

# Studies on *Lactobacillus casei* DN-114 001 from Danone

## Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomized, double-blind, placebo-controlled trial

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*British Medical Journal*, July 2007; [bmj.com](http://bmj.com)

**Abstract.** Objective: To determine the efficacy of a probiotic drink containing *Lactobacillus* for the prevention of any diarrhoea associated with antibiotic use and that caused by *Clostridium difficile*. Design: Randomized, double-blind, placebo-controlled study. Participants: 135 hospital patients (mean age 74) taking antibiotics. Exclusions included diarrhoea on admission, bowel pathology that could result in diarrhoea, antibiotic use in the previous four weeks, severe illness, immunosuppression, bowel surgery, artificial heart valves, and history of rheumatic heart disease or infective endocarditis. Intervention: Consumption of a 100 g (97 ml) drink containing *Lactobacillus casei*, *L bulgaricus*, and *Streptococcus thermophilus* twice a day during a course of antibiotics and for one week after the course finished. The placebo group received a longlife sterile milkshake. Main outcome measures: Primary outcome: occurrence of antibiotic-associated diarrhoea. Secondary outcome: presence of *C difficile* toxin and diarrhoea. Results: 7/57 (12%) of the probiotic group developed diarrhoea associated with antibiotic use compared with 19/56 (34%) in the placebo group ( $p=0.007$ ). Logistic regression to control for other factors gave an odds ratio 0.25 (95% confidence interval 0.07 to 0.85) for use of the probiotic, with low albumin and sodium also increasing the risk of diarrhoea. The absolute risk reduction was 21.6% (6.6% to 36.6%), and the number needed to treat was 5 (3 to 15). No one in the probiotic group and 9/53 (17%) in the placebo group had diarrhoea caused by *C difficile* ( $p=0.001$ ). The absolute risk reduction was 17% (7% to 27%), and the number needed to treat was 6 (4 to 14). Conclusion: Consumption of a probiotic drink containing *L casei*, *L bulgaricus*, and *S thermophilus* can reduce the incidence of antibiotic-associated diarrhoea and *C difficile*-associated diarrhoea. This has the potential to decrease morbidity, healthcare costs, and mortality if used routinely in patients aged over 50.

## The effect of supplementation with milk fermented by *Lactobacillus casei* (strain DN-114 001) on acute diarrhoea in children attending day care centres

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*Int. J. Clin. Pract.*, Apr.-May 1999; 53 (3): 179-184

**Summary.** The objective of this study was to determine if supplementation with milk fermented by yogurt cultures and *Lactobacillus casei* (strain DN-114 001) could lessen acute diarrhoea in healthy children. The study was conducted over six months, with 287 children aged 18.9 (SD 6.0) months, comprising three periods of one-month supplementation, each month being followed by one month without supplementation. Subjects were supplemented daily with either 125 g or 250 g (according to age) of one of three tested dairy products: standard yogurt, milk fermented by yogurt cultures and *Lactobacillus casei* ( $10^8$  cfu/ml), or a jellied milk (control product). A daily record was kept of the number and type of stools. Although the incidence of diarrhoea was not shown to be different between the groups, the severity of diarrhoea over the six-month study was significantly decreased (4.3 days) with the supplementation of *L.casei* fermented milk compared with the jellied milk (8.0 days) ( $p=0.009$ ).

## Multicentric study of the effect of milk fermented by *Lactobacillus casei* on the incidence of diarrhoea

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*Int. J. Clin. Pract.*, Nov. 2000; 54 (9): 568-571

**Summary.** The aim of this study was to determine if supplementation of healthy children with milk fermented by yogurt cultures and *Lactobacillus casei* strain DN-114 001 could affect the incidence of acute diarrhoea when compared with traditional yogurt. The study was a multicenter, randomized, double-blind trial, conducted over four months, on 928 children aged, at inclusion, 6-24 months. The study consisted of two periods: supplementation and observation. Subjects were supplemented daily with 100 g of one of the two dairy products being tested: standard yogurt and milk fermented by yogurt cultures and *Lactobacillus casei* ( $10^8$  cfu/ml). Frequency or duration of any diarrhoea episode was evaluated. As far as frequency was concerned there was a statistically significant difference between the groups, the incidence of diarrhoea being significantly reduced by supplementation with *L.casei* fermented milk (15.9%) compared with yogurt (22%) ( $p=0.03$ ). These results suggest an additional benefit of *L.casei* in acute diarrhoea in children compared with standard yogurt.



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### Feasibility studies to control acute diarrhoea in children by feeding fermented milk preparations Actimel and Indian Dahi

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*European Journal of Clinical Nutrition*, 2002; 56, Suppl. 4, S56-S59

The aim of this work was to study feasibility of diarrhoea control in children (6 months to 5 years of age) by feeding fermented milk preparations. The design used was a randomized controlled clinical trial and the study was carried out at the Delhi University College Hospital providing tertiary care, and a nearby community centre Nand Nagri, a resettlement colony in East Delhi. Children suffering from acute diarrhoea (75 patients from the hospital and 75 from the community) were allocated to three groups by double-blind technique. Group 1 was given a fermented milk, Actimel, containing  $10^8$  of each *Lactobacillus casei* DN-114001, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* per gram. Group 2 was given Indian Dahi (Lf 40) containing  $10^8$  of each *Lactococcus lactis*, *Lactococcus lactis cremoris* and *Leuconostoc mesenteroides cremoris* per gram. Group 3 was given ultra-heat-treated yoghurt preparation (no live bacteria). Actimel was also used as a starter to prepare the curd in order to study the preventive effect of diarrhoea in children in a community. In the hospital study Indian Dahi and Actimel administration reduced mean duration of diarrhoea by 0.3 and 0.6 day ( $P < 0.001$ ), respectively. The corresponding figures in the community study were 0.2 and 0.5 day ( $P < 0.05$ ), respectively. The families using Actimel as a starter showed a reduction in diarrhoeal morbidity episodes by 40% of the children tested in a 3-month follow-up. In conclusion, Actimel, fermented milk containing *Lactobacillus casei* DN-114001, and Indian Dahi can significantly reduce the duration of diarrhoea in children, the former preparation being superior.

