

Lactobacillus casei strain Shirota (Yakult)

Research Update for Healthcare Professionals

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Lactobacillus casei strain Shirota – *Research Update*

IMPROVEMENT IN INTESTINAL HEALTH

1. Dysregulated Circulating Dendritic Cell Function in Ulcerative Colitis Is Partially Restored by Probiotic Strain *Lactobacillus casei* Shirota

Mann ER *et al* (2013) *Mediators Inflamm.* 2013:573576.
doi: 10.1155/2013/573576. Epub 2013 Jul 18.

Background. Dendritic cells regulate immune responses to microbial products and play a key role in ulcerative colitis (UC) pathology. We determined the immunomodulatory effects of probiotic strain *Lactobacillus casei* Shirota (LcS) on human DC from healthy controls and active UC patients.

Methods. Human blood DC from healthy controls (control-DC) and UC patients (UC-DC) were conditioned with heat-killed LcS and used to stimulate allogeneic T cells in a 5-day mixed leucocyte reaction.

Results. UC-DC displayed a reduced stimulatory capacity for T cells ($P < 0.05$) and enhanced expression of skin-homing markers CLA and CCR4 on stimulated T cells ($P < 0.05$) that were negative for gut-homing marker $\beta 7$. LcS treatment restored the stimulatory capacity of UC-DC, reflecting that of control-DC. LcS treatment conditioned control-DC to induce CLA on T cells in conjunction with $\beta 7$, generating a multihoming profile, but had no effects on UC-DC. Finally, LcS treatment enhanced DC ability to induce TGF β production by T cells in controls but not UC patients.

Conclusions. We demonstrate a systemic, dysregulated DC function in UC that may account for the propensity of UC patients to develop cutaneous manifestations. LcS has multifunctional immunoregulatory activities depending on the inflammatory state; therapeutic effects reported in UC may be due to promotion of homeostasis.

2. Effects of antibiotic therapy on the gastrointestinal microbiota and the influence of *Lactobacillus casei*.

Pirker *et al* (2012) *Food & Agric.Immunol.*
DOI:10.1080/09540105.2012.689816.

This study involved 678 hospital patients (mean age 71) who were on a range of single or multiple antibiotic regimes. One group (n=340) consumed a daily fermented milk drink containing *L. casei* Shirota during the antibiotic treatment and for three days after this ceased. (At the time of probiotic intervention, all patients and staff on the ward received probiotic.) A control group of patients also on antibiotics (n=338), did not receive the probiotic; these patients were matched to the probiotic group in terms of age, sex, antibiotics, disease severity, length of hospital stay, use of proton pump inhibitors.

There was no exclusion of any particular antibiotic. Antibiotics taken included penicillins, cephalosporins, quinolones, clindamycin, vancomycin, and were multiple as well as single antibiotic regimes.

During probiotic intake, all patients on the ward were offered probiotic, as well as the staff. During periods on the wards of no probiotic intake, neither staff nor patients took probiotic.

RESULTS

AAD*: 17/340 (5%) in LcS group *vs* 63/338 (18.6%) control group (*P* less than 0.001)

CDI*: 1/340 (0.3%) in LcS group *vs* 21/338 (6.2%) control group (*P* less than 0.001)

Faecal analysis showed that antibiotic therapy decreased abundance of total bacteria, *Bifidobacterium* spp, *Clostridium* clusters IV and XI whereas Enterobacteriaceae increased. LcS intervention reduced the observed antibiotic-induced decrease in abundance of total bacteria and *Bifidobacterium*. LcS intervention also increased *Lactobacillus* abundance.

*Antibiotic-associated diarrhoea = AAD; *C. difficile* infection = CDI

3. *Lactobacillus casei* Shirota modulation of ammonia metabolism in physical exercise.

Fuskevåg O-M *et al* (2012) *Int. J. Probiotics & Prebiotics* 7(1):13-16.

INTRODUCTION: During exercise, ammonia is generated as a natural metabolic waste product, normally excreted via the kidneys after conversion into urea in the liver. If the exercise is strenuous, blood ammonia levels can build up and exceed the liver's capacity to remove it. This can cause ammonia levels to increase in the brain, and if this happens the ammonia is removed through the astrocytes through the enzymatic activity of glutamine synthetase, which combines ammonia with glutamate to produce glutamine. Osmotic stress may be induced by the subsequent increase in intracellular glutamine, however, leading to water accumulation, potentially mild encephalopathy and impaired cognitive function.

