant difference between survival of mice orally administered *L. plantarum* and sham-treated mice ($P=0·13$).

Bacterial clearance from spleens and livers

To verify the capacity of the immune system to eliminate *L. monocytogenes* from spleens or livers, these organs were homogenized and viable bacteria were isolated. The recovery of viable *L. monocytogenes* from spleens was significantly lower ($P<0·05$) in *L. plantarum*-treated mice compared with sham-treated mice (i.e. 10-, 5-fold at 1 and 2 d after the challenge, respectively), whereas it was significantly higher ($P<0·05$) in *L. plantarum*-treated mice compared with sham-treated mice (i.e. 8-, 75-fold at 3 and 4 d after the challenge, respectively) (Fig. 3(A)). Finally, *L. monocytogenes* was not detected in spleens of both experimental and control groups at day 5 after the challenge. Bacterial recovery from livers was similar at day 1 of experimental infection in both the experimental and control groups, whereas the counts of *L. monocytogenes* were significantly higher ($P<0·05$) in *L. plantarum*-treated mice compared with sham-treated mice (i.e. 5-, 6- and 10-fold at 2, 3 and 4 d after the challenge, respectively) (Fig. 3(B)). At day 5 post-infection, the bacteria were completely eliminated from livers of *L. plantarum*-treated mice, whereas 10^2 c.f.u. were counted in the livers of the control group, although this micro-organism was absolutely eliminated from liver at day 7 after the challenge (Fig. 3(B)).

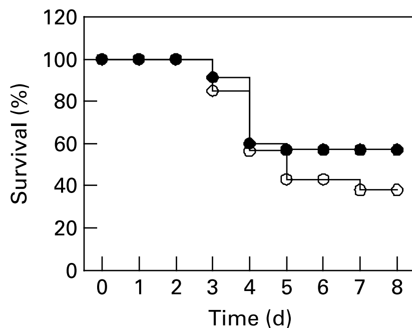

Fig. 2. Measurement of survival percentage of mice orally administered *Lactobacillus plantarum* and challenged with *Listeria monocytogenes*. Balb/c mice were treated with *L. plantarum* or received PBS (sham-treated mice) for 4 weeks (fifteen in each group) and subsequently infected with *L. monocytogenes* (10^6 colony forming units (c.f.u.)/ ml). ●, Represents the survival percentages of mice treated with *L. plantarum* (experimental mice); ○, represents the survival percentages of sham-treated mice. The data represent the pooled result of three experiments. For details of animals and procedures, see Materials and methods.

Measurement of pro-inflammatory cytokine production

In general, the oral administration of *L. plantarum* to mice experimentally infected with *L. monocytogenes* produced a reduction of IL-1β and IL-6 concentration in the serum of mice treated with *L. plantarum* (Fig. 4). The alteration of both IL-1β and IL-6 production depends on the treatment ($P<0·05$) and on the time of challenge with *L. monocytogenes* ($P<0·05$). IL-1β production in the *Lactobacillus*-treated group was significantly reduced before infection with *L. monocytogenes* (day 0), compared with sham-treated mice ($P<0·05$), but levels of this cytokine increased at day 2 post-infection. Again, the secretion of this cytokine was significantly reduced with regard to control mice at 4 and 6 d after the challenge with *L. monocytogenes* ($P<0·01$) (Fig. 4(A)). On the other hand, the production of another pro-inflammatory cytokine such as IL-6 was quantified in the serum from these mice. The levels of this cytokine decreased progressively in comparison with the values from the control group at day 4 after the challenge with *L. monocy-*

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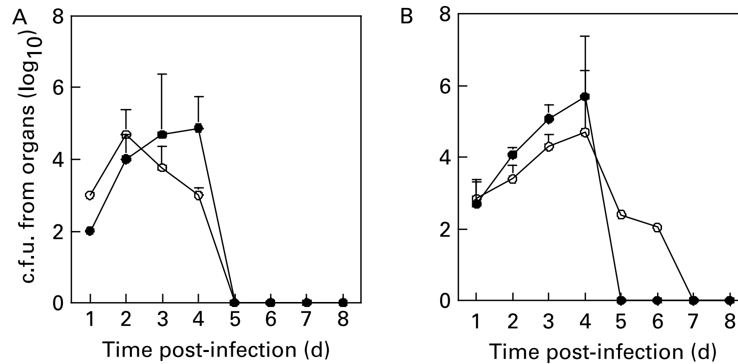

Fig. 3. Recovery of viable *Listeria monocytogenes* from spleens (A) and livers (B) of mice orally fed with *Lactobacillus plantarum* or treated with PBS (sham-treated mice). Balb/c mice were treated with *L. plantarum* (●) for 4 weeks (five in each time point) and subsequently infected with *L. monocytogenes* (10^5 colony forming units (c.f.u.)/ml). Sham-treated mice received orally sterile PBS (○) for 4 weeks (five in each time point) and subsequently infected with *L. monocytogenes* (10^5 c.f.u./ml). The number of bacterial colonies was counted and the results were expressed as $\log_{10}$ viable bacteria. Results are means with their standard errors of the means of two identical experiments. Data were analysed by two-way ANOVA. Mean values with different superscript letters were considered to be significantly different ($P<0.05$). For details of animals and procedures, see Materials and methods.