### Effect of fermented milk containing the probiotic *Lactobacillus casei* DN-114 001 on winter infections in free-living elderly subjects: a randomized, controlled pilot study

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*Journal of Nutrition, Health and Aging*, 2003; 7 (2): 75-77

Abstract: Probiotics are being increasingly studied for their ability to enhance host resistance to, and recovery from, infection. The probiotic strain *Lactobacillus casei* DN-114 001 has previously been shown to reduce the incidence and duration of episodes of diarrhoea in children. Our controlled pilot study aimed to evaluate the effect of supplementation for 3 weeks with milk fermented with yoghurt cultures and *L. casei* DN-114 001 on the incidence and severity of winter infections (gastrointestinal and respiratory) in elderly people. We found no difference in the incidence of winter infections between groups. However, duration of all pathologies was significantly lower in the treatment group ( $7.0 \pm 3.2$  days,  $n=180$ ) than in the control group ( $8.7 \pm 3.7$  days;  $n=180$ ) ( $p=0.024$ ), as was maximal temperature ( $38.3 \pm 0.5^\circ\text{C}$  treatment group vs.  $38.5 \pm 0.6^\circ\text{C}$  control;  $p=0.01$ ). The potential for a 20% reduction in the duration of winter infections that we have found warrants further investigation on a larger scale.

### Food supplementation with milk fermented by *Lactobacillus casei* DN-114 001 protects suckling rats from rotavirus-associated diarrhea

C. Guérin-Danan, J.C. Meslin, A. Chambard, A. Charpilienne, P. Relano, C. Bouley, J. Cohen and C. Andrieux

*J. Nutr.*, Jan. 2001; 131 (1): 111-117

Abstract. Group A rotavirus is the leading cause of diarrhea among children aged 3-36 mo worldwide. Introducing fermented milk products into the infant diet has been proposed for the prevention or treatment of rotavirus diarrhea. The preventive effect of milk fermented by the *Lactobacillus casei* strain DN-114 001 was studied in a model of germ-free suckling rats supplemented daily from d 2 of life and infected with SA11 rotavirus at d 5 (RF group). One group was supplemented with nonfermented milk (RM) and two uninfected groups (CM and CF) received either nonfermented or fermented milk. Frequency and severity of diarrhea were observed. Rats were killed at various times from 0 to 120 h postinfection (p.i.). Bacteria were measured in the intestine, and rotavirus antigens were detected by ELISA in fecal samples and in different parts of the intestine. Histologic observations were made, including vacuolation, morphology of intestinal villi and number of mucin cells. RM rats had diarrhea for 6 d; compared with the CM group, they had alterations of the intestinal mucosa characterized by cellular vacuolation 48 and 72 h p.i. and a lower number of sulfated mucin cells 72 and 96 h p.i. ( $P < 0.05$ ). Early supplementation with fermented milk significantly decreased the clinical signs of diarrhea from 24 to 144 h p.i. ( $P < 0.05$ ) and prevented rotavirus infection in all sections of the intestine. Histologic lesions of the small intestine were greatly reduced ( $P < 0.05$ ) and the number of mucin cells remained unchanged. The data are discussed with respect to the possibility of reducing rotavirus diarrhea in young children by consumption of fermented milk.

### Enhancement of host resistance against *Salmonella typhimurium* in mice fed a diet supplemented with yogurt or milks fermented with various *Lactobacillus casei* strains

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*Int. J. Immunotherapy*, 1995; XI (4): 153-161

Summary: The aim of this study was first to compare the effects of milks fermented with various *Lactobacillus casei* strains on the survival rate of mice infected with *Salmonella typhimurium*. Secondly, the relationship between this protective effect and the non-specific immune response was studied. For each experiment, mice were randomized into groups of eight animals. Animals were supplemented with one of the following preparations for 7 days: milk fermented with one of the three *L. casei* strains (Danone strain 001 (LAB-1), LAB-2 and Yakult), yogurt (YF), a mixture of LAB-1 and YF (LAB-1+YF) or milk. The survival of the animals after single oral *S. typhimurium* infection (LD50 dosage) was monitored for 2 weeks. All fermented milks exhibited a protective effect against *S. typhimurium* infection. The highest protection was obtained with LAB-1 and that of LAB-1 + YF was significantly higher than that obtained with other treatments. Circulating IgA levels,  $\beta$ -glucuronidase activity of peritoneal macrophages and phagocytosis index were significantly enhanced in animals supplemented with LAB-1 and Yakult. This study shows that for one bacteria species, various strains exhibit different effects on protection and on immune parameters. The hierarchy established for survival rates does not entirely correlate with the effects noted on immune parameters. This indicates that stimulation of non-specific immunity, especially on macrophage activity, is not the only mechanism involved in protection against intestinal infections.

