

Orally Administrated *Lactobacillus gasseri* TMC0356 and *Lactobacillus* GG Alleviated Nasal Blockage of Guinea Pig with Allergic Rhinitis

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Abstract: *Lactobacillus* GG (LGG) and *L. gasseri* TMC0356 (TMC0356) were investigated for their ability to alleviate nasal blockage associated with allergic rhinitis using a guinea pig model. The increases in sRaw at 10 min and 5 hr after the exposure of the nasal mucosa to OVA were significantly alleviated in the guinea pigs orally administrated with LGG and TMC0356 compared with those of the control ($P<0.05$ and $P<0.01$). The total numbers of leukocytes, particularly eosinophils and neutrophils from the nasal cavity lavage fluid, and the OVA-specific IgE concentration in the serum were also decreased in the guinea pigs orally administrated with LGG and TMC0356, although the decreases were not statistically significant. These results suggest that LGG and TMC0356 can alleviate antigen-induced nasal blockage in early-phase and late-phase inflammatory responses associated with allergic rhinitis.

Key words: *Lactobacillus*, Nasal blockage, Allergic rhinitis, Guinea pig

Allergic rhinitis is an inflammation of the nasal mucosa caused by IgE-mediated hypersensitivity to environmental allergens (1, 14, 18). The occurrence of allergic rhinitis, particularly seasonal allergic rhinitis caused by two kinds of major allergens (i.e., Cry j 1, Cry j 2) in the Japanese cedar (*Cryptomeria japonica*) pollen, is becoming a serious problem in terms of public health in Japan (21, 27). Allergic rhinitis can lead to a marked reduction in the quality of life of both patients and their families, which negatively affects work productivity, school performance and social activities.

Although the specific mechanism behind the epidemiological increase in the incidence of allergy such as allergic rhinitis still remains unclear, a very hygienic environment with low microbial load and few fecal and oral contaminants might provide less stimulation to the gut immunity system, favoring allergy-prone immunity (20, 25, 26). Accumulating scientific lines of evidence indicate that abnormal intestinal microbes might be one of the important causes of the pathology of various allergic diseases (2–4, 7, 10). Therefore, the intestinal microbiota has been considered as one of the prospective targets in the management of allergic disorders including allergic rhinitis. Probiotic bacteria can offer a

safe and practical means of modulating the structure and function of the human intestinal microbiota. There has been much interest in the potential of using probiotic bacteria for treating allergic diseases (6, 10, 22, 23). Lactobacilli have received considerable attention as probiotics. Emerging lines of evidence from recent clinical and animal studies have supported the idea that lactobacilli, particularly some selected strains, can modify the immune responses of the host by which these bacteria are believed to have beneficial effects against allergic diseases (5, 7, 8). This study aimed to obtain additional lines of evidence of the beneficial effects of lactobacilli against allergic rhinitis and the underlying mechanisms for such effects.

Lactobacillus GG (ATCC 53103) (LGG) was supplied by Valio (Helsinki, Finland). *L. gasseri* TMC0356 (TMC0356) was stored in the Technical Research Laboratory of Takanashi Milk Products Co., Ltd. (Yokohama, Japan). Lactobacilli were routinely cultured at 37 °C for 18 hr in MRS broth (Becton Dickinson, Sparks, Md., U.S.A.). After incubation, the bacteria were collected by centrifugation (7,000×g), and washed three times with sterilized 0.85% NaCl. The washed bacteria were lyophilized and kept at –80 °C until use.

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Abbreviations: CFU, colony-forming unit; IFN, interferon; Ig, immunoglobulin; IL, interleukin; OVA, ovalbumin; PBS, phosphate-buffered saline.