togenes ($P<0.01$) (Fig. 4(B)). Finally, the production of another important pro-inflammatory cytokine such as TNF-α was also assessed. Results from values of mice orally treated with *L. plantarum* have not indicated any significant differences with regard to values from non-treated mice in the different time intervals of infection, although an important increase of TNF production was observed at day 2 of challenge in both sham- and *L. plantarum*-treated mice ($P<0.05$) (Fig. 4(C)).

Discussion

Health benefits attributable to the consumption of LAB have been extensively reported in recent years[22]. Based on these properties, probiotics have been applied as micro-organisms that exert a favourable influence on the host by improving the indigenous microbiota[2,23]. It is well recognized that the organisms most commonly used as probiotics are LAB (lactobacilli and bifidobacteria), which appear to be promising candidates in clinical practice for the treatment of disorders caused by intestinal abnormal microbiota[24]. Hence, probiotics constitute a valuable option that may contribute to improve gut mucosal barrier functions[5], because antigenic components from lactobacilli can enter epithelia cells, establishing contact with immune cells and stimulating the intestinal mucosal immune system without safety problems[25].

In addition, LAB act as immunomodulatory agents capable of exerting a crucial role in the host immune defence against infectious micro-organisms. Therefore, an initial objective in the present study was focused on the examination of *in vivo* action of *L. plantarum* and its ability to modify host resistance against an infectious micro-organism. For this reason, we have used a murine model infected with *L. monocytogenes*, a Gram positive pathogen that serves as an important model for understanding host immune resistance against intracellular bacteria. In addition, from a clinical point of view, *L. monocytogenes* exerts an adverse effect in elderly, newborns and immunocompromised individuals, who are more susceptible to infection by this micro-organism.

Before arriving in the intestinal tract, probiotic micro-organisms must resist transit through the stomach[26]; therefore, resistance to low pH and bile are considered as essential properties of probiotics. Despite the reported beneficial effects of oral probiotics on the immune system functions, in the present study, the oral administration of an acid- and bile-resistant strain of *L. plantarum* has not produced an efficient elimination of *L. monocytogenes* from both the liver and spleen of mice, suggesting an absence of a protective activity against this pathogen after oral administration of *L. plantarum*. In fact, an early investigation suggested that *L. plantarum* together with other species of lactobacilli do not enhance the immune resistance against *L. monocytogenes* infection[27], but it prevents colonization of pathogenic micro-organisms by competitive inhibition for microbial adhesion sites[28]. However, this strain has been recently reported to promote adhesion to epithelial cells, although it was unable to establish a continuous colonization in the gastrointestinal tract[29]. In the current study, the impact of *L. plantarum* on the survival against a lethal dose of *L. monocytogenes* has produced a non-statistical significant increase of survival in comparison with values from sham-treated mice. Similarly, other early studies also reported an enhancement of resistance to *L. monocytogenes* infection in mice that received an intravenous or a subcutaneous injection of *L. casei*[30,31].

In spite of the fact that the oral administrations of another LAB, such as *L. casei*, significantly improves host resistance against oral *L. monocytogenes* infection[32], the present findings show that the oral administration of *L. plantarum* is unable to ameliorate the elimination of *L. monocytogenes* from spleens and livers of mice experimentally infected. Indeed, the present data have shown that spleen weight was initially reduced before the inoculation of *L. monocytogenes* and after the challenge with this pathogen. In addition, a recent study has indicated that *in vitro* cultures of *L. plantarum* inhibit the growth of *L. monocytogenes*, but the presence of *L. plantarum* in the gut of gnotobiotic rats facilitates *L. monocytogenes* colonization[33]. This fact corroborates the reduced capacity of *L. plantarum* to stimulate host immune response.

Numerous investigations have reported that LAB are involved in an increase of cytokine secretion[2,34,35]. Nonetheless, *L. plantarum* administered to Balb/c by an oral route for 4 weeks significantly decreases the production of

