

The effect of the sympathetic nervous system on splenic natural killer cell activity in mice administered the *Lactobacillus pentosus* strain S-PT84

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Splenic sympathetic nerve activity (SNA) modulates cellular immune functions such as splenic natural killer cell activity. *Lactobacillus pentosus* strain S-PT84 enhances splenic natural killer cell activity. Here, we examined whether S-PT84 affects splenic natural killer activity through splenic SNA in BALB/c mice. Splenic SNA was significantly decreased following the administration of S-PT84. This phenomenon was inhibited by pretreatment with thioperamide (histamine H₃ receptor antagonist), suggesting that S-PT84 directly affected splenic SNA. Thioperamide also inhibited the increase in splenic natural killer activity by S-PT84. Thus, the change in splenic natural killer activity by S-PT84 may be partially modulated through SNA. *NeuroReport* 24:988–991 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The immune system and the nervous system affect each other [1]. Kimura *et al.* [2] reported that pinching of the hindpaws in rats increases splenic sympathetic nerve activity (SNA) and reduces splenic natural killer (NK) cell activity. Katafuchi *et al.* [3] also showed that the reduction in splenic NK activity induced by stimulation of the splenic sympathetic nerve is completely blocked by β -adrenergic receptor antagonists. Some studies have suggested that the increase in tumor volume is suppressed when splenic SNA is decreased by ingestion of amino acids or peptides [4,5]. Recently, Kakutani *et al.* [6] reported that enzymatically synthesized glycogen enhances splenic NK activity by acting on autonomic nerves. Thus, modulation of splenic SNA by food ingredients may affect splenic NK activity.

Lactic acid bacteria have been used widely as health food materials and have several beneficial effects including immunomodulatory activity [7,8]. Recently, Ueno *et al.* [9] reported that a heat-killed *Lactobacillus* strain maintains intestinal homeostasis and improves intestinal disorders. Some reports have described a preventive effect on chronic diarrhea [10] or a protective effect against viral infection [11] by ingesting a dead or an inactive *Lactobacillus* strain. Thus, not only live bacteria but also dead bacteria have

beneficial effects on health. Indeed, we showed that both live and heat-killed *Lactobacillus pentosus* strain S-PT84 stimulate interleukin-12 and interferon- γ production from immune cells *in vitro* and enhance splenic NK activity *in vivo* [12]. We also showed that heat-killed S-PT84 induces cytokine production through interactions between dendritic cells and NK cells and that Th1 cytokine (interleukin-12 or interferon- γ) production occurs through toll-like receptor 2 or 4 in immune cells [13]. Recently, we reported that a low-molecular-weight fraction of culture supernatant of S-PT84 affects brown adipose tissue SNA and gastric vagal nerve activity [14]. In contrast, it is not clear whether S-PT84 bacterial cells affect SNA, and whether another mechanism is involved in the increase in splenic NK activity by ingestion of S-PT84 cells. Some reports have shown that renal or adrenal SNA is modulated by lactic acid bacteria [15,16].

Here, we investigated whether modulation of splenic SNA by administration of S-PT84 cells affects splenic NK activity.

Materials and methods

Preparation of *Lactobacillus pentosus* strain S-PT84

S-PT84 was cultured in de Man, Rogosa, Sharpe (MRS) broth (Difco Laboratories, Detroit, Michigan, USA) at 37°C for 24 h. Cultured S-PT84 cells were collected by centrifugation at 9190g for 10 min, washed twice with sterile saline, and then washed with distilled water. After cell counting, the S-PT84 suspension was heat killed at 95°C for 5 min and lyophilized.

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Animals

BALB/c female mice (8 weeks old) were obtained from Japan SLC Inc. (Hamamatsu, Japan) and kept under constant temperature and humidity with a 12-h light-dark cycle. Food (AIN-93M; Oriental Yeast Co., Ltd, Tokyo, Japan) and tap water were freely available. The mice were acclimated to the environment for at least 1 week before the experiment. Experiments were approved by the Institutional Animal Care and Use Committee of ANBAS Corporation and the Animal Care and Use Committee of Suntory Holdings Limited according to the guidelines for animal experiments prescribed by the Science Council of Japan on 1 June 2006.