Successful treatment with a phenylacetate precursor has been used in patients suffering urea cycle disorders and raised levels of ammonia in the blood. Phenylacetate forms a stable conjugate with glutamine to make phenylglutamine (PAG) which can be readily excreted via the kidneys.

RATIONALE: As lactobacilli can metabolise phenylalanine into phenylacetate, the study was based on the hypothesis that supplementation with *L. casei* Shirota could help to regulate ammonia metabolism in healthy people undertaking strenuous exercise via the natural generation of phenylacetate by the probiotic. This would enable ammonia, trapped as glutamine, to be excreted as phenylacetylglutamine via the kidneys.

METHOD: An open-label, pilot, proof of principle study was conducted in 20 male football players assigned to consume either probiotic (*L. casei* Shirota; minimum 6.5×10^9 twice per day) or no supplementation. The players undertook an exhaustive routine designed to exercise all the major muscle blocks (two cycles of a 9-station static exercise program with a one minute rest between the two cycles; water supplied ad libitum). The players provided a four hour timed urine sample after the exercise program. Both the exercise program and urine sampling were repeated after one month. Urine samples were measured for phenylacetylglutamine and ammonia and corrected by creatinine levels.

RESULTS: The results (expressed as the difference in urinary levels for each volunteer between the two sampling points) showed that phenylacetylglutamine

significantly increased in the probiotic group (2.98 ± 1.04 vs -0.911 ± 0.477 ; P less than 0.01) and, while not reaching statistical significance, their ammonia levels were also lower compared to the control group (0.953 ± 0.868 vs 1.486 ± 0.865 ; $P=0.064$).

CONCLUSIONS: The researchers concluded that probiotic supplementation with a probiotic *Lactobacillus* strain appeared to help regulate exercise-generated ammonia in young health sportsmen.

IMMUNE MODULATION

1. Oral Delivery of a Probiotic Induced Changes at the Nasal Mucosa of Seasonal Allergic Rhinitis Subjects after Local Allergen Challenge: A Randomised Clinical Trial

Ivory K *et al* (2013) *PLoS ONE* 8(11): e78650.

Abstract

Objective: To determine effects of probiotic consumption on clinical and immunological parameters of seasonal allergic rhinitis (SAR) in an out-of-season single nasal allergen challenge.

Methods: In a study registered at ClinicalTrials.gov (NCT01123252), a 16-week dietary intervention was undertaken in 60 patients with allergic rhinitis (.16 years old). Using a double-blinded, placebo-controlled anonymised design, the patients were divided equally into two groups. One group was given a dairy drink containing *Lactobacillus casei* Shirota to ingest daily while the other consumed a similar drink without bacteria. Participants attended the clinic on two consecutive days before the intervention and then again at the end of the study period. On the first day of each 2-day visit, following clinical examination, assessments were made of total nasal symptoms scores and peak nasal inspiratory flow. Nasal scrapings, nasal lavage and blood were collected for laboratory analyses of cellular phenotypes, soluble mediator release and in vitro responses to pollen allergen. These procedures were repeated 24 hours following nasal allergen challenge.

Results: Prior to and following intervention there were no detectable differences between study groups in measured clinical outcome. After intervention, there were differences between groups in their percentages of CD86+ epithelial cells ($p = 0.0148$), CD86+CD252+ non-epithelial cells ($p = 0.0347$), sIL-1RII release ($p = 0.0289$) and IL-1b ($p = 0.0224$) levels at the nasal mucosa. Delivery of probiotic also suppressed production of sCD23 ($p = 0.0081$), TGF- β ($p = 0.0283$) and

induced increased production of IFN- γ ($p = 0.0351$) in supernatants of cultured peripheral blood.

Conclusions & Clinical Relevance: This study did not show significant probiotic-associated changes with respect to the primary clinical endpoint. An absence of overt clinical benefit may be due to an inability of single nasal challenges to accurately represent natural allergen exposure. Nevertheless, oral delivery of probiotics produced changes of the immunological microenvironment at the nasal mucosa in individuals affected by SAR.

2. Decreased duration of acute upper respiratory tract infections with daily intake of fermented milk: A multicenter, double-blinded, randomized comparative study in users of day care facilities for the elderly population.

