

Effects of *Lactobacillus acidophilus* Strain L-55 on Experimental Allergic Rhinitis in BALB/c Mice

Yosuke SUNADA,^a Syoji NAKAMURA,^a and Chiaki KAMEI^{*,b}

^aFundamental Laboratory, Ohayo Dairy Products Co., Ltd.; Okayama 701–1221, Japan; and ^bDepartment of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; Okayama 700–8530, Japan. Received June 27, 2007; accepted September 4, 2007; published online September 6, 2007

We investigated the effect of *Lactobacillus acidophilus* strain L-55 isolated from infant feces on experimental allergic rhinitis in BALB/c mice. The heat-treated cells of strain L-55 were orally administered for 4 consecutive weeks to mice sensitized by ovalbumin (OVA), and nasal symptoms (sneezing and nasal rubbing) induced by OVA challenge were evaluated. Strain L-55 at doses of 1 and 10 mg cells/mouse significantly inhibited nasal symptoms by repeated administration over a period of 2 weeks. Furthermore, we measured the level of OVA-specific IgE titers in the serum by passive cutaneous anaphylaxis (PCA) reaction. PCA titers in the sera from mice administered strain L-55 were significantly lowered compared with the control. These results suggest that oral administration of strain L-55 may be useful for alleviating the nasal symptoms of allergic rhinitis.

Key words allergic rhinitis; lactic acid bacteria; *Lactobacillus acidophilus* strain L-55

Lactic acid bacteria, which are Gram-positive bacteria, are industrially important microorganisms used for the production of various fermented foods. Recently, probiotic lactic acid bacteria defined as “living microorganisms that benefit the health of the host by conditioning the intestinal environment”¹⁾ have attracted the attention of many scientists, and began to be applied in the dairy industry. For example, the intake of fermented milk containing probiotic lactic acid bacteria is known to improve the balance of the intestinal microflora by both increasing beneficial bacteria and reducing harmful bacteria in the intestine. Moreover, the immunomodulating effects of lactic acid bacteria have also been reported^{2,3)} and some specific strains of lactic acid bacteria are recognized to alleviate allergic diseases such as allergic rhinitis.^{4–6)} Although the etiology of allergic disease is still unclear, disturbance of the intestinal microflora, especially endogenous lactic acid bacteria, has been regarded as one of the important reasons related to allergic diseases.^{7–9)} Accordingly, improvement of the intestinal environment by the intake of probiotic lactic acid bacteria is considered effective for modulation of the immune system.¹⁰⁾

Lactobacillus acidophilus strain L-55, which was originally isolated from healthy infant feces, is probiotic lactic acid bacteria used as a starter strain for the fermentation of dairy products. Strain L-55 possesses adherent ability to Caco-2 cells derived from the human enteric epithelium¹¹⁾; therefore, it may be capable of regulating the immune system. The purpose of the present study was to examine the inhibitory effect of oral administration of strain L-55 on experimental allergic rhinitis in BALB/c mice.

MATERIALS AND METHODS

Animals Female BALB/c mice (6 weeks old) were purchased from Japan SLC, Inc., Shizuoka, Japan. The animals were housed in an air-conditioned room and maintained at 24±2 °C with 55±15% humidity. They were given standard laboratory rodent chow (Oriental Yeast, Tokyo) and water *ad libitum*. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at

Okayama University Advanced Science Research Center.

Preparation of Bacterial Cells Strain L-55 was originally isolated from human feces at the Fundamental Laboratory, Ohayo Dairy Products Co., Ltd. (Okayama, Japan). After the cultivation of strain L-55 in 5 l of MRS broth (Difco, Detroit, MI, U.S.A.) at 37 °C for 18 h, cells were harvested by centrifugation at 3000×*g* for 20 min. The cells were washed twice with sterile distilled water, and resuspended in 500 ml of sterile distilled water. The obtaining cell suspension was boiled at 100 °C for 10 min, and then lyophilized.

Reagents Reagents were obtained from the sources shown in parentheses: ovalbumin (OVA, Sigma, St. Louis, MO, U.S.A.), pertussis toxin (Sigma) and aluminum hydroxide hydrate gel suspension (LSL Co., Tokyo, Japan). These reagents were dissolved in saline. Tranilast (Kissei Pharmaceuticals Co., Ltd., Nagano, Japan) was used as a positive control in this study.