### The association of yogurt starters with *Lactobacillus casei* DN-114 001 in fermented milk alters the composition and metabolism of intestinal microflora in germ-free rats and in human flora-associated rats

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*J. Nutr.*, 1997; 127: 2260-2266

Abstract. The aim of this study was to compare the effects of milk and of various fermented milks on the composition and metabolic activities of the intestinal microflora. Groups of eight rats were fed for 6 wk a diet containing 30% non fermented milk (M), yogurt (Y), milk fermented with *Lactobacillus casei* (LcFM) or milk fermented with the association of *L. casei* DN-114 001 and yogurt starters (LcYFM). In the first study, the survival of the lactic acid bacteria from the fermented milks was assessed by bacterial enumeration in feces of germ-free rats (GF rats) fed milk or fermented milks. The metabolic activities of the lactic acid bacteria were studied in these rats by measurement of glycolytic activities and products of bacterial fermentation, i.e., acetate and lactate (isoforms L and D). In a second study, the effects of fermented milks on the composition and metabolism [gas, glycolytic activities, short-chain fatty acid (SCFA), alcohol and ammonia] of human flora were studied using human flora-associated rats (HF rats). In GF rats, the survival of *L. casei* in the feces did not differ between those fed the LcFM and LcYFM diets. *L. bulgaricus* was detected in the feces of the rats fed Y, whereas *Streptococcus thermophilus* was found in the feces of the LcYFM group. In HF rats, fecal concentration of Bifidobacteria was greater in the LcFM group than in others.  $\beta$ -glucuronidase (EC 3.2.1.31) activity was lower in rats fed LcFM and Y than in those fed M and LcYFM, whereas  $\beta$ -galactosidase (3.2.1.23),  $\alpha$ -glucosidase (EC 3.2.1.20) and  $\beta$ -glucosidase (EC 3.2.1.21) activities were higher in the LcYFM group compared with the others. Methane excretion was higher in rats fed Y than in the other groups. Cecal SCFA concentrations did not differ in LcFM, Y and M groups, but total SCFA, acetate, propionate and butyrate were significantly greater in the LcYFM group. These results suggest that milk fermented with the combination of *L. casei* and yogurt starters leads to specific effects that are different from the simple addition of the effects found with yogurt and milk fermented with *L. casei*. These specific effects are potentially beneficial to human health.

### Milk fermented with yogurt cultures and *Lactobacillus casei* compared with yogurt and gelled milk: influence on intestinal microflora in healthy infants

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*Am. J. Clin. Nutr.*, 1998; 67: 111-117

Abstract. Ingestion of fermented dairy products induces changes in the equilibrium and metabolism of the intestinal microflora and may thus exert a healthful influence on the host. We compared the effects of consumption of a traditional yogurt, a milk fermented with yogurt cultures and *Lactobacillus casei* (YC), and a nonfermented gelled milk on the fecal microflora of healthy infants. Thirty-nine infants aged 10-18 mo were randomly assigned to one of three groups in which they received 125 g/d of one of the three products for 1 mo. The following indexes were not modified during the supplementation period or for 1 wk after the end of supplementation: total number of anaerobes, bifidobacteria, bacteroides, and enterobacteria; pH; water content; concentrations of acetate, butyrate, propionate, and lactate; and bacterial enzyme activity of  $\beta$ -galactosidase and  $\alpha$ -glucosidase. In contrast, in the yogurt group the number of enterococci in fecal samples increased ( $P<0.05$ ), whereas the percentage of branched-chain and long-chain fatty acids, which are markers of proteolytic fermentation, decreased ( $P<0.05$ ). In the YC group, the percentage of children with  $>6 \log_{10}$  colony-forming units lactobacilli/g feces increased ( $P<0.05$ ) whereas the potentially harmful enzyme activity of  $\beta$ -glucuronidase and  $\beta$ -glucosidase decreased ( $P<0.05$ ). These decreases were particularly marked in those infants in the YC group in whom activity of the enzymes was initially unusually high.

### Diet supplemented with yoghurt or milk fermented by *Lactobacillus casei* DN-114 001 stimulates growth and brush-border enzyme activities in mouse small intestine

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*Digestion*, 1998; 59: 349-359

Abstract. The nutritional benefits of lactic acid bacteria in fermented dairy products have been well documented, especially in terms of weight gain and feed efficiency, but not in terms of small intestine adaptation. The effects of a diet supplemented (30% wt/wt) with milk fermented either by *Lactobacillus casei* DN-114 001 or yoghurt for 3 or 15 days were investigated in the small intestine of mice by morphometry, kinetic analysis and determination of brush-border enzyme activities. Results were compared with those obtained with standard or milk isocaloric diets. Cell proliferation and villous area were significantly increased in the proximal intestine of mice fed the fermented-milk-supplemented diets for 3 days and were associated with hypertrophy and hyperplasia of Paneth and goblet cells. Lactase-specific activity was increased by fermented milk diets at days 3 and 15, whereas there was no variation in maltase-specific activity. Alkaline phosphatase-specific activity was increased after 3 days of the three tested diets in the whole intestine, and after 15 days in the proximal intestine. Aminopeptidase activity was increased in the distal part of the intestine after 3 days of the 3 diets. Our findings suggest that diets supplemented with fermented milks have a positive effect on the trophicity of the mucosa in the small intestine of mice.

### Modulation of proliferation, second messenger levels, and morphotype expression of the rat intestinal epithelial cell line IEC-6 by fermented milk

K. Thoreux, F. Senegas-Balas, F. Bernard-Perrone, S. Giannarelli, G. Denariaz, C. Bouley and D. Balas  
*J. Dairy Sci.*, 1996; 79: 33-43

Abstract. Trophic effects of milk fermented with *Lactobacillus helveticus*, *Lactobacillus paracasei ssp paracasei*, *Bifidobacterium* sp., or the combination of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (yogurt) were studied on the IEC-6 intestinal epithelial cell line. Incorporation of [methyl- $^3\text{H}$ ]thymidine, mitochondrial dehydrogenase activities, cyclic AMP production, and differentiation of levels of the IEC-6 strain were evaluated between the 15<sup>th</sup> and the 30<sup>th</sup> passage in culture. All fermented and unfermented milks enhanced trophic responses of IEC-6 cells in a dose-dependent manner. Compared with the corresponding milks, supernatant fractions were more effective in stimulating mitochondrial dehydrogenase response. Fermented milk supernatants were also more effective than the corresponding unfermented fractions. Increases in DNA synthesis and cyclic AMP confirmed the activation observed with mitochondrial dehydrogenase. Yogurt induced the more trophic response with an increased number of the more differentiated cell morphotype. Fermentation with *L. casei* also demonstrated an important trophic adaptation of IEC-6 cells. Milk processing by lactic acid bacteria enhanced trophic and proliferation responses of intestinal epithelial cell line IEC-6. These results suggested that IEC-6 cells could represent an accurate and easy *in vitro* model for testing the trophic quality of various nutrients and for an optimization of physiological digestive functions.

