The effect of orally administered viable probiotic and dairy lactobacilli on mouse lymphocyte proliferation

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Abstract

Four common Lactobacillus strains were screened for their effects on proliferation of mouse splenic lymphocytes. Mice received perorally 10^9 viable bacteria kg^-1 body weight for 7 days. Lactobacillus acidophilus treatment enhanced ex vivo basal proliferation (by 43%) and B-cell response at suboptimal and optimal concentrations of lipopolysaccharide (LPS) (by 27–28%). Conversely, Lactobacillus casei, Lactobacillus gasseri and Lactobacillus rhamnosus inhibited both basal proliferation (by 14–51%) and mitogen-stimulated lymphoproliferation, particularly at supra-optimal concentrations of concanavalin A (by 43–68%) and LPS (by 23–62%). Therefore, these Lactobacillus strains demonstrate strain-specific effects on B- and T-cells and may also alter the splenocyte sensitivity to the cytotoxic effects of mitogens. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Immunomodulation in humans by orally administered micro-organisms, particularly by those considered probiotic, has received increasing attention. Probiotics are defined as microbial feed or food supplements which beneficially influence the host [1]. Of the strains currently considered as ‘successful’ probiotics, most belong to the Lactobacillus genus [2]. Various immune responses have been reported to be influenced by probiotics and these immunomodulatory effects have been proposed for several potential applications. These include the prevention of infectious diarrhoea, management of hypersensitivity reactions and tumour suppression [2–4]. At present, there is little knowledge concerning the dose response relationships for the immunomodulatory effects of different probiotic strains or on the mechanism of these effects. Consequently, immunomodulation by probiotics can yet not be performed in a controlled manner, thus limiting the clinical applications [3].

In this study, we examined four different Lactobacillus strains commonly used in dairy fermentation.
The objective for the study was to make a preliminary evaluation of the effects that these strains have on the systemic immunity, using the parameters of unstimulated and mitogen-induced splenic lymphocyte proliferation. All of the four strains selected for testing in this study represent different Lactobacillus species and also include Lactobacillus casei Shirota (YIT 9018) (LS), which is one of the most studied probiotic bacteria.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (Monash University, Clayton, Vic., Australia), 8–10-weeks old, were housed in plastic cages in an air-conditioned room and given free access to food and water. This study was performed under the approval of RMIT-University Animal Experimentation Ethics Committee Project number 9608.

2.2. Micro-organisms and feeding procedure

Strains of Lactobacillus acidophilus (ATCC 4356) (LA) and Lactobacillus gasseri (ATCC 33323) (LG) were obtained from the culture collection of the Department of Medical Laboratory Science (RMIT-University, Melbourne, Vic., Australia). Lactobacillus rhamnosus strain LC705 (DSM7061) (LC) was obtained from Valio (Helsinki, Finland). LS was isolated from a commercially available fermented milk drink, Yakult®. All bacteria were grown from an inoculation, in de Man, Rogosa and Sharpe broth (Oxoid Hampshire, UK) for 18–22 h at 37°C. After cultivation, bacteria were harvested by centrifugation (5 min, 2000 g) and washed three times with sterile saline.

For assessment of the number of viable bacteria, a sample of each culture was analysed by flow cytometry (Coulter Elite®), as previously described [5]. SYTOX® (a fluorescent green nucleic acid dye staining the DNA of cells with compromised membranes) was used to evaluate bacterial viability. The bacterial suspensions were diluted to the desired concentration with sterile saline and administered orally (at a dose of 10⁹ viable bacteria kg⁻¹ body weight, in a volume of 1 ml kg⁻¹) to mice with a stainless steel oesophageal gavage needle (22 gauge × 38 mm, with a ball diameter of 1.025 mm) and a 1-ml syringe. Control mice were treated in a similar manner but received only sterile saline.