Sensitization The experimental procedure for the animal model of allergic rhinitis is summarized in Fig. 1. The mice were sensitized by an injection of 0.2 ml of physiological saline containing OVA (0.1 mg), aluminum hydroxide gel (1 mg) and pertussis toxin (300 ng) into the peritoneal cavity on the first day. At intervals of 5 d, they were boosted by a subcutaneous injection of 0.1 ml of physiological saline containing OVA (50 µg) in the back. From 18 d after the first immunization, daily intranasal sensitization with OVA (50 µg)

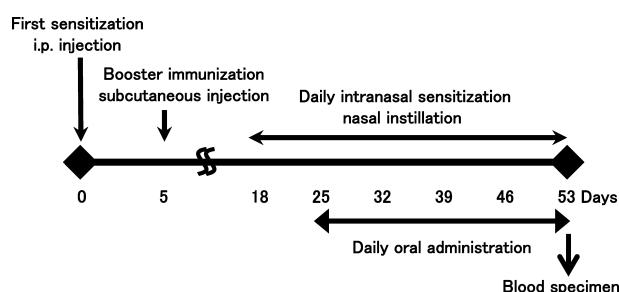


Fig. 1. Schedule of Sensitization of Mice, Oral Administration of the Test Sample, and Collection of Blood Specimen

* To whom correspondence should be addressed. e-mail: kamei@pheasant.pharm.okayama-u.ac.jp

was performed for 5 weeks.

Oral Administration to Mice Cells of strain L-55 were orally administered to mice *via* a feeding tube placed in the esophagus. Before administration, an adequate amount of lyophilized cells was suspended in distilled water. Mice received 0.3 ml of the suspension containing 1 mg (dose of 1 mg/mouse) or 10 mg (dose of 10 mg/mouse) of cells. As a control, 0.3 ml of distilled water was administered to mice. Furthermore, mice as a positive control received tranilast at a dose of 300 mg/kg. The administration was carried out once daily for 4 consecutive weeks from day 25 to day 53 (Fig. 1).

Evaluation of Nasal Symptoms Nasal symptoms were evaluated according to the method of Takubo *et al.*¹²⁾ Cells of strain L-55 and tranilast were suspended in distilled water and administered orally 1 h before the start of behavioral observation. Before the observation, the animals were placed into an observation cage (32×22×10 cm) for about 10 min for acclimatization. After nasal instillation of 1 μ l of OVA solution (50 mg/ml) into the bilateral nasal cavities, the animals were placed into an observation cage (one animal/cage), and sneezing and nasal rubbing behaviors were counted for 60 min. Nasal symptoms were observed on day 25, 32, 39, 46 and 53. Strain L-55 and tranilast were administered orally every day from day 25 to day 53.

Titration of Passive Cutaneous Anaphylaxis Reaction

The degree of OVA-specific IgE production in the serum was measured by passive cutaneous anaphylaxis (PCA) reaction. Blood specimens of sensitized mice were drawn from the cava abdominalis on day 53 (Fig. 1) and centrifuged (755×g for 10 min at 4 °C) to prepare the sera. Serum samples were stored at -20 °C until measurement. Serially diluted sera were injected intradermally in a volume of 0.1 ml into the shaved backs of normal rats. Histamine as a positive control was also injected in the same manner. After 48 h, saline containing OVA (2.5 mg/ml) and Evans blue (10 mg/ml) were injected into the tail vein at a dose of 0.2 ml/100 g body weight. After 30 min, the rats were sacrificed, the dorsal back skin was peeled off, and the diameter of the blue spot on the underside of the skin was measured. The PCA titer was ex-

pressed as the maximum dilution of the antiserum that gave a passive reaction of more 5 mm in diameter in the dorsal skin.

Statistical Analysis Data are presented as the means $\pm$ standard error of means (S.E.M.). Statistical analyses were one-way analysis of variance with Dunnett's test. A probability value of less than 0.05 was considered significant.

RESULTS

Effects on Nasal Rubbing Figure 2 shows the effect of strain L-55 on nasal rubbing induced by antigen. The nasal rubbing of the control group increased gradually depending on daily intranasal sensitization. Strain L-55 caused no significant inhibition of nasal rubbing by single administration (data not shown) and repeated administration for 1 week even at a dose of 10 mg/mouse; however, at doses of 1 and 10 mg/mouse, it caused significant inhibition of nasal rubbing by repeated administration over a period for 2 weeks. Tranilast used as a positive control also caused significant inhibition of nasal rubbing over 2 weeks. The effects of L-55 groups were almost the same as that of the tranilast-treated group.

