Note

Effects of a Fermented Milk Drink Containing Lactobacillus casei Strain Shirota on the Immune System in Healthy Human Subjects

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Nine healthy volunteers drank fermented milk containing $4 \times 10^9$ live cells of Lactobacillus casei strain Shirota daily for 3 weeks, and their NK activity and other immunological functions were measured. NK activity significantly increased ($p < 0.01$) 3 weeks after the start of intake and remained elevated for the next 3 weeks. The effect was particularly prominent in low-NK-individuals.

Key words: fermented milk; immune system; NK cell activity; Lactobacillus casei strain Shirota

Lactic acid bacteria are commonly used for the manufacture of fermented milk products and are known to have excellent nutritional and flavoring effects. Recent studies have revealed that some strains of lactic acid bacteria have health-promoting effects, through improvement of the intestinal microflora and modulation of the immune system.1,2 Lactobacillus casei strain Shirota (LcS) has been shown to have potent anti-tumor effects in rodents.3,4 Immunomodulatory effects of this bacterium also have been well documented in animal models.5 In humans, a LcS preparation has been shown to prevent the recurrence of superficial bladder cancer and a possible effect on the immune system has been suggested.6,7 Furthermore, habitual intake of lactic acid bacteria containing LcS has been shown to reduce the risk of bladder cancer recently and an effect on the immune system of healthy people has also been suggested,8 however, the precise mechanisms of action remain to be discovered.9

We examined the effects of drinking fermented milk containing LcS on the immune system of healthy individuals. All experiments were done in accordance with the guidelines of the Helsinki Declaration and the ethical committee for clinical experiments of Juntendo University School of Medicine. Subjects who were healthy but had relatively low levels of NK cell activity, below 45% cytotoxicity,10 were adopted as volunteers. They ranged in age from 20 to 40 ($32.67 \pm 6.65$ (mean $\pm$ SD)). The subjects were separated into two groups, an experimental group and a control group. The experimental group consisted of males and 4 females, and they each drank one bottle of Yakult 400® (containing 2.5 g of nonfat dry milk, 14.4 g of sugars, flavors, and at least $4 \times 10^9$ live LcS cells per bottle) after lunch every day for 3 weeks. The control group consisted of 3 males and 5 females, and they each drank the same amount of unfermented milk having a basic composition similar to that of Yakult 400® except for the presence of the bacteria. Blood samples were collected at 5 times, before drinking the milk product, 1 week and 3 weeks after the start of intake, and 3 weeks and 2 months after the period of intake.

It is known that NK cells play a critical role in immune surveillance against tumor development and viral infections11,12 and that intestinal microflora can modulate the NK cell activity.13-15 It is also shown that oral administration of heat-killed LcS cells improves the NK cell activity in tumor-bearing mice.16 In this study, we examined the natural killer (NK) cell activity of peripheral blood mononuclear cells (PBMC) at each time to investigate the influence of intake of LcS on the healthy human immune system. NK cell activity was measured by the europium (Eu) release assay as described previously.17,18

Figure 1 shows the changes in NK cell activity in the experimental group and the control group. In the experimental group, the level of NK cell activity was increased 1 week after the start of intake of the fermented milk drink ($p = 0.0598$), and was significantly increased 3 weeks after the start of intake ($p = 0.0050$), compared with the value before intake. The NK cell activity remained elevated for the next 3 weeks ($p = 0.0221$). Two months after the period of intake, the activity was found to have returned to almost the same level as that before intake. On the other hand, the level of NK cell activity in the control

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Abbreviations: NK, natural killer; LcS, Lactobacillus casei strain Shirota; Eu, europium
group did not significantly change at any time compared with the level before intake. These findings suggest that the continuous intake of the fermented milk containing LcS is effective to augment NK cell activity in healthy people.

We next studied the relationship between the magnitude of the increase in NK cell activity that occurs upon intake of the fermented milk drink for 3 weeks and the NK cell activity before intake in the experimental group. As shown in Fig. 2, the magnitude of the increase in NK cell activity that occurred as a result of intake of the fermented milk drink was inversely correlated with the levels of NK cell activity before intake \( (r = -0.7652, p = 0.0163) \). This finding demonstrates that the effect of the fermented milk drink is particularly prominent in individuals who have low levels of NK cell activity. This suggests the possibility that LcS may be effective for improvement of NK cell activity in patients with autoimmune disease or cancer,\(^9\) or elderly persons,\(^9\) who usually have low levels of NK cell activity.

The frequency of CD3\(^+\)CD16\(^+\)CD56\(^+\) NK cells among the peripheral blood mononuclear cells (PBMC) was measured by flow cytometry. However, comparing the values before and after intake of the fermented milk drink in the experimental group, no change was recognized (data not shown). From this result, it seems that the augmentation of NK cell activity by intake of LcS may depend upon an increase in activity per NK cell rather than an increase in the number of NK cells.

An effect of LcS on T cells was not recognized. The frequency of CD4\(^+\) cells and that of CD8\(^+\) cells among the PBMC were measured by flow cytometry. However, comparing the values before and after intake, there was no change in either of the groups. The serum concentrations of IFN\(\alpha\) and IFN\(\gamma\) were also measured at each time point by enzyme-linked immunosorbent assay (ELISA). However, the concentration of IFN\(\alpha\) did not change and IFN\(\gamma\) could not be detected at all. Although we could detect no other change than NK cell activity, augmentation of NK cell activity may have been mediated by IL-12 and IFN\(\gamma\). It is reported that LcS activates macrophages to induce IL-12 and that this cytokine in turn activates T cells to secrete IFN\(\gamma\).\(^{20,21}\)

It is known that bad lifestyle habits, including smoking and mental stress, exert a negative influence on NK cell activity, but balanced nutrition, physical exercise, and other aspects of good lifestyles are associated with high levels of NK cell activity.\(^{22,23}\) The results of this study strongly suggest that drinking fermented milk containing LcS can influence the levels of NK cell activity in healthy people. Augmentation of NK cell activity in individuals who have low levels of NK cell activity would be important to maintain a healthy life and prevent diseases.

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References