### Host-pathogens cross-talk. Indigenous bacteria and probiotics also play the game

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*Biology of the Cell*, 2003; Vol. 95, Issue 8, pp. 503-6

Abstract. Microflora-born bacteria or probiotic strains are able to modulate host-pathogens interactions in the gut. *In vivo* and *in vitro* studies indicate that species-specific modulations of intestinal cell glycosylation may represent a simple, general and efficient mechanism to adapt the host defense toward pathogens.

### ***Lactobacillus casei* DN-114 001 inhibits the increase of paracellular permeability in enteropathogenic *Escherichia coli*-infected T84 cells**

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*Research in Microbiology*, 2005; Vol. 156, Issue 2, pp. 256-262

Abstract. Probiotics are living microorganisms, which, when ingested in adequate amounts, exert health benefits toward the host. For instance, probiotics might act through reinforcement of the intestinal epithelial barrier function. The goal of the present study was to determine whether *Lactobacillus casei* DN-114 001 could abrogate the increase in paracellular permeability induced by enteropathogenic *Escherichia coli*. We used the human colon T84 cell line infected with a wild type enteropathogenic *E. coli* (strain E2348/69). Paracellular permeability was followed by monitoring transepithelial electrical resistance variations and by observing Zonula Occludens-1 distribution. Two infection procedures were used; co-incubation (the pathogenic and probiotic strains were simultaneously incubated with T84 cells) and post-infection (the probiotic was added in the presence of pathogenic bacteria, 3 hours after the beginning of the infection). We also investigated the effect of *L. casei* on enteropathogenic *E. coli* adhesion. *L. casei* DN-114 001 inhibited in a dose-dependent-manner, the decrease in enteropathogenic *E. coli*-induced transepithelial electrical resistance, and Zonula Occludens-1 redistribution using two different infection procedures. However, *L. casei* did not inhibit pathogenic strain adhesion. *L. casei* DN-114 001 inhibited the increase in EPEC-induced paracellular permeability. This property could partially explain the previously observed health benefits of this probiotic for human natural defenses, such as those associated with prevention of diarrhea.

### ***Lactobacillus casei* DN-114 001 inhibits the ability of adherent-invasive *E. coli* isolated from Crohn's disease patients to adhere to and to invade intestinal epithelial cells**

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*Appl. Environ. Microbiol.*, 2005 Jun.; 71(6):2880-7

Ileal lesions in 36.4% of patients with Crohn's disease are colonized by pathogenic adherent-invasive *Escherichia coli*. The aim of this study was to determine the *in vitro* inhibitory effects of the probiotic strain, *Lactobacillus casei* DN-114 001, on adhesion to and invasion of human intestinal epithelial cells by adherent-invasive *E. coli* isolated from Crohn's disease patients. The experiments were performed with undifferentiated Intestine-407 cells and with undifferentiated or differentiated Caco-2 intestinal epithelial cells. Bacterial adhesion to and invasion of intestinal epithelial cells were assessed by counting CFU. The inhibitory effects of *L. casei* were determined after co-incubation with adherent-invasive *E. coli* or after preincubation of intestinal cells with *L. casei* prior to infection with adherent-invasive *E. coli*. Inhibitory effects of *L. casei* on adherent-invasive *E. coli* adhesion to differentiated and undifferentiated intestinal epithelial cells reached 75% to 84% in co-incubation and 43% to 62% in preincubation experiments, according to the cell lines used. Addition of *L. casei* culture supernatant to the incubation medium increased *L. casei* adhesion to intestinal epithelial cells and enhanced the inhibitory effects of *L. casei*. The inhibitory effects on *E. coli* invasion paralleled those on adhesion. This effect was not due to a bactericidal effect on adherent-invasive *E. coli*, or a cytotoxic effect on epithelial intestinal cells. As *Lactobacillus casei* DN-114 001 strongly inhibits interaction of adherent-invasive *E. coli* with intestinal epithelial cells, this finding suggests that the probiotic strain could be of therapeutic value in Crohn's disease.

### **The effect of fermented milk containing *Lactobacillus casei* on the immune response to exercise**

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*Training and Rehab.*, 2000; 9(3), 209-223

There is evidence that exhaustive exercise produces depression of the immune system, especially on the number and activity of Natural Killer (NK) cells. On the other hand, fermented milk has been shown to moderate the immune response by inducing NK activity. The present work was carried out to determine if a *Lactobacillus casei* (LC) fermented milk supplemented diet would provide protection of the immune system against an exercise induced immune system depression of NK cells. Twenty-five athletes were selected out of 94 for their significant decrease in NK cell concentration compared with a normal basal concentration in plasma 2 h after an exercise stress test. Subjects ingested a daily fermented milk diet with LC for one month and a standard milk diet also for one month. After each phase of dieting, a subject was investigated before, 5 min and 2 h after an exercise stress test, testing for NK cells and IL-1 $\beta$ , IL-6, IL-2, IFN $\gamma$  IgA, IgM, IgG, NK cells, CD8, CD4, CD3 and sIL-2 receptor. A significant smaller decrease of NK cell concentration after 2 h was found in the fermented milk feeding phase vs. the standard milk period.