Five-week-old male Hartley strain guinea pigs (body weight, 304–327 g) were purchased from Japan SLC (Hamamatsu, Japan). They were kept in individual stainless cages on a 12-hr light-dark cycle, and were allowed tap water *ad libitum*. Temperature and humidity were controlled at 22 ± 3 C and $50 \pm 20\%$, respectively. The guinea pigs were initially given a standard diet (RC4, Oriental Yeast, Tokyo) for 7 days, and were divided into two groups (control group and lactobacilli group) on the basis of their mean body weight.

Each group was given either the control diet (RC4) or a lactobacilli diet (control diet containing 0.2% lyophilized LGG cells and 0.2% lyophilized TMC0356 cells) for 3 weeks. The number of viable cells of the tested bacteria in the lactobacilli diet was $<10^3$ CFU/g, because of the heat treatment for the pelletization of the diet. On day 7 after grouping the animals, the guinea pigs were subcutaneously injected with 1 mg of OVA (Sigma Grade V; Sigma Chemical, St. Louis, Mo., U.S.A.) and 10 mg of Aluminum hydroxide Gel (Alum) (Cosmo Bio, Tokyo) in sterilized 0.85% NaCl in a total volume of 1 ml. On day 14, 20 μ l of 10 mg/ml OVA in saline was administrated into both anterior nares of the guinea pigs.

Seven days after the second immunization with OVA, nasal blockage was induced by the administration of 10 μ l of 20 mg/ml OVA in saline into both anterior nares of the guinea pigs. Before OVA administration and at some points after the administration, specific airway resistance (sRaw) was measured using a two-chambered double-flow plethysmograph system according to the method of Pennock et al. (15). sRaw was used to indicate the degree of nasal blockage in sensitized guinea pigs (13). In brief, an unanesthetized guinea pig was placed in a two-chambered box with its neck extending through the partition of the two-chambered box, and sRaw was measured using the noninvasive airway resistance data analyzer Pulmos-I (M.I.P.S., Osaka, Japan). The mean of 100 consecutive respiration data was determined as the measurement at each time point. The increase in the ratio of sRaw was calculated by the following formula:

% of increase in sRaw = $[(A - B) / B] \times 100$. *A* is the sRaw at each time point and *B* is the sRaw before induction.

All animal experiments were performed in accordance with the Guidelines for the Use and Care of Laboratory Animals (The Prime Minister's Office, Announcement No. 6, Japan, 1980).

For the determination of the number of inflammatory cells, nasal cavity lavage fluid (NCLF) was collected. The guinea pigs were anesthetized by the intraperitoneal injection of sodium pentobarbital (32 mg/kg

body weight) and fixed in a supine position. A cannula was inserted into the trachea on the lung side for the maintenance of breathing. The esophagus was ligated, and the oral cavity was filled with a petrolatum-soaked absorbent cotton. A second cannula was inserted into the trachea in the posterior part of the nasal cavity to allow perfusion of the nasal mucosa. The nasal mucosa was perfused with physiologic saline from the second cannula at a rate of 1.0 ml/min. The perfusate was collected for 10 min and centrifuged ($240 \times g$, 10 min, 4 C). The cell pellet was suspended with 1.0 ml of physiologic saline. For determining the number of total leukocytes, a portion of the cell suspension was stained with Turk's solution followed by counting of the total number of leukocytes under a light microscope using a hemocytometer. A portion of the cell suspension was also stained with Wright-Giemsa's staining solution and 100 leukocytes were microscopically counted and classified into eosinophil, neutrophil, lymphocyte, and monocyte/macrophage.