Fujita R et al (2013). Am J Infect Control. 41(12):1231-5.

Abstract

BACKGROUND:

There is insufficient evidence of preventive effect of probiotics on upper respiratory tract infections (URTIs) in an elderly population.

METHODS:

We conducted a multicenter, double-blinded, randomized, placebo-controlled parallel group study. Elderly persons had participated who used day care at 4 facilities in Tokyo. We used fermented milks containing *Lactobacillus casei* strain Shirota (LcS) and placebo drinks as test drinks.

RESULTS:

A total of 154 subjects was analyzed. The number of persons diagnosed with an acute URTIs was almost identical in both groups (LcS: 31, placebo: 32), whereas the number of acute URTIs events (LcS: 68, placebo: 51) and the symptom score (LcS: 425, placebo: 396) were both higher in the LcS group. Permutation tests performed using the total number of acute URTIs infection events/total days of observation and the total symptom score/total days of observation found no statistically significant difference respectively (P values of .89 and .64, respectively). Comparing the mean duration of infection per infection event found a shorter mean duration in the LcS group (LcS: 3.71 days, placebo: 5.40 days), and the difference was statistically significant.

CONCLUSION:

The results suggest that fermented milk containing LcS probably reduces the duration of acute URTIs.

3. Probiotic upregulation of peripheral IL-17 responses does not exacerbate neurological symptoms in experimental autoimmune encephalomyelitis mouse models.

Kobayashi T *et al* (2012) *Immunopharmacol. Immunotoxicol.*34(3):423-33.

Abstract

CONTEXT:

It is of great importance to evaluate the safety of probiotics in dysregulated immune conditions, as probiotics can possibly modulate immune functions in the host.

OBJECTIVE:

We tried to confirm the safety of using *Lactobacillus casei* strain Shirota (LcS) to help prevent autoimmunity in the central nervous system.

METHODS:

We used two chronic experimental autoimmune encephalomyelitis (EAE) models, a relapse and remission type EAE model in SJL/J mice and a durable type model in C57BL/6 mice. LcS was administered from 1 week before antigen sensitization until the end of the experiments, and neurological symptoms and histopathological changes of the spinal cord were observed. Immunological parameters were also examined in the SJL/J mouse model.

RESULTS:

LcS administration did not exacerbate neurological symptoms or histopathological changes of the spinal cord in either model but instead tended to improve neurological symptoms in the SJL/J mouse EAE model. LcS administration transiently upregulated IL-17 production by antigen-stimulated lymphocytes of draining lymph nodes 7 days after sensitization. Enhanced production of IL-10 and an increase in the percentage of CD4(+)CD25(+) T regulatory cells were also observed at the same sites. Strong expression of IL-17 mRNA was detected in the spinal cord of mice that displayed severe neurological symptoms on day 12, but this expression was not enhanced by LcS administration.

CONCLUSION:

These results demonstrate that LcS does not exacerbate, but instead may improve EAE depending on the immunization conditions, and that IL-17 responses at peripheral sites may not always result in a worsening of autoimmune diseases.

OTHERS

1. Short communication: Effect of supplementation with *Lactobacillus casei* Shirota on insulin sensitivity, β -cell function, and markers of endothelial function and inflammation in subjects with metabolic syndrome--a pilot study.

Tripolt NJ *et al* (2013) *J. Dairy Sci.* 96(1):89-95.

Abstract

Based on animal studies, intake of probiotic bacteria was suggested to improve insulin sensitivity by reducing endotoxemia and inflammation. The objective of this study was to determine the effects of supplementation with the probiotic strain *Lactobacillus casei* Shirota (LcS) over 12 wk on insulin sensitivity, β -cell function, inflammation, and endothelial dysfunction parameters in subjects with metabolic syndrome. In a randomized-controlled study, 30 subjects with metabolic syndrome either received *Lactobacillus casei* Shirota 3 times daily for 12 wk or served as controls with standard medical therapy. Fasting blood samples were taken and a 75-g oral glucose tolerance test was performed to derive indices for insulin sensitivity and β -cell function. In addition, parameters to assess endothelial function and inflammation markers were determined. Even though the insulin sensitivity index significantly improved after 3 mo of probiotic supplementation (0.058 ± 0.021 vs. 0.038 ± 0.025), the change was not significantly different compared with the control group. No improvements were seen in additional indices of insulin sensitivity (quantitative insulin sensitivity check index, insulin sensitivity by oral glucose tolerance test, and homeostasis model assessment for insulin resistance) and β -cell function (first and second phase insulin secretion, and homeostasis model assessment for β -cell function). Probiotic supplementation resulted in a significant reduction in soluble vascular cell adhesion molecule-1 (sVCAM-1) level ($1,614 \pm 343$ vs. $1,418 \pm 265$ ng/mL). No significant changes in parameters used to assess low-grade inflammation or endothelial dysfunction were observed. Intake of LcS for 12 wk in subjects with metabolic syndrome did not improve insulin sensitivity, β -cell function, endothelial function, or inflammation markers in this trial.

