Effects of milk fermented by Lactobacillus helveticus R389 on immune cells associated to mammary glands in normal and a breast cancer model.

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Abstract

Antitumour activity is an effect attributed to probiotics and fermented foods. Here, the immune cells in mammary glands and cytokine concentration in serum were analyzed using mice fed with milk fermented by Lactobacillus helveticus R389 or L89 (proteolytic-deficient variant), injected or not with breast tumour cells. Mice were fed 7 days with fermented milk, injected with breast tumour cells and 4 days post-injection, they received fermented milk. IgA, CD4, CD8, cytokines and Bcl-2 positive cells in mammary glands and cytokine in serum were determined. Mice fed with L. helveticus R389 fermented milk and injected with tumour cells increased IgA and CD4 positive cells in mammary glands (tumour control increased CD8 + cells). Mice from fermented milk control groups (without tumour cell injection) did not show changes in immune cell or cytokine positive cell numbers. IL-10 increases and IL-6 decreases were more pronounced in mice fed with milk fermented by L. helveticus R389 than in the other groups.

This study demonstrated the immunoregulatory capacity of milk fermented by L. helveticus R389 on the immune response in mammary glands in presence of a local pathology (breast tumour). Orally administered fermented products could be used to modify the immune cell activation in distant mucosal sites and maintain these cells alert, but local stimulus was necessary to produce the activation of a local immune response in mammary glands, which could modulate the immune-endocrine relationship in these glands.
Abstract

Previous studies have shown that compounds released during milk fermentation by Lactobacillus helveticus are implicated in the antitumour effect of this product. Here the effects of the consumption, during 2 or 7 days, of kefir or kefir cell-free fraction (KF) on the systemic and local immune responses in mammary glands and tumours using a murine hormone-dependent breast cancer model were studied. In the tumour control group, mice did not receive these products. At the end of the feeding period, mice were injected subcutaneously with tumour cells in the mammary gland. Four days post-injection, they received kefir or KF on a cyclical basis. Rate of tumour development, cytokines in serum; mammary gland tissue, and tumour isolated cells were monitored. Two-day cyclical administration of both products delayed tumour growth. Both kefir and KF increased IL-10 in serum and decreased IL-6(+) cells (cytokine involved in oestrogen synthesis) in mammary glands. Two-day cyclical administration of KF increased IL-10(+) cells in mammary glands and in tumours and decreased IL-6(+) cells in tumour. This study demonstrated the modulatory capacity of KF on the immune response in mammary glands and tumours and the importance of the administration period to obtain this effect.


Study of immune cells involved in the antitumor effect of kefir in a murine breast cancer model.

Abstract

Administration of kefir and a kefir cell-free fraction (KF) to mice injected with breast tumor cells produced, locally in the mammary gland, different profiles of cells secreting cytokines. Here, the immune cell populations in mammary glands affected by the cyclic consumption of kefir or KF for 2 or 7 d were evaluated using a breast tumor model.
Apoptosis was also assayed as another mechanism involved in tumor growth delay. The rate development of tumor cells, IgA(+) cells, and CD4+ and CD8+ T lymphocytes was monitored in mammary gland tissues. The number of Bcl-2(+) cells in the mammary gland was compared with the apoptosis observed in the tumor. Two-day cyclical administration of both products delayed tumor growth and increased the number of IgA(+) cells in the mammary gland. Changes in the balance between CD4+ and CD8+ cells in the mammary gland were observed in mice from the group fed KF cyclically for 2 d, such that the number of CD4+ cells increased when the number of CD8+ cells remained constant. Mice that received 2-d cyclic administration of KF showed significant increases in the number of apoptotic cells and decreases in Bcl-2(+) cells in the mammary gland, compared with the tumor control group. The present study allows a better understanding of the mechanisms (immune and nonimmune) involved in the antitumor effect observed in mice administered kefir or KF. The importance of nonmicrobial components released during milk fermentation to obtain the beneficial antitumor effects is also reported.


Immunomodulating capacity of commercial fish protein hydrolysate for diet supplementation.

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Abstract

Dietary proteins harbour bioactive peptides that can be released by a fermentation process. Fish proteins are a valuable and little-exploited source of potentially active biopeptides. The aim of this research was to evaluate the effects of a commercially available fermented fish protein concentrate (Seacure) (FPC) derived from a fermentation process, on the mucosal immune response in a murine model. BALB/c mice received the FPC or the non-fermented powder at different concentrations (0.20, 0.25 or 0.30mg/ml) for 2, 5 or 7 consecutive days. At the end of each feeding period, histological studies of the gut were carried out and the phagocytic activity of peritoneal macrophages, the number of IgA+ cells in the small intestine lamina propria and bronchial tissue and the number of IL-4+, IL-6+, IL-10+, IFNgamma+ and TNFalpha+ cells in the small intestine lamina propria were determined. Different accumulative doses of FPC did not induce any inflammatory immune response and the normal morphology of the small intestine
was not affected. Phagocytic activity of peritoneal macrophages was enhanced following FPC administration at 0.3mg/ml for 7 consecutive days. The number of IgA+ cells increased in the small intestine lamina propria but not in the bronchial tissue. IL-4, IL-6 and IL-10 were all significantly increased in the lamina propria of the small intestine of animals that received FPC. At the same time, some pro-inflammatory cytokines such as IFNgamma and TNFalpha also increased, but the intestinal homoeostasis was maintained and no tissue damage was observed. We conclude that FPC is an immunomodulating food with a demonstrated capacity to enhance non-specific host defense mechanisms.