Determination of splenic sympathetic nerve activity

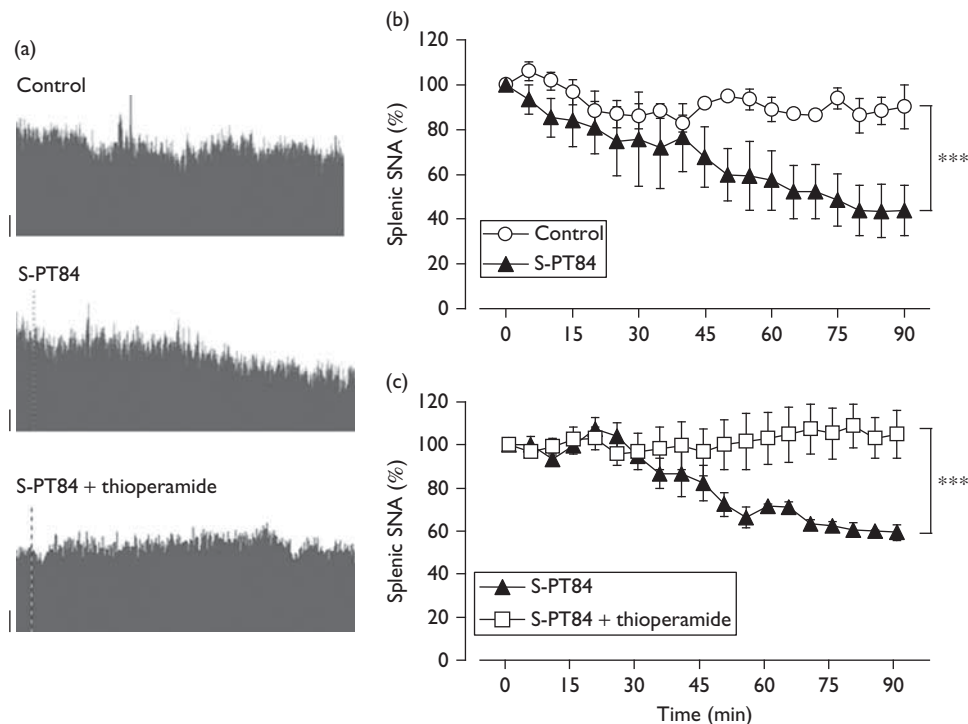
On the experimental day, food was removed 3 h before surgery. Mice were anesthetized with an intraperitoneal injection of 1 g/kg urethane. Mice were cannulated intraduodenally to administer saline or S-PT84. To record the neural activity, the sympathetic nerve innervating the spleen was ligated at its distal end and hooked up to a pair of silver wire electrodes. S-PT84 solution was prepared with sterile saline at 3×10^8 cells/ml. Mice were intraduodenally administered 100 μ l saline or S-PT84 solution.

Thioperamide (thioperamide maleate; Sigma-Aldrich Corp., St Louis, Missouri, USA) was prepared with sterile saline at 2 mg/ml and a subcutaneous injection (5 ml/kg) was administered 10 min before administration of S-PT84. Changes in SNA following administration of saline or S-PT84 were determined electrophysiologically over the next 90 min. The electrical signals from the electrodes were amplified, filtered, and monitored on an oscilloscope. The raw nerve activity was converted into standard pulses using a window discriminator. The data were sampled using a Power-Lab analog-to-digital converter (Power-Lab model 4sp, AD Instruments, Colorado Springs, Colorado, USA) and stored on hard disks for off-line analysis. Splenic SNA measured during each 5-min period after oral administration of saline or S-PT84 was averaged after digital signal processing analysis.

Determination of splenic natural killer activity

Next, we determined splenic NK activity to confirm the effect of S-PT84 and thioperamide at 90 min, after which splenic SNA was changed by S-PT84 administration. Mice were orally administered 100 μ l saline or S-PT84 solution as described above. Thioperamide was prepared as above and administered intraperitoneally (5 ml/kg) 10 min be-

Fig. 1



Changes in splenic sympathetic nerve activity (SNA) in BALB/c mice. (a) Representative recordings of splenic SNA in mice administered saline, S-PT84, or S-PT84/thioperamide. Vertical scale bars to the left of the recordings represent neural discharge rates of 100 spikes/5 s. (b) Effect of S-PT84 administration on splenic SNA. (c) Effect of thioperamide on the decrease in splenic SNA by S-PT84. Data (mean \pm SEM) are expressed as percentages relative to values at 0 min. $n=3$ mice/group. *** $P<0.001$ between the control and the S-PT84 groups and the S-PT84 and S-PT84 + thioperamide groups.

fore oral administration of S-PT84. Ninety minutes after administration of saline or S-PT84, mice were anesthetized with diethyl ether and killed by exsanguination. The spleens were aseptically removed and splenocytes were prepared after lysis of erythrocytes. Splenic NK activity was determined as described previously [12].

Statistical analysis

Data are presented as the mean \pm SEM. The Mann-Whitney *U*-test was used to detect statistically significant differences between baseline (0 min) splenic SNA values in each group. Analysis of variance with repeated measures was used to evaluate group differences in SNA. Two-way analysis of variance was used to determine statistically significant differences for NK activity comparison. *P*-values less than 0.05 were considered significant.

Results

Changes in splenic sympathetic nerve activity in BALB/c mice

Basal levels of splenic SNA were as follows: control group, 235.1 ± 55.7 spikes/5 s ($n = 3$), and S-PT84 group, 200.1 ± 74.0 spikes/5 s ($n = 3$). These differences were not significant. Representative data are shown in Fig. 1a. Splenic SNA values were expressed as the percentage of baseline values (Fig. 1b). Splenic SNA in the S-PT84 group was gradually and significantly reduced compared with splenic SNA in the control group ($P < 0.001$).