Effects on Sneezing As shown in Fig. 3, strain L-55 caused no inhibition of sneezing by single administration (data not shown) and repeated administration for 1 week; however, at doses of 1 and 10 mg/mouse, it caused significant inhibition of sneezing by repeated administration over a period of 2 weeks. Tranilast at a dose of 300 mg/kg also caused significant inhibition of sneezing by repeated administration for 4 weeks.

Effects on the Titration of OVA-Specific IgE IgE titers in the serum were measured by PCA reaction. The results are shown in Fig. 4. After repeated administration for 4 weeks, strain L-55 caused a significant decrease of PCA titers compared with the control group. These results indicate that repeated administration of strain L-55 inhibited the production of antigen-specific IgE in sensitized mice.

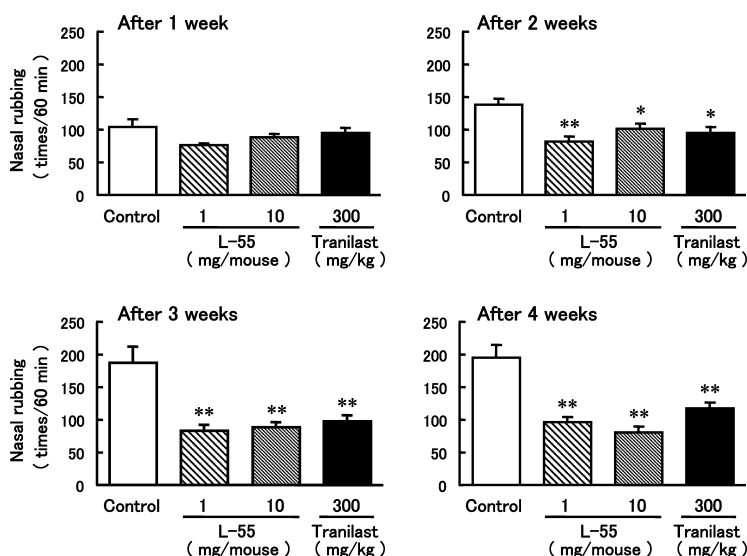


Fig. 2. Effects of Consecutive Administration of Strain L-55 on Nasal Rubbing in Sensitized BALB/c Mice

Strain L-55 and tranilast were administered daily for 4 weeks, and nasal rubbing was observed every week for 60 min. Each column and vertical bar shows the means $\pm$ S.E.M. ($n=6-9$). *, ** Significantly different from the control group at $p<0.05$ and $p<0.01$, respectively.

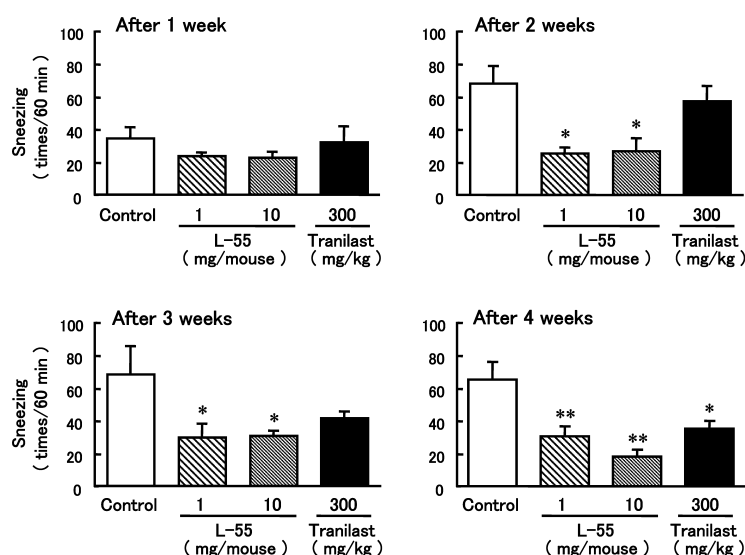


Fig. 3. Effects of Consecutive Administration of Strain L-55 on Sneezing in Sensitized BALB/c Mice

Strain L-55 and tranilast were administrated daily for 4 weeks, and sneezing was observed every week for 60 min. Each column and vertical bar shows the means $\pm$ S.E.M. ($n=6-9$). *, ** Significantly different from the control group at $p<0.05$ and $p<0.01$, respectively.