2.3. Splenocytes

Mice were killed by cervical dislocation. Spleens were removed and placed in RPMI 1640 media (ICN Biomedicals, Aurora, OH, USA) with HEPES buffer (20 mM), sodium bicarbonate (24 mM) and gentamicin (100 µg ml⁻¹) (Sigma Chemical, St. Louis, MO, USA). The splenocytes were isolated by injecting the media into the spleen, after which the spleens were teased apart. The splenic cell suspension was filtered through a nylon mesh (pore size 250 µm) and the cells were pelleted by centrifugation (200 × g for 5 min at 4°C). To remove erythrocytes, the splenocytes were incubated for 3 min in lysing buffer (155 mM ammonium chloride, 10 mM potassium bicarbonate and 127 µM EDTA) following a dual wash (200 × g for 5 min at 4°C). For the mitogenesis assay, the viable splenic lymphocytes in 3 ml were diluted with foetal calf serum (FCS) (CSL Biochemicals, Parkville, Vic., Australia)-enriched media (10% FCS) to 1 × 10⁶ cells ml⁻¹. Trypan blue exclusion was used to check the viability of the immune cells.

2.4. Lymphocyte proliferation assay

The splenic immune cell suspensions were incubated at 37°C (with 5% CO₂ in air) in 96-well round-bottom microtitre plates (Greiner, Frickenhausen, Austria) with concanavalin A (Con A) or lipopolysaccharide (LPS). Suboptimal, optimal and supra-optimal mitogen concentrations were used (i.e. 2.5, 5, 10 µg ml⁻¹ of Con A and 12.5, 25, 50 µg ml⁻¹ of LPS). The rate of spontaneous lymphoproliferation was assessed by incubating the immune cell suspension without mitogen. After a 24-h incubation period, cells were pulsed for 48 h with tritiated thymidine (5⁰⁻²H, Amersham International, Amersham, UK) (0.5 µCi well⁻¹) and harvested onto glass filter mats (Flow Laboratories Australasia, North Ryde, N.S.W., Australia) using a 12-well semi-automatic cell harvester (Skatron, Lier,
Radioactivity incorporated to the newly dividing cells was measured with a liquid scintillation counter (LKB Wallac, Turku, Finland), using ACS-II scintillation fluid (Amersham International, Amersham, UK).

2.5. Statistical analyses

The results are presented as relative proliferation indices (i.e. % mean dpm in cell cultures of treated mice per mean dpm in cell cultures of simultaneously tested control mice). Analysis of variance was used to evaluate the statistical significance of the differences between the treatment groups. The statistical significance of each treatment group in comparison to control animals was tested at the $P = 0.05$ level, using a two-tailed $t$-test.

3. Results

3.1. Somatic index

The average spleen somatic index (spleen to body weight ratios) and lymphocyte yields obtained from the spleens of each treatment group are presented in Table 1 and expressed as % control. No statistically significant effects on these two parameters were observed with any bacterial strain using this administration procedure. However, there appeared to be a slight reduction in spleen weight following LC treatment.

3.2. Lymphoproliferative responses

The various lactobacilli treatments demonstrated a significant strain dependency in their effects on the ex vivo proliferation of both resting and mitogen-stimulated lymphocytes (Table 2). The spontaneous lymphoproliferation was decreased by LC, LS and LG. Conversely, LA treatment appeared to enhance this parameter. T-cell proliferation was decreased by LG at the suboptimal concentration of Con A and by LG, LS and LC at the supra-optimal mitogen concentration. B-cell proliferation was decreased by LS, LG and LC at the supra-optimal concentration of LPS, while LC treatment alone reduced lymphoproliferation at the suboptimal LPS concentration. In this study, LA had no significant effect on mitogen-stimulated lymphoproliferation.