Next, we examined the effect of thioperamide, a histamine H₃ receptor antagonist that inhibits the decrease in SNA induced by several food ingredients [4,5], on the decrease in splenic SNA by S-PT84. Basal levels of splenic SNA were as follows: S-PT84 group, 257.7 ± 9.0 spikes/5 s ($n = 3$), and S-PT84 + thioperamide group, 245.2 ± 10.1 spikes/5 s ($n = 3$). These differences were not significant. The decrease in splenic SNA by S-PT84 was significantly inhibited by thioperamide ($P < 0.001$; Fig. 1c).

Changes in splenic natural killer activity in BALB/c mice

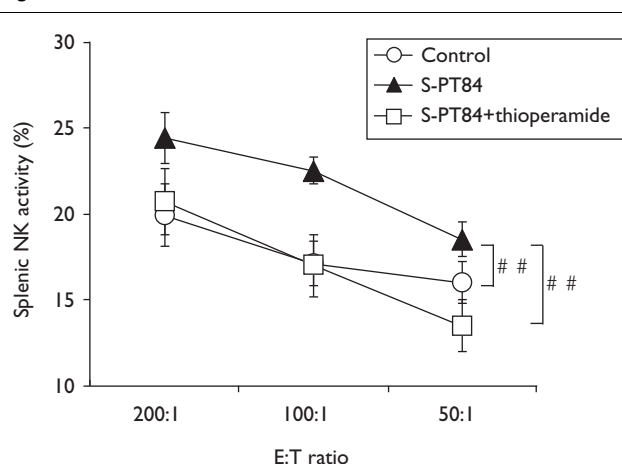
Splenic NK activity was determined 90 min after administration of S-PT84 when splenic SNA was decreased significantly and was increased significantly by S-PT84 ($P < 0.01$; Fig. 2).

We examined the effect of thioperamide on splenic NK activity enhanced by S-PT84. The enhancement of splenic NK activity by S-PT84 was inhibited significantly by pretreatment with thioperamide ($P < 0.01$; Fig. 2). We examined the effect of single thioperamide administration on splenic NK activity, but thioperamide exerted no effect on splenic NK activity (data not shown).

Discussion

The spleen is a crucial secondary lymphoid organ that is densely innervated by sympathetic nerve fibers; the

Fig. 2



Changes in splenic NK activity in BALB/c mice. Effect of oral administration of S-PT84 and effect of thioperamide on splenic NK activity enhanced by S-PT84 administration. Each group consists of three to six mice. Statistically significant differences between the control and S-PT84 group. $^{##}P < 0.01$, between S-PT84 group and S-PT84 + thioperamide group. $^{###}P < 0.01$ (two-way analysis of variance). NK, natural killer.

splenic nerve contains $\sim 98\%$ sympathetic axons [17,18]. Splenic NK activity is well known to be decreased when splenic SNA is increased [2,3]. Some reports have shown that renal or adrenal SNA is decreased by ingestion of *Lactobacillus johnsonii* [15,16] and that prevention of hyperexcitability in colonic dorsal root ganglion neurons is induced by ingestion of *Lactobacillus reuteri* [19].

In the present study, splenic SNA was significantly reduced by administration of heat-killed *L. pentosus* strain S-PT84, indicating that S-PT84 has a splenic SNA-suppressive effect. This is the first report showing that splenic SNA is reduced by administration of *Lactobacillus* cells.

Recently, Logan *et al.* [20] reported that rhythmic noradrenaline input to the spleen acts as an entrainment cue that modulates the molecular clock in splenic NK cells, suggesting that modulation of splenic SNA affects splenic NK cell function. Splenic NK activity was also significantly elevated by a single dose of S-PT84, suggesting that this increase was likely induced by suppression of splenic SNA.

Tanida *et al.* [15,21] reported that thioperamide eliminates the decreases in both renal SNA and mean arterial pressure by *Lactobacillus johnsonii* La 1 or anserine. The decreases in white adipose tissue SNA by orexin-A and in cutaneous arterial SNA by urea are also reversed by thioperamide [22,23]. Thus, suppression of SNA may be mediated by histaminergic H₃ receptors. Thus, we used thioperamide to examine the mechanism of the decrease in splenic SNA. Suppression of splenic SNA by S-PT84 was blocked by treatment with thioperamide. Further-

more, the enhancement of splenic NK activity by S-PT84 was also eliminated by thioperamide injection. These results suggest that S-PT84 enhances splenic NK activity by decreasing splenic SNA.

Meltzer *et al.* [24] reported that muramyl dipeptide increased sleep and indicated central nervous system-depressant effects in rats. Recently, Tohno [25] reported the possibility that L-muramyl dipeptide modulates intestinal nerve activity, not D-muramyl dipeptide. Gram-positive bacteria including S-PT84 have this component in cell walls; therefore, cell components may participate in the mechanism of S-PT84 that decreased SNA, but the details are unclear.

Conclusion

S-PT84 increased splenic NK activity not only by cytokine induction as reported previously but also by modulating splenic SNA. Further studies are required to elucidate the details of the effects of S-PT84 on both the immune system and the nervous system.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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