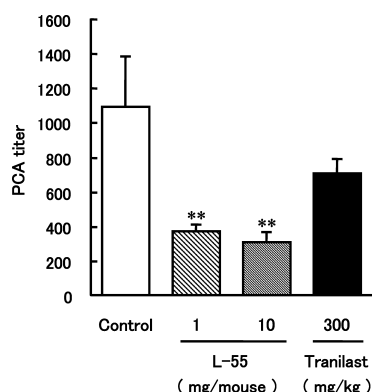


Fig. 4. Effects of Consecutive Administration of Strain L-55 on the Titration of PCA in the Serum

Strain L-55 and tranilast were administrated daily for 4 weeks. Sera were collected on day 53, and the level of OVA-specific IgE was determined by PCA reaction. Each column and vertical bar shows the means $\pm$ S.E.M. ($n=8-10$). ** Significantly different from the control group at $p<0.01$.

DISCUSSION

Recent studies have demonstrated that the oral administration of specific lactic acid bacterial strains could stimulate the immune system.^{13,14} For instance, Fujiwara *et al.*¹⁵ reported that *Lactobacillus paracasei* strain KW3110 inhibited serum IgE elevation in the mouse allergy model. Strain L-55 used in this study had adherent ability to Caco-2 cells derived from human enteric epithelium.¹¹ From these results, we expected that strain L-55 might regulate the immune system; therefore, we studied the effect of strain L-55 on some allergic rhinitis models in BALB/c mice.

In this study, strain L-55 showed no significant inhibition of nasal symptoms (sneezing and nasal rubbing) at doses of 1 and 10 mg/mouse by single administration; however, it has been reported that natural products, such as Brazilian propolis¹⁶ and Lo Han Kuo,¹⁷ exert a stronger anti-allergic effect by consecutive administration; therefore, we studied the effect of strain L-55 on nasal symptoms by repeated administration. As a result, it was found that strain L-55 significantly

inhibited nasal symptoms by repeated administration for 2 or 4 weeks (Figs. 2, 3). From these results, strain L-55 may need repeated administration to modulate the immune system. On the other hand, no clear dose-dependent effect was found with strain L-55 on nasal symptoms. As shown in this study, a dose of 1 mg/mouse seemed enough to inhibit nasal symptoms by repeated administration of 2 weeks.

It goes without saying that the symptoms of allergic rhinitis are caused by activation of mucosal mast cells of the nostril. This activation of mast cells is initiated by binding of antigen to IgE, and the cross-linkage of IgE at the surface of mast cells triggers the release of many chemical mediators, such as histamine, leukotrienes and prostaglandins. Some investigations indicate that histamine caused sneezing and nasal rubbing by its binding to H_1 -receptors on sensory nerve endings.¹⁸⁻²¹ As a matter of course, there are some findings that histamine H_1 -receptor antagonists inhibited the symptoms of sneezing and nasal rubbing of allergic rhinitis model in mice and rats.^{22,23} Moreover, other investigators have shown that the suppression of antigen-specific IgE production inhibited the nasal symptoms of an allergic rhinitis model.²⁴ Because, it is well known that IgE is one of the risk factors for the allergic rhinitis. As shown in this study, strain L-55 inhibited the production of antigen-specific IgE in sensitized mice (Fig. 4). From these findings, therefore, it seems likely that strain L-55 has an anti-allergic effect through the inhibition of antigen-specific IgE production. Although tranilast, an antiallergic drug that inhibits the release of chemical mediators from mast cells, also caused significant inhibition of nasal symptoms, the drug caused no OVA-specific IgE lowering activity. Therefore, it is clear from the present data that the mechanism of an inhibition on allergic rhinitis by strain L-55 was different from that of tranilast.

In the intestinal immune system, two types of helper T cells, classified Th1 cell and Th2 cell, influence the production of IgE. For example, Th1 cells produce interferon- γ which is suppressible cytokine for the production of IgE, but Th2 cells produce interleukin (IL)-4 and IL-5 which are inducible cytokines for the production of IgE. In addition, it is

well known that the disturbance of equilibrium between Th1 cell and Th2 cell causes various immunological diseases such as allergic diseases. It has been reported that some specific lactic acid bacteria strains increase the levels of some cytokines produced by Th1 cells and decrease those of some cytokines produced by Th2 cells and consequently inhibit IgE production.^{15,25)}

The model of allergic rhinitis used in this study is well recognized to be useful for evaluating the efficacy of anti-allergic drugs on allergic rhinitis.¹²⁾ Our results intensively indicated that this model is also available for the evaluation of the anti-allergic effect of lactic acid bacteria.