The guinea pigs were bled from the abdominal aorta after the collection of NCLF. Serum samples were obtained after incubation at room temperature and centrifugation at $1,820 \times g$ for 15 min, and the serum samples were stored at -80 C until immunoglobulin analysis. Serum total IgE was determined using a commercial ELISA kit (Guinea Pig OVA-IgE Test kit, Dainippon Sumitomo Pharma, Osaka, Japan). To determine serum OVA-specific IgG1 and IgG2 titers, diluted samples were added to microtiter plates previously coated with OVA, and were incubated for 2 hr at room temperature. After washing three times with PBS containing 0.05% Tween 20, goat anti-guinea pig IgG1 and IgG2 conjugated to HRP (Serotec, Ltd., Oxford, U.K.) were added to the wells, and incubated for 1 hr at room temperature. After washing six times, tetramethylbenzidine (TMB) substrate solution was added to the plate and incubated for 15 min at room temperature. After adding 2 N H_2SO_4 to the wells, antibody titer was read using a microtiter plate reader, and OVA-specific IgG1 and IgG2 titers were expressed as the absorbance at 450 nm. The statistical significance of the differences between the groups was determined using an unpaired Student's *t*-test or Welch's *t*-test after analysis using the *F*-test.

There were no significant differences in the level of food intake or body weight between the control group and the lactobacilli group (Data not shown). No abnormalities in appearance or other clinical signs were observed in any of the tested guinea pigs. The sRaw (cmH₂O·sec, $M \pm SE$) before of OVA administration in the control and the lactobacilli group was 1.10 ± 0.05 and 1.16 ± 0.05 , respectively, and the difference was not

statistically significant. The topical administration of OVA to anterior nares of the sensitized guinea pigs induced an immediate increase in the sRaw after 10 min in the early phase (Fig. 1). The high sRaw decreased after 30 min of the OVA administration. The sRaw in the lactobacilli group was significantly lower than that in the control group after 10 min of the OVA administration ($P<0.05$). The sRaw of the control group increased again after 4 and 5 hr, and decreased after 6 hr of the OVA administration in the late phase. The same increase of sRaw was not observed in the guinea pigs fed with lactobacilli. The sRaw was significantly different between the guinea pigs fed with lactobacilli and those fed with control diet after 5 hr of the OVA administration ($P<0.01$). AUC_{4-6hr} based on the area under the response curve (AUC) for sRaw from 4 to 6 hr in the lactobacilli group was also significantly

lower than that in the control group (Data not shown).

The numbers of inflammatory cells from the NCLF sampled from the guinea pigs after sRaw measurement are shown in Fig. 2. The number of eosinophils was the highest among the different leukocytes (WBCs). The number of eosinophils in the lactobacilli group was lower than that of the control. However such difference was not statistically significant. Serum OVA-specific IgE, IgG1 and IgG2 titers in the serum samples collected from the tested guinea pigs after sRaw measurement were analyzed (Fig. 3). The serum OVA-specific IgE titer of the tested guinea pigs fed with lactobacilli was lower than that of the control group. However, such difference was not statistically significant. No significant differences were found in serum OVA-specific IgG1 or IgG2 titers between the control and lactobacillus groups.