4. Discussion

Lymphoproliferation is commonly examined when analysing the efficacy of an immunosuppressive or immuno-enhancing therapy, when testing chemicals for their immunotoxic potential and when monitoring congenital immunological defects. In this study,
oral treatment with LS, LG and LC inhibited lymphocyte proliferation, which suggests that these bacteria may have potential for use in the management of hypersensitivity responses. This postulation correlates with recent reports of the oral LS treatment reducing IgE production in mice [6,7]. However, the use of these strains as probiotics in an immunodeficient host should be carefully assessed to avoid exacerbation of their immunosuppressed status. In contrast, the LA treatment tended to enhance basal and LPS-stimulated mitogenesis, although this effect did not reach statistical significance. Nevertheless, other dosage regimes of LA should be investigated in order to further evaluate its potential as an immunomodulator.

The present study demonstrated that splenic lymphocytes isolated from mice treated with LC, LG or LS were more vulnerable to the inhibitory effects of supra-optimal concentrations of LPS. Previously, the reduced proliferative B-cell response to high concentrations of this mitogen has been connected to activation of the nitric oxide pathway by tumour necrosis factor-α (TNF-α) [8]. Endogenous TNF levels have also been suggested to mediate LPS-induced apoptotic cell death in DMSO-pretreated human myelomonocytic cells [9]. Furthermore, TNF-α production is enhanced by exposure to lactic acid bacteria [10,11] and therefore, the increased susceptibility of B-cells to high concentrations of LPS may be mediated, at least in part, by this cytokine. As LPS plays a major role in the pathogenesis of Gram-negative bacterial sepsis and endotoxic shock [12], the effect of lactobacilli treatments on lymphocyte responsiveness should be investigated in disease states which have an increased risk of sepsis, such as ulcerative colitis, in order to determine whether probiotic dosing is contra-indicated in these cases.

Administration of LC, LG and LS also appeared to reduce the resistance of the splenic immune cells to supra-optimal concentrations of Con A. Small increases in the relative concentration of Con A to the immune cell number can result in a ‘switchover’ from mitogenicity to cytotoxicity [13]. It has been hypothesised that T-lymphocytes respond to the binding of this mitogen by producing a burst of reactive oxygen species (such as superoxide or hydrogen peroxide), which, if sufficiently elevated and prolonged, will result in cytotoxicity [13]. Further evidence suggests that the cytotoxicity induced by Con A results in ultrastructural changes consistent with apoptotic processes [14] and has consequently been suggested as a useful model for the study of lymphocyte apoptosis in AIDS [13]. The enhancement of the inhibitory effects of high mitogen concentrations by LC, LG and LS treatments warrants further investigation to determine whether the ingestion of lactic acid bacteria can alter the susceptibility of lymphocytes to apoptosis. Furthermore, it would also be useful to study the effects of lactic acid bacteria on the susceptibility of host lymphocytes to known cytoto-, endo- and immunotoxins.

Apart from the biological significance, the observation that some of these lactobacilli treatments inhibit basal lymphoproliferation highlights the need for appropriate reporting of such mitogenesis studies. It is common practise to present lymphoproliferation results only as ‘stimulation indices’ (SI) (i.e. the ratio of mitogen-stimulated proliferation to unstimulated proliferation), as has been applied previously in some probiotic-related studies [15,16]. However, if treatment with a probiotic strain inhibits the basal lymphoproliferation, but not mitogen-stimulated proliferation, the resultant enhancement of SI is artifactual. Therefore, the background values associated with each SI should always be presented to enable adequate evaluation of the published studies.

In conclusion, this study demonstrated that the oral administration of different dietary lactobacilli results in strain-specific effects on the ex vivo proliferative activity of splenic B- and T-lymphocytes in both unstimulated and mitogen-stimulated cultures. These findings suggest that the immune effects of one *Lactobacillus* organism cannot easily be extrapolated from the effects of another *Lactobacillus* strain or species, even if closely related. Furthermore, additional studies are needed to examine the optimal dose and treatment period regimes, before these lactobacilli can be used appropriately for their immunomodulatory effects in therapeutic trials.

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References