### **The effect of milk fermented by yogurt cultures plus *Lactobacillus casei* DN-114 001 on the immune response of subjects under academic examination stress**

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*Eur. J. Nutr.*, 2004; 43(6): 381-9

Summary. Background: A suppressed immune response has been documented in students under examination stress. Aim of the study: The current study aimed to evaluate the effect of milk fermented with yogurt cultures plus *Lactobacillus casei* DN-114 001 (Actimel®) on the immune system of subjects under academic examination stress. Methods: University students were allocated to one of two groups, receiving during 6 weeks (3 weeks prior to, as well as the 3-week duration of the examination period) either: a) a glass of semi-skimmed milk each day (control group,  $n = 63$ ) or b) two 100mL portions per day of fermented milk (treatment group,  $n = 73$ ). Anxiety and immunological measurements were monitored at baseline (Phase 0) and study end (Phase 1). Results: The results were expressed as the differences between the data obtained from Phase 0 and Phase 1. This was calculated by subtracting Phase 1 results from the Phase 0 and it is denominated "Treatment effect." Mean ( $\pm$ SE) anxiety increased significantly ( $P < 0.05$ ) over the 6-week study in all students, from  $40.74 \pm 2.50$  to  $61.19 \pm 2.64$  (in percentiles). There was no significant treatment effect since this increase was similar in the control and the treatment groups ( $21.65 \pm 5.09$  vs  $19.14 \pm 3.67$ , respectively). However, there was a significant treatment effect ( $P < 0.05$ ) on the mean change in absolute number of lymphocytes during the 6-week study, which decreased in the control group ( $-0.04 \pm 0.12$  cells  $\times 10^3/\text{mm}^3$ ) and increased in the treatment group ( $0.37 \pm 0.11$  cells  $\times 10^3/\text{mm}^3$ ). There was also a significant treatment effect ( $P < 0.05$ ) on the change in absolute numbers of CD56 cells during the 6-week study. Mean absolute CD56 cells significantly decreased ( $P < 0.05$ ) in the control group ( $-51.97 \pm 21.33$  cells/ $\text{mm}^3$ ), while remaining similar in the treatment group ( $17.29 \pm 17.27$  cells/ $\text{mm}^3$ ). During the study, mean serum cortisol increased  $4.30 \pm 0.98$   $\mu\text{g}/\text{dL}$  in the control group, and  $1.75 \pm 1.05$   $\mu\text{g}/\text{dL}$  in the treatment group and no significant differences were found between both values ( $P = 0.062$ ). Conclusions: Milk fermented with yogurt cultures plus *Lactobacillus casei* DN-114 001 was able to modulate the number of lymphocytes and CD56 cells in subjects under academic examination stress.

### Monocyte function in healthy middle-aged people receiving fermented milk containing *Lactobacillus casei*

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*The Journal of Nutrition, Health & Aging*, 2004; Vol 8, (4); pp. 208-211

Abstract: Background: Lactic acid bacteria have been suggested as a dietary strategy to enhance immune system activity. Objective: The aim of the current work was to test the effects of a *Lactobacillus casei* fermented milk consumption on monocyte activity of middle-aged volunteers. Design: Forty-five healthy volunteers, 24 women and 21 men (aged: 51–58 years), were randomized in two groups to receive three cups per day of a fermented milk containing *L. casei* DN-114 001 ( $10^8 - 10^{10}$ /g) ( $n = 23$ ), or placebo ( $n = 22$ ), during 8 weeks. White blood cell count and the oxidative burst capacity of monocytes and granulocytes were examined with a FACScalibur. Measurements were performed at baseline and after the nutritional intervention, at day fifty-six. Results: After the trial, no changes in immune cell proportions were detected in both groups, as well as in monocyte activity after the placebo consumption ( $p = 0.625$ ). However, volunteers included in the probiotic-treated group increased ( $p = 0.029$ ) their oxidative burst capacity of monocytes, and this increment inversely and significantly correlated with the intensity registered at baseline ( $r = -0.653$ ,  $p = 0.004$ ). Conclusions: Results showed that daily intake of fermented milk containing *Lactobacillus casei* was able to modulate the oxidative burst capacity of monocyte subset in healthy middle-aged people, particularly in subjects with lower initial levels. Thus, this nutritional strategy could be considered to maintain immune competence in ageing.

### Daily ingestion of fermented milk containing *Lactobacillus casei* DN-114 001 improves innate-defense capacity in healthy middle-aged people

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*J. Physiol. Biochem.*, 2004; Vol. 60, (2); pp. 85-92

Different lactic acid bacteria have often been administered as a dietary means to enhance immune system activity. Based on this statement, the aim of the current work was to test the effects of a *Lactobacillus casei* DN-114 001 fermented milk consumption on the immune response capacity in middle-aged volunteers. Forty-five healthy volunteers, 24 women and 21 men (aged: 51-58 years), were randomized into two groups to receive three cups per day of a *L. casei* DN-114 001 ( $10^8-10^{10}$  ufc/g) fermented milk ( $n = 23$ ), or placebo ( $n = 22$ ), during an 8-week period. Measurements were performed before (day 0), and after the nutritional intervention (day 56). After the trial, no changes in immune cell proportions were detected, but the probiotic-treated group increased oxidative burst capacity of monocytes (probiotic group:  $p = 0.029$ ; placebo group:  $p = 0.625$ ), as well as NK cells tumoricidal activity (probiotic group:  $p = 0.023$ ; placebo group:  $p = 0.125$ ). Results showed that daily intake of fermented milk containing *Lactobacillus casei* DN-114 001 could have a positive effect in modulating the innate immune defense in healthy middle-aged people.