In conclusion, the data presented in this paper indicate that the oral administration of strain L-55 inhibited the nasal symptoms induced by antigen challenge in a model of allergic rhinitis. Most commonly, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum* and *Bifidobacterium longum* have been used as probiotics in humans. Many species of lactic acid bacteria have no tolerant to acid and can not penetrate through the intestinal tract. However, it is well known that *L. acidophilus* has the ability to survive under acidic conditions. Moreover, *L. acidophilus* strain L-55 has adherent ability to Caco-2 cells derived from human enteric epithelium.¹¹⁾ Therefore, it is expected that strain L-55 is able to penetrate through the human stomach and digestive system, and colonize in the gut. In addition, it seems likely that strain L-55 may modulate the immuno system more effectively than the other strains.¹⁰⁾ Strain L-55 which was isolated from healthy infant feces and used for the fermentation of dairy products is cheap and safe, and has no severe adverse effects. From above findings, it may be useful as not only pharmaceutical applications but also a preventative diet against allergic rhinitis.

REFERENCES

- Fuller R., *J. Appl. Bacteriol.*, **66**, 365—378 (1989).
- Haller D., Blum S., Bode C., Hammer W. P., Schiffrin E. J., *Infect. Immun.*, **68**, 752—759 (2000).
- Meydani S. N., Ha W.-K., *Am. J. Clin. Nutr.*, **71**, 861—872 (2000).
- Ishida Y., Nakamura F., Kanzato H., Sawada D., Hirata H., Nishimura A., Kajimoto O., Fujiwara S., *J. Dairy Sci.*, **88**, 527—533 (2005).
- Peng G. C., Hsu C. H., *Pediatr. Allergy Immunol.*, **16**, 433—438 (2005).
- Wang M. F., Lin H. C., Wang Y. Y., Hsu C. H., *Pediatr. Allergy Immunol.*, **15**, 152—158 (2004).
- Börkstén B., Naaber P., Sepp E., Mikelsaar M., *Clin. Exp. Allergy*, **29**, 342—346 (1999).
- Laiho K., Hoppu U., Ouwehand A. C., Salminen S., Isolauri E., *Br. J. Nutr.*, **88**, S19—S27 (2002).
- Noverr M. C., Huffnagle G. B., *Clin. Exp. Allergy*, **35**, 1511—1520 (2005).
- Schiffrin E. J., Brassart D., Servin A. L., Rochat F., Donnet-Hughes A., *Am. J. Clin. Nutr.*, **66**, 515S—520S (1997).
- Sunada Y., Nakamura S., Tomochika K., *Bacterial Adherence Biofilm*, **17**, 1—5 (2003).
- Takubo M., Inoue T., Jiang S., Tsumuro T., Yatsuzuka R., Segawa S., Watari J., Kamei C., *Biol. Pharm. Bull.*, **29**, 689—692 (2006).
- Bengmark S., *Nutrition*, **14**, 585—594 (1998).
- Dugas B., Mercenier A., Lenior-Wijnkoop I., Arnaud C., Dugas N., Postaire E., *Immunol. Today*, **20**, 387—390 (1999).
- Fujiwara D., Inoue S., Wakabayashi H., Fujii T., *Int. Arch. Allergy Immunol.*, **135**, 205—215 (2004).
- Shinmei Y., Hossen M. A., Okihara K., Sugimoto H., Yamada H., Kamei C., *Int. Immunopharmacol.*, **4**, 1431—1436 (2004).
- Hossen M. A., Shinmei Y., Juang S., Takubo M., Tsumuro T., Murata Y., Sugiura M., Kamei C., *Biol. Pharm. Bull.*, **28**, 238—241 (2005).
- Ohtsuka H., Okuda M., *Arch. Otorhinolaryngol.*, **233**, 227—235 (1981).
- Raphael G. D., Meredith S. D., Baraniuk J. N., Druce H. M., Banks S. M., Kaliner M. A., *Am. Rev. Respir. Dis.*, **139**, 791—800 (1989).
- Shelton D., Eiser N., *Clin. Otolaryngol. Allied. Sci.*, **19**, 45—49 (1989).
- Wang D., Smits J., Waterschoot S., Clement P., *Allergy*, **52**, 162—167 (1997).
- Kayasuga R., Sugimoto Y., Watanabe T., Kamei C., *Int. Immunopharmacol.*, **2**, 745—750 (2002).
- Sugimoto Y., Kawamoto E., Chen Z., Kamei C., *Immunopharmacology*, **48**, 1—7 (2000).
- Tsumuro T., Ogawa M., Minami K., Takubo M., Rahman A., Fujii Y., Kamei C., *Eur. J. Pharmacol.*, **524**, 155—158 (2005).
- Matsuzaki T., Yamazaki R., Hashimoto S., Yokokura T., *J. Dairy Sci.*, **81**, 48—53 (1998).