2. Reduction of aflatoxin level in aflatoxin-induced rats by the activity of probiotic *Lactobacillus casei* strain Shirota.

Nikbakht Nasrabadi E *et al* (2013) *J. Appl. Microbiol.* 114(5):1507-15.

Abstract

AIMS:

Aflatoxin B1 (AFB1) is considered as the most toxic food contaminant, and microorganisms, especially bacteria, have been studied for their potential to reduce

the bioavailability of mycotoxins including aflatoxins. Therefore, this research investigated the efficacy of oral administration of *Lactobacillus casei* Shirota (LcS) in aflatoxin-induced rats.

METHODS AND RESULTS:

Sprague Dawley rats were divided into three groups of untreated control, the group induced with AFB1 only, and the group given probiotic in addition to AFB1. In the group induced with AFB1 only, food intake and body weight were reduced significantly. The liver and kidney enzymes were significantly enhanced in both groups induced with AFB1, but they were lower in the group given LcS. AFB1 was detected from all serum samples except for untreated control group's samples. Blood serum level of AFB1 in the group induced with AFB1 only was significantly higher than the group which received probiotic as a treatment ($P < 0.05$), and there was no significant difference between the control group and the group treated with probiotic.

CONCLUSIONS:

LcS supplementation could improve the adverse effect of AFB1 induction on rats' body weight, plasma biochemical parameters and also could reduce the level of AFB1 in blood serum.

SIGNIFICANCE AND IMPACT OF THE STUDY:

This study's outcomes contribute to better understanding of the potential of probiotic to reduce the bioavailability of AFB1. Moreover, it can open an opportunity for future investigations to study the efficacy of oral supplementation of probiotic LcS in reducing aflatoxin level in human.

3. Inhibitory activity of fermented milk with *Lactobacillus casei* strain Shirota against common multidrug-resistant bacteria causing hospital-acquired infections.

Tiengrim S and Thamlikitkul V. (2012) *J. Med. Assoc. Thai.* 95 Suppl 2:S1-5.

Abstract

OBJECTIVE:

To determine inhibitory activity of fermented milk with *Lactobacillus casei* strain Shirota (FMLC) against common multi-drug-resistant (MDR) bacteria causing hospital-acquired infections.

MATERIAL AND METHOD:

Time-kill methods of FMLC and cell-free filtered fluid of FMLC (CF-FMLC) against *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, ESBL-producing *Escherichia coli* & *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* were conducted. The control solutions were Mueller Hinton broth (MHB)

and distilled water. The mixtures of FMLC, CF-FMLC, MHB and DW with 10⁵ to 10⁶ CFU/ml of each bacterium were prepared and incubated at 35 degrees C. Each mixture was quantified for viable bacteria at 0, 1, 3, 6 and 24 hr after incubation onto brain heart infusion agar plates. The inoculated agar plates were incubated at 35 degrees C for 24-48 hr. Bacterial colonies on agar plates were counted and compared among the mixtures.

RESULTS:

Log CFUs of each organism in MHB and distilled water after incubation were increased from 5.1-6.3 at 0 h to 6.4- > 11 at 24 hr. Log CFUs of each organism in FMLC and CF-FMLC after incubation with study bacteria for 0, 1, 3, 6 and 24 hr were decreased to undetectable amounts at 24 hr.

CONCLUSION:

FMLC and CF-FMLC exerted slow inhibitory activity against MDR bacteria resulting in eradication of all study bacteria at 24 hr. Such inhibitory effects were probably due to the products of the milk fermented by *Lactobacillus casei* strain Shirota. Clinical study is needed to determine if consumption of FMLC can prevent and treat colonization and infection with MDR bacteria in hospitalized patients.