### Increased mucosal TNF $\alpha$ production in Crohn's disease can be downregulated *ex vivo* by probiotic bacteria

N. Borruel, M. Carol, F. Casellas, M. Antolín, F. de Lara, E. Espín, J. Naval, F. Guarner and JR. Malagelada.  
*Gut*, 2002; 5: 659-664

Background & Aims: TNF $\alpha$  plays a key role in the pathogenesis of intestinal inflammation in Crohn's disease. The effect of bacteria on TNF $\alpha$  release by intestinal mucosa was investigated. Methods: Ileal specimens were obtained at surgery from 10 patients with Crohn's disease (ileal stricture) and five disease controls undergoing right hemicolectomy (caecal cancer). Mucosal explants from each specimen were cultured for 24 hours with either non-pathogenic *E. coli*, *Lactobacillus casei* DN-114 001, *L. bulgaricus* LB10, or *L. crispatus* (each study contained blank wells with no bacteria). Tissue and bacteria viability was confirmed by LDH release and culture. Concentrations of TNF $\alpha$  was measured in supernatants, and the phenotype of the intestinal lymphocytes was analyzed by flow cytometry. Results: Coculture of mucosa with bacteria did not modify LDH release. Release of TNF $\alpha$  by inflamed Crohn's disease mucosa was significantly reduced by coculture with *L. casei* or *L. bulgaricus*; changes induced by *L. crispatus* or *E. coli* were not significant. The effect of *L. casei* and *L. bulgaricus* was not prevented by protease inhibitors. Coculture with *L. casei* and *L. bulgaricus* reduced the number of CD4 cells, as well as TNF $\alpha$  expression among intraepithelial lymphocytes from Crohn's disease mucosa. None of the bacteria induced changes in non-inflamed mucosa. Conclusions: Probiotics may interact with immunocompetent cells using the mucosal interface and modulate locally the production of proinflammatory cytokines.

### Effects of nonpathogenic bacteria on cytokine secretion by human intestinal mucosa

N. Borruel, F. Casellas, M. Antolín, M. Llopis, M. Carol, E. Espin, J. Naval, F. Guarner and JR. Malagelada.  
*Am. J. Gastro.*, 2003; 98[4]: 865-870

Objective: The human intestine harbors a complex microbial ecosystem, and the mucosa is the interface between the immune system and the luminal environment. The aim of this study was to elucidate whether host-bacteria interactions influence mucosal cytokine production. Methods: Macroscopically normal colonic specimens were obtained at surgery from 8 patients with neoplasm, and inflamed ileal specimens from 2 patients with Crohn's disease. Mucosal explants were cultured for 24 h with either nonpathogenic *Escherichia coli* ECOR-26, *Lactobacillus casei* DN-114 001, *Lactobacillus casei* DN-114 056, *Lactobacillus casei* ATCC-334 or *Lactobacillus bulgaricus* LB-10. Each study included blank wells with no bacteria. Tissue and bacteria viability was confirmed by LDH release and culture. Concentration of TNF $\alpha$ , TGF $\beta$ 1, IL-8 and IL-10 was measured in supernatants. In parallel experiments, neutralizing anti-TNF $\alpha$  antibody was added to the culture. Results: Co-culture of mucosa with bacteria did not modify LDH release. Co-culture with *Lactobacillus casei* strains significantly reduced TNF $\alpha$  release, whereas *E. coli* increased it. These effects were observed both in normal and inflamed mucosa. In combination studies, DN-114 001 prevented TNF $\alpha$  stimulation by *E. coli*. *L. casei* DN-114 001 also reduced IL-8 release via a TNF $\alpha$ -independent pathway. *L. casei* DN-114 056 or *E. coli* increased IL-10 release in the presence of neutralizing anti-TNF $\alpha$ . Conclusions: Nonpathogenic bacteria interact with human intestinal mucosa and can induce changes in cytokine production that are strain-specific.

### **Lactobacillus casei reduces CD8<sup>+</sup> T cell-mediated skin inflammation**

Ludvine Chapat, Karine Chemin, Bertrand Dubois, Raphaëlle Bourdet-Sicard and Dominique Kaiserlian  
*European Journal of Immunology*, 2004; 34(9): 2520-2528

Probiotics, including Lactobacilli, have been postulated to alleviate allergic and inflammatory diseases, but evidence that they exert an anti-inflammatory effect by immune modulation of pathogenic T cell effectors is still lacking. The aim of this study was to examine whether *L. casei* could affect antigen-specific T cell mediated skin inflammation. To this end, we used contact hypersensitivity to the hapten DNFB, a model of allergic contact dermatitis mediated by CD8<sup>+</sup> CTL and controlled by CD4<sup>+</sup> regulatory T cells. Daily oral administration of a fermented milk containing *L. casei* or *L. casei* alone decreased skin inflammation by inhibiting the priming/expansion of hapten-specific IFN-gamma-producing CD8<sup>+</sup> effector T cells. The down-regulatory effect of the probiotics required the presence of CD4<sup>+</sup> T cells, which control the size of the hapten-specific CD8<sup>+</sup> T cell pool primed by skin sensitization. *L. casei* cell wall was as efficient as live *L. casei* to regulate both the CHS response and the hapten-specific CD8<sup>+</sup> T cell response, suggesting that cell wall components contribute to the immunomodulatory effect of *L. casei*. This study provides the first evidence that oral administration of *L. casei* can reduce antigen-specific skin inflammation by controlling the size of the CD8<sup>+</sup> effector pool.

### **Effect of fermented milk containing probiotic bacteria in the prevention of an enteroinvasive Escherichia coli infection in mice.**

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*J. Dairy Res.*, 2005; May ;72(2): 243-9.