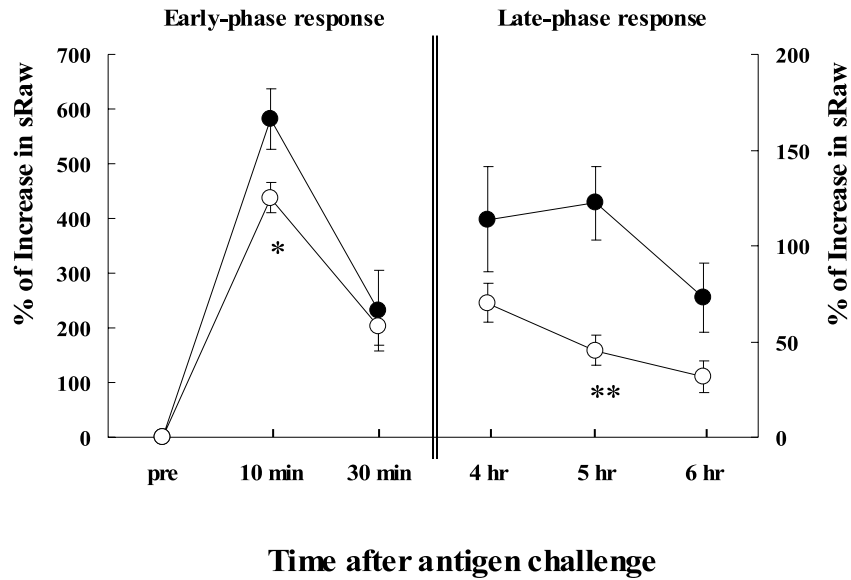


Fig. 1. Effects of lactobacilli on antigen-induced specific airway resistances (sRaw) in sensitized guinea pigs. Closed and open circles represent control and lactobacilli groups, respectively. Data are expressed as mean $\pm$ SEM ($n=11$). An asterisk indicates a significant difference from control group (** $P<0.01$, * $P<0.05$).

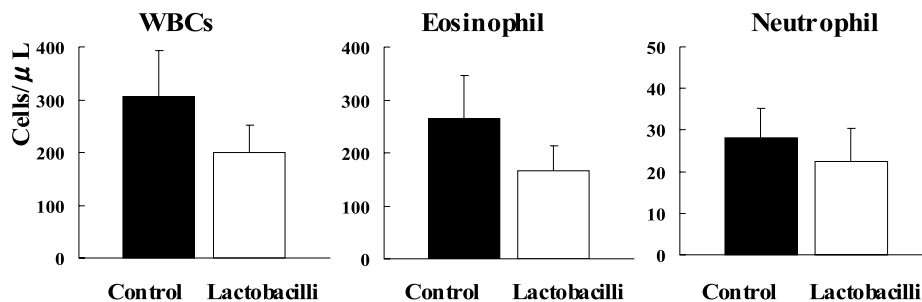


Fig. 2. Effects of lactobacilli on numbers of inflammatory cells from nasal cavity lavage fluid (NCLF) sampled from sensitized guinea pigs. Data are expressed as mean $\pm$ SEM ($n=11$). No significant difference was found between the control and lactobacilli groups.