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**Effects of the oral administration of the exopolysaccharide produced by Lactobacillus kefiranofaciens on the gut mucosal immunity.**

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**Abstract**

The probiotic effects ascribed to lactic acid bacteria (LAB) and their fermented dairy products arise not only from whole microorganisms and cell wall components but also from peptides and extracellular polysaccharides (exopolysaccharides) produced during the fermentation of milk. There is a lack of knowledge concerning the immune mechanisms induced by exopolysaccharides produced by lactic acid bacteria, which would allow a better understanding of the functional effects described to them. The aim of this study was to investigate the in vivo immunomodulating capacity of the exopolysaccharide produced by Lactobacillus kefiranofaciens by analyzing the profile of cytokines and immunoglobulins induced at the intestinal mucosa level, in the intestinal fluid and blood serum. BALB/c mice received the exopolysaccharide produced by L. kefiranofaciens for 2, 5 or 7 consecutive days. At the end of each period of administration, control and treated mice were sacrificed and the numbers of IgA+ and IgG+ cells were determined on histological slices of the small and large intestine by immunofluorescence. Cytokines (IL-4, IL-6, IL-10, IL-12, IFNgamma and TNFalpha) were also determined in the gut lamina propria as well as in the intestinal fluid and blood serum. There was an increase of IgA+ cells in the small and large intestine lamina propria, without change in the number of IgG+ cells in the small intestine. This study reports the effects of the oral
administration of the exopolysaccharide produced by L. kefiranofaciens in the number of IgA+ cells in the small and large intestine, comparing simultaneously the production of cytokines by cells of the lamina propria and in the intestinal fluid and blood serum. The increase in the number of IgA+ cells was not simultaneously accompanied by an enhance of the number of IL-4+ cells in the small intestine. This finding would be in accordance with the fact that, in general, polysaccharide antigens elicit a T-independent immune response. For IL-10+, IL-6+ and IL-12+ cells, the values found were slightly increased compared to control values, while IFN gamma+ and TNF alpha+ cells did not change compared to control values. The effects observed on immunoglobulins and in all the cytokines assayed in the large intestine after kefiran administration were of greater magnitude than the ones observed in the small intestine lamina propria, which may be due to the saccharolytic action of the colonic microflora. In the intestinal fluid, only IL-4 and IL-12 increased compared to control values. In blood serum, all the cytokines assayed followed a pattern of production quite similar to the one found for them in the small intestine lamina propria. We observed that the exopolysaccharide induced a gut mucosal response and it was able to up and down regulate it for protective immunity, maintaining intestinal homeostasis, enhancing the IgA production at both the small and large intestine level and influencing the systemic immunity through the cytokines released to the circulating blood.

_Milk fermented by Lactobacillus helveticus R389 and its non-bacterial fraction confer enhanced protection against Salmonella enteritidis serovar Typhimurium infection in mice._

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**Abstract**

Bacterial infections in the gastrointestinal tract represent a major global health problem, even in the presence of normally effective mucosal immune mechanisms. Milk fermented by Lactobacillus helveticus R389 (FM) or its non-bacterial fraction obtained by milk fermentation at controlled pH 6 (NBF) are able to activate the small intestine mucosal immune response according to previous studies. In this work we aimed at comparing their protection capacity against an infection by Salmonella enteritidis serovar Typhimurium and at studying the mechanisms
involved. In a completely randomized design, BALB/c mice received FM or NBF for 2, 5 or 7 consecutive days, followed by a single oral challenge with S. Typhimurium (10^7 cells/mouse). The increase in the number of IgA+ cells in the lamina propria of the small intestine, after the feeding periods, was accompanied by an increase in the luminal content of total S-IgA. However, no antibodies were produced against the NBF. In mice given the FM or the NBF for 7 consecutive days, lower levels of liver colonization on day 7 post-challenge with S. Typhimurium, higher luminal contents of specific anti-Salmonella S-IgA, higher percentages of survival to infection and lower numbers of MIP-1alpha+ cells in the lamina propria were observed. In this work we observed that in both the FM or the NBF there are active principles that confer enhanced protection against S. Typhimurium infection. However, the mechanisms underlying mucosal immunomodulation and protection are different. In those mechanisms, the mucosal immune response would seem to be more involved than the competitive or exclusion mechanisms between L. helveticus R389 and S. enteritidis serovar Typhimurium.