This study investigated the protective capacity of the oral administration of fermented milk containing the probiotic strains; *Lactobacillus casei*, *Lb. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, against enteroinvasive *Escherichia coli* infection in a murine (BALB/ c mice) model. Mice were fed for 2, 5 or 7 consecutive days with fermented milk diluted to a concentration of viable *Lb. casei*, *Lb. delbrueckii* subsp. *bulgaricus* and *Strep. thermophilus* of 10(7) cfu/ml. Phagocytic activity of peritoneal macrophages and the number of IgA<sup>+</sup> cells in small and large intestine were determined at the end of the feeding periods. For the preventive effect against *Esch. coli*, animals were fed for 5 days (selected dose). Mice were challenged with an infective dose of enteroinvasive *Esch. coli* of 10(8) cfu/mouse. The colonization of liver and spleen and the secretory IgA specific for the pathogen in the intestinal fluid were determined (ELISA test). Results showed that the unspecific immune response enhanced itself after 5 consecutive days of the administration of this fermented milk (increase in the percentage of phagocytosis and number of IgA<sup>+</sup> cells in the small intestine). Treated animals showed less *Esch. coli* colonization of liver than control mice and a higher secretory anti-*Esch. coli* IgA in the intestinal fluids. These results suggest that the protection against enteroinvasive *Esch. coli* infection observed for the fermented milk containing probiotic bacteria may be associated with an enhance of the intestinal mucosa immunity.

### **Mucosal colonisation with Lactobacillus casei mitigates barrier injury induced by exposure to trinitrobenzene sulphonic acid.**

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*Gut*, 2005; Jul; 54(7): 955-9.

BACKGROUND: Trinitrobenzene sulphonic acid (TNBS) induces chronic transmural inflammatory lesions in the rat colon. Injury is facilitated by barrier disruption and invasion of commensal bacteria. However, certain bacteria have shown anti-

inflammatory properties in in vitro models. AIM: To investigate in vivo the anti-inflammatory effect of *Lactobacillus casei* DN-114 001. METHODS: Rats with a colonic segment excluded from faecal transit were surgically prepared. After washing the lumen with antibiotics, the excluded segment was recolonized (control group: standard flora of rat origin; test group: standard flora and *L. casei*). Microbial colonisation was confirmed by culture of segment washing, and colitis was then induced by instillation of TNBS. One day after, intestinal lesions were blindly graded by macro- and microscopic scores, and myeloperoxidase activity measured in tissue homogenates. Translocation of bacteria to mesenteric lymph nodes, spleen and liver was investigated. RESULTS: Test rats showed a smaller area of mucosal injury than control rats (p<0.05). Maximum depth lesion scores were similar in both groups but myeloperoxidase activity was lower in test than in control rats (p<0.05). Remarkably, bacterial translocation was quantitatively lower (p<0.01) and less frequent (p<0.05) in test than in control rats. CONCLUSION: In rats colonised with *L. casei*, mucosal injury, inflammatory response, and barrier disruption after TNBS challenge were attenuated. Bacterial communities colonising the mucosa can modify inflammatory responses to luminal challenges.

### **Anti-Inflammatory Effect of Lactobacillus casei on Shigella-Infected Human Intestinal Epithelial Cells.**

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*Journal of Immunology*, 2006; 176: 1228-1237.

Shigella invades the human intestinal mucosa, thus causing bacillary dysentery, an acute recto-colitis responsible for lethal complications, mostly in infants and toddlers. Conversely, commensal bacteria live in a mutualistic relationship with the intestinal mucosa that is characterized by homeostatic control of innate responses, thereby contributing to tolerance to the flora. Cross-talk established between commensals and the intestinal epithelium mediate this active process, the mechanisms of which remain largely uncharacterized. Probiotics such as *Lactobacillus casei* belong to a subclass of these commensals that modulate mucosal innate responses and possibly display anti-inflammatory properties. We analyzed whether *L. casei* could attenuate the pro-inflammatory signaling induced by *Shigella flexneri* after invasion of the epithelial lining. Cultured epithelial cells were infected with *L. casei*, followed by a challenge with *S. flexneri*. Using microarray DNA chips, we observed that *L. casei* down-regulated the transcription of a number of genes encoding pro-inflammatory effectors such as cytokines and chemokines and adherence molecules induced by invasive *S. flexneri*. This resulted in an anti-inflammatory effect that appeared mediated by the inhibition of the NF-kappaB pathway, particularly through stabilization of I-kappaBalpha. In a time-course experiment using GeneChip hybridization analysis, the expression of many genes involved in ubiquitination and proteasome processes were modulated during *L. casei* treatment. Thus, *L. casei* has developed a sophisticated means to maintain intestinal homeostasis through a process that involves manipulation of the ubiquitin/ proteasome pathway upstream of I-kappaBalpha.

### **Lactobacillus casei is able to survive and initiate protein synthesis during its transit in the digestive tract of human flora-associated mice**

R. Oozeer, N. Goupil-Feuillerat, C.A. Alpert, M. van de Guchte, J. Anba, J. Mengaud and G. Corthier  
*Appl. Envir. Microbiol.*, 2002; 68: 3570-3574

Live *Lactobacillus casei* is present in fermented dairy products and has beneficial properties for human health. In the human digestive tract, the resident flora generally prevents the establishment of ingested lactic acid bacteria, the presence of which is therefore transient. The aim of this work was to determine if *L. casei* DN-114 001 survives during transit and how this

bacterium behaves in the digestive environment. We used the human flora-associated (HFA) mouse model. *L. casei* DN-114 001 was genetically modified by the introduction of *erm* and *lux* genes encoding erythromycin resistance and luciferase, respectively. For this modified strain (DN-240 041) light emission, related to luciferase expression, could easily be detected in the contents of the digestive tract. When inoculated in the digestive tract of HFA mice, *L. casei* (DN-240 041) survives but is eliminated with the same kinetics as an inert transit marker, indicating that it does not establish itself. In pure culture of *L. casei*, luciferase activities were high in the exponential and early stationary growth phases but decreased to become undetectable 1 day after inoculation. Viability was only slightly reduced even after more than 5 days. After transit in HFA mice, luciferase activity was detected even when 5 days old *L. casei* cultures were given to the mice. In culture, the luciferase activity could be restored after 0.5 to 7 hours of incubation in fresh medium or milk containing glucose, unless protein synthesis was inhibited by the addition of chloramphenicol or rifampicin. These results suggest that in HFA mice *L. casei* DN-240 041 and thus probably *L. casei* DN-114 001 is able to initiate new protein synthesis during its transit with the diet. The beneficial properties of *L. casei* fermented milk for human health might be related to this protein synthesis in the digestive tract.