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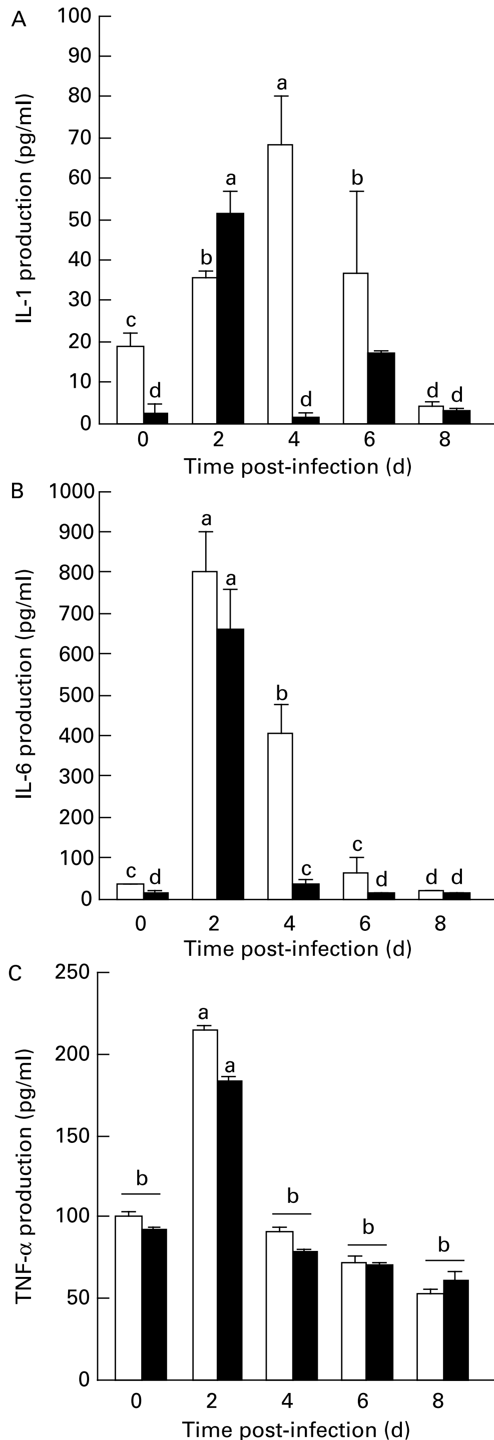

Fig. 4. Measurement of IL-1β, IL-6 and TNF-α production from the sera of peripheral blood of mice orally administered *Lactobacillus plantarum* or PBS (sham-treated mice) and challenged with *Listeria monocytogenes*. BALB/c mice (five in each time point) received sterile PBS (□) or were orally administered *L. plantarum* (■) for 4 weeks and subsequently were challenged with *L. monocytogenes* (10^5 colony forming units (c.f.u.)/ml). The production of IL-1β (A), IL-6 (B) and TNF-α (C) from the animal sera was measured from 0 to 8 d after experimental infection. Quantification of IL-1β, IL-6 and TNF-α levels in experimental samples were made by extrapolation from ELISA results, using various concentrations of rIL-1β, rIL-6 and r-TNF-α as a standard, respectively. Results are means with their standard errors of the means of two identical experiments. Data were analysed by two-way ANOVA. Mean values with different superscript letters were considered to be significantly different ($P < 0·05$). For details of animals and procedures, see Materials and methods.

pro-inflammatory cytokines such as IL-1β and IL-6, but does not alter the secretion of TNF-α. We hypothesize that *L. plantarum* could modify cytokine profile reducing T-helper 1 cell response.

According to the results, *L. plantarum* does not enhance immune resistance against an intravenous infection with the intracellular pathogen *L. monocytogenes*. In addition, the production of pro-inflammatory cytokines such as IL-1β or IL-6 was strongly inhibited in mice orally fed with *L. plantarum*, whereas TNF-α production was not altered after the oral administration of *L. plantarum*. Based on the present results, we conclude that the *L. plantarum* strain isolated from kefir is characterized by showing a resistance to bile salts and acids, without conferring a determinant protective role, because it does not appear to participate in the elimination of *L. monocytogenes*. However, the reduction of IL-1 and IL-6 levels leads to the hypothesis that *L. plantarum* may represent an important model to reduce inflammatory disorders. On the basis of this last argument, we suggest that further studies should determine the action of *L. plantarum* and its application in clinical practice as an immunomodulatory micro-organism that diminishes the inflammatory response without severely reducing the host resistance to the infection. Hence, *L. plantarum* is not related to an impairment of host resistance against *L. monocytogenes* because it does not exert adverse effects on survival and on the clearance of *L. monocytogenes* from liver and spleen. However, oral administration of *L. plantarum* is associated with an important reduction of pro-inflammatory IL secretion. Indeed, *L. plantarum* and other lactobacilli species have been reported as probiotics able to strengthen anti-inflammatory cytokine synthesis, decreasing expression of pro-inflammatory interferon-γ and stimulating secretory IgA production[15,36,37].

Taking into account the high incidence of allergies, inflammatory bowel disease, urogenital infections and other disorders in the gut and urogenital tract, the action of probiotic micro-organisms may play an essential role in contributing to their resolution. Therefore, further studies are needed to determine the influence of an oral administration of *L. plantarum* in the production of cytokines with an anti-inflammatory function and to understand the impact of probiotic treatment upon the modulation of host immune resistance against an infectious micro-organism.

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