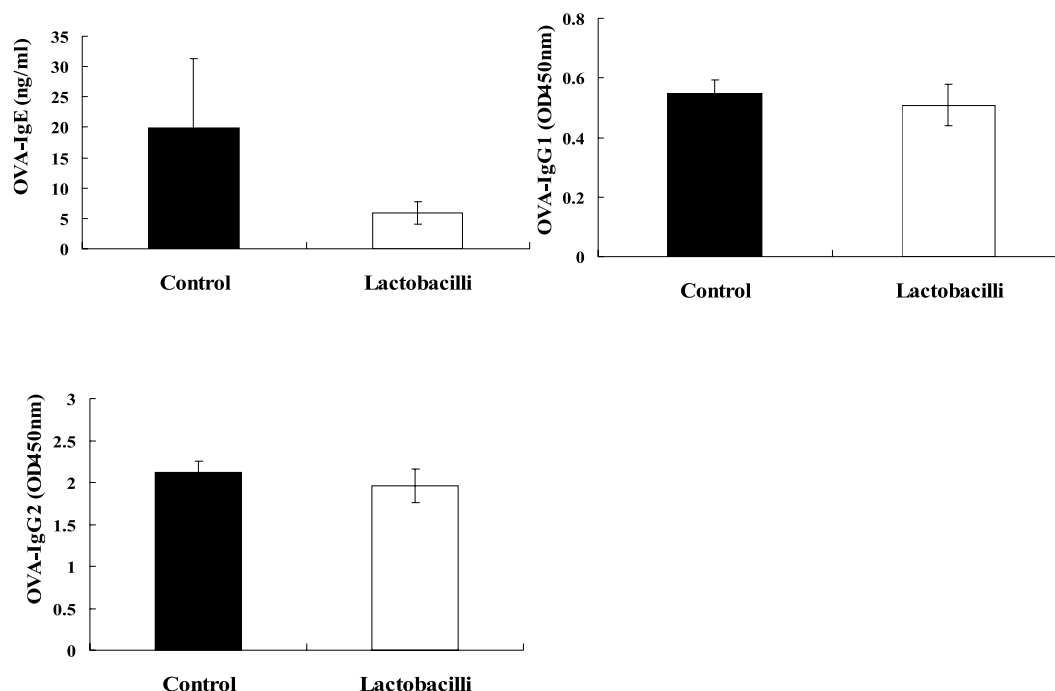


Fig. 3. Effects of lactobacilli on serum ovalbumin (OVA)-specific IgE, IgG1 and IgG2 titers in sensitized guinea pigs. Data are expressed as mean $\pm$ SEM ($n=11$). No significant difference was found between the control and lactobacilli groups.

The immune response associated with allergic rhinitis can be classified as immediate or early-phase response and late-phase response (1, 14, 18). The former is mediated by the actions of released preformed chemical mediators such as histamine after the IgE-dependent activation of nasal mucosal mast cells and other inflammatory cells. The latter is induced by the infiltration of eosinophils, neutrophils, basophils, T lymphocytes and macrophages into the nasal mucosa. The inflammatory mediators derived from these cells induce chronic allergic diseases. Nasal blockage is one of the main symptoms in both the early and late phases of inflammation associated with allergic rhinitis. Sneezing and watery rhinorrhea can be suppressed by antihistaminics, the widely used antiallergic drugs. However, nasal blockage is not relieved by these antiallergic drugs and can only be relieved by α -adrenoceptor stimulatory decongestants and corticosteroids, which unfortunately induce tachyphylaxis when repeatedly used over a short period and other well-known side effects, respectively (13). Therefore, a novel treatment for nasal blockage associated with allergic rhinitis is needed in the management of allergic rhinitis.

In this study, LGG and TMC0356 were examined for their effects against nasal blockage associated with allergic rhinitis using a well established analysis system (12, 13). In this analysis system, the degree of nasal blockage of antigen-sensitized guinea pigs can be mea-

sured quantitatively using a two-chambered double-flow plethysmograph system by detecting nasal airway resistance in unanesthetized guinea pigs (15). This animal model has been well used to analyze the pathological events involved in nasal blockage associated with allergic rhinitis and to test the efficacy of candidate chemical agents as rhinitis drugs (12, 13, 15). The oral administration of LGG and TMC0356 significantly improved the antigen-induced nasal blockage of the tested guinea pigs with allergic rhinitis. This is, to the best of our knowledge, the first study to demonstrate quantitatively and qualitatively the effects of lactobacilli on alleviating nasal blockage associated with allergic rhinitis. These results indicate that the alleviation of the characteristic nasal blockage in allergic rhinitis might be one of the underlying mechanisms behind the possible benefits of lactobacilli as observed in several clinical studies (5, 11), although such effect of lactobacilli may be different for each strain.

In contrast to the clear results of the nasal blockage experiments, oral administration of LGG and TMC0356 did not significantly change the titers of serum OVA-specific IgG1, IgG2 and IgE or the numbers of inflammatory cells from NCLF, although LGG and TMC0356 modified some immune parameters of host animals in other animal and human studies (8, 9, 11, 16, 17, 19, 24). Therefore, it still remains unclear how each of the ingested bacteria implicated and whether

the combination of these bacteria synergistically influenced the immunity of host animal by which nasal blockage associated with allergic rhinitis in this animal model is alleviated. Considering the complexity of the immunity related to the nasal blockage, the test parameters used in this study might not be appropriate enough to monitor or trace the immune actions of LGG and TMC0356 against the nasal blockage, and there might be more underlying mechanisms by which these strains implicate the immunity of host animals. Therefore, new immunological parameters and test models are still necessary to gain an insight into cross reactions between these bacteria and the immunity of host animals, and get a complete picture of the immune modulatory effects of probiotic strains.

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