#### **Initiation of protein synthesis by a labeled derivative of the *Lactobacillus casei* DN-114 001 strain during its transit from the stomach to the cecum in human-microbiota-associated mice**

R. Oozeer, D.D.G Mater, N. Goupil-Feuillerat, G. Corthier  
*Appl. Envir. Microbiol.*, 2004; 70(12): 6992-6997

Although studies on the survival of bacteria in the digestive tract have been reported in the literature, little data are available on the physiological adaptation of probiotics to the digestive environment. In previous work, a transcriptional fusion system (i.e., luciferase genes under the control of a deregulated promoter) was used to demonstrate that a derivative of the *Lactobacillus casei* DN-114 001 strain, ingested in a fermented milk and thus exhibiting initially a very weak metabolic activity, synthesized proteins *de novo* after its transit in the human-microbiota-associated mice digestive tract. Using the same genetic system and animal model, we here investigate, for the first time, the ability of *Lactobacillus casei* to reinstate synthesis in the different digestive tract compartments. In this study, most ingested *L. casei* cells transited from the stomach to the duodenum-jejunum within 1 hour post-ingestion. No luciferase activity was observed in these digestive tract compartments over the first hour. At later times, the bulk of bacteria had transited to the ileum and the cecum. Luciferase synthesis was detected at the ileal level between 1.5 - 2.0 hours and from 1.5 hours to at least 6.0 hours in the cecum where the activity remained at a maximum level. These results demonstrate that ingested *Lactobacillus casei* (derivative of DN-114 001 strain) administered via a fermented milk has already reinstated protein synthesis when it reaches the ileal and cecal compartments.

#### **Differential activities of four *Lactobacillus casei* promoters during bacterial transit through the gastrointestinal tracts of human-microbiota-associated mice**

R. Oozeer, JP. Furet, N. Goupil-Feuillerat, J. Anba, J. Mengaud, G. Corthier  
*Appl. Envir. Microbiol.*, 2005; 71(3): 1356-63

In a previous study using fusion of the deregulated lactose promoter, *lacTp\**, and reporter genes, we suggested that *L. casei* could initiate *de novo* protein synthesis during intestinal transit. In order to confirm this finding and extend it to other promoters, we adopted a quantitative reverse transcriptase PCR approach combined with a transcriptional fusion system consisting

of luciferase genes under the control of four promoters (*ccpA*, *dlt*, *ldh* and *lacT\**) from *L. casei* DN-114 001. Promoter expression was monitored during cell growth and variable luciferase activities were detected. In three-day cultures, all the genetically modified strains survived but without exhibiting luciferase activity. Luciferase mRNA levels determined by RT-QPCR analysis (RNA/CFU) were not significant. The cultures were administered to human-microbiota-associated mice and the feces were collected 6 hours later. *L. casei* promoters *lacTp\** and *ldhp* initiated mRNA synthesis during gastrointestinal transit. The promoters, *ccpAp* and *dltp*, exhibited no luciferase activity, nor was *de novo* synthesized luciferase mRNA detected in the feces. *L. casei* seems to adapt its physiology to the gastrointestinal tract environment by modulating promoter activities. The approach (fecal transcriptional analysis) described herein may, moreover, be of value in studying gene expression of transiting bacteria in human fecal specimens.

#### **Survival of *Lactobacillus casei* in the human digestive tract after consumption of fermented milk.**

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*Appl. Envir. Microbiol.*, 2006; Aug; 72(8): 5615-7

A human trial was carried out to assess ileal and faecal survival of the probiotic bacterium *Lactobacillus casei* DN-114 001 ingested in fermented milk. Results showed that survival rates were up to 51.2% in the ileum and 28.4% in faeces. It is concluded that *L. casei* can survive transit through the human gut.

#### **The intestine and its microflora are partners for the protection of the host: report on the Danone Symposium "The Intelligent Intestine," held in Paris, June 14, 2002**

Pierre Bourlioux, Berthold Koletzko, Francisco Guarner and Véronique Braesco  
*American Journal of Clinical Nutrition*, October 2003; Vol. 78, No. 4, 675-683

Abstract. The intestine is an extremely complex living system that participates in the protection of the host through a strong defense against aggressions from the external environment. This defensive task is based on 3 constituents that are in permanent contact and dialog with each other: the microflora, mucosal barrier, and local immune system. We review herein current knowledge about these important functions. The gut microflora play a major role against exogenous bacteria through colonization resistance, but the mechanism of action is not yet established, although it is linked to the bacteria colonizing the gut. This colonization involves bacteria-bacteria dialog, bacteria-mucins interactions, and bacteria-colonocytes cross-talk associated with environmental factors. The intestinal mucosa is a cellular barrier and the main site of interaction with foreign substances and exogenous microorganisms. It is a complex physicochemical structure consisting of a mucous layer linked to cellular and stromal components that participate in the defense of the host through mucosal blood flow, mucosal secretions, epithelial cell functionals, surface hydrophobicity, and defensin production. The intestine is the primary immune organ of the body represented by the gut-associated lymphoid tissue through innate and acquired immunity. This immune system can tolerate dietary antigens and the gut-colonizing bacteria and recognizes and rejects enteropathogenic microorganisms that may challenge the body's defenses. In cooperation with these endogenous barriers, some in-transit bacteria, such as probiotics, can act as partners of the defense system of the intestine.