Inhibitory Effect of *Lactobacillus gasseri* TMC0356 and *Lactobacillus* GG on Enhanced Vascular Permeability of Nasal Mucosa in Experimental Allergic Rhinitis of Rats

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**Note**

The nasal vascular permeability of ovalbumin (OVA)-sensitized Brown Norway rats was evaluated by analyzing a brilliant blue concentration in perfusate from the nose after exposure of the nasal mucus to OVA. Oral administration of *Lactobacillus* GG and *L. gasseri* TMC0356 significantly inhibited the increase in nasal vascular permeability (*P* < 0.01). The serum IgE of the tested rats also decreased, although the change was not statistically significant. These results indicate that *Lactobacillus* GG and *L. gasseri* TMC0356 might alleviate nasal allergic symptoms by suppressing the increase in nasal vascular permeability caused by local inflammation associated with allergic rhinitis.

**Key words:** *Lactobacillus*; probiotic; allergic rhinitis; vascular permeability

Allergic rhinitis is an inflammation of the nasal mucosa caused by IgE-mediated hypersensitivity to environmental allergens. There are two types of allergic rhinitis, seasonal allergic rhinitis (more commonly referred to as hay fever) and perennial allergic rhinitis, which occurs year-round. There has been enormous interest in the search for alternative solutions in the treatment and prevention of allergic rhinitis, which significantly decreases the quality of life and impairs social and work function.

Probiotics are defined as microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host. Recently, selected probiotic strains altered allergic disease in several well-designed clinical studies while they persisted in the human intestinal epithelium. However, the underlying mechanisms by which probiotic strains can influence allergic disease remain unclear. The present study aimed to find additional evidence to support the effect of probiotic lactic acid bacteria on allergic rhinitis in an experimental animal model. In the present study, the rats were fed *Lactobacillus* GG and *L. gasseri* TMC0356 and immunized subcutaneously with food antigen ovalbumin (OVA). The nasal vascular permeability of the tested rats was analyzed after exposure of their nasal mucus to OVA. In parallel, the serum IgE of the tested rats was also tested by the ELISA method.

*Lactobacillus* GG (ATCC 53103) was supplied by Valio (Helsinki, Finland). *L. gasseri* TMC0356 were stored in the Technical Research Laboratory of Takanashi Milk Products Co., Ltd. (Yokohama, Japan). Lactobacilli were routinely cultured at 37°C for 18 h in MRS broth (Becton, Dickinson, Sparks, MD). 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.

Four-week-old male Brown Norway rats were purchased from Charles River, Japan (Yokohama, Japan). They were kept in individual stainless cages on a 12-h light-dark cycle, and were allowed tap water ad libitum. The temperature and humidity were controlled at 22°C ± 2 and 50 ± 20%. The rats were initially given a standard diet (MF, Oriental Yeast, Tokyo) for 7 d, 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 (MF) or a lactobacilli diet (control diet containing 0.1% lyophilized *Lactobacillus* GG cells and 0.1% lyophilized *L. gasseri* TMC0356 cells) for 4 weeks.

At 7 and 14 d after division into the two groups, the rats were subcutaneously injected with 1 mg of OVA (Sigma Grade V; Sigma Chemical, St. Louis, MO) and 10 mg of Aluminum hydroxide Gel (Alum) (Cosmo Bio, Tokyo) in sterilized 0.85% NaCl in a total volume of 1 ml. Two weeks after a second immunization with

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**Abbreviations:** OVA, ovalbumin; IL, interleukin; Ig, immunoglobulin
OVA and Alum, the rats were anesthetized with an i.p. injection of pentobarbital (50 mg/kg) and fixed in a supine position (Fig. 1A). Changes in vascular permeability were measured according to the method described by Kojima et al.,\textsuperscript{10} with modifications.\textsuperscript{11,12} A cannula was inserted into the trachea on the lung side for maintenance of breathing. The other side of the cannula was connected to an artificial respirator (Shinano SN-480-7, Tokyo) at a frequency 60 times/min. The oral cavity was filled with 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. Brilliant Blue 6B (1%, 5 ml/kg) was given i.v. by the jugular vein, and then the nasal mucosa was perfused with either 0.85% NaCl or 0.85% NaCl containing OVA (3%) from the cannula according to this timetable shown in Fig. 1B. The perfusate was collected for 50 min at 10-min intervals, centrifuged (1,800 × g, 10 min) and assayed photochemically (OD 620 nm) for dye concentration. Total dye content was calculated by dye concentration and perfusate content. 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).

The rats were bled from the abdominal aorta after the nasal vascular permeability test. Serum was obtained after incubation in ice-temperature storage and centrifugation at 1,800 × g for 15 min. Serum samples were stored at −80 °C until IgE analysis. Serum total IgE of the tested rats was analyzed with a commercial ELISA kit (Rat IgE Test Kit, Morinaga Institute of Biological Science, Yokohama, Japan). In order to examine the serum OVA-specific IgE titer of the tested rats, biotinylated ovalbumin (US Biological, Swampscott, MA) and HRP-streptavidin conjugate (Zymed Laboratories, San Francisco, CA) were used as the second antibody instead of the enzyme-linked sheep anti-rat IgE antibody in the same commercial ELISA kits. OVA-specific IgE titer was expressed as the absorbance at 450 nm.

The statistical significance of the differences between the groups was calculated using an unpaired Student’s\textsuperscript{t} test or Welch’s\textsuperscript{t} test after calculation by the F-test. There were no significant differences in 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 rats. Topical administration of OVA to the nasal cavity of anesthetized rats resulted in increased nasal vascular permeability (the amounts of dye in the

**Fig. 1.** Scheme and Time Table to Test Nasal Vascular Permeability in Rats.  
A, The anesthetized rat was fixed in a supine position. Two cannulas were inserted into the trachea, which were connected with an artificial respirator and infusion pump. B, The nasal cavity was perfused with either 0.85% NaCl or 0.85% NaCl containing OVA (3%) from the cannula according to this timetable.
perfusate) at 20 min or more after dye administration (Fig. 2A). The increased nasal vascular permeability reached a maximum at 30 min after dye administration. Nasal vascular permeability in the lactobacilli group was significantly lower than in the control group at 20 min \( (P < 0.01) \). Nasal vascular permeability in the lactobacilli group at 30 min after dye administration was about 50% of that of the control group \( (P < 0.05) \). Nasal vascular permeability continued to be different significantly between the lactobacilli group and control group till 40 min. Figure 2B shows the AUC\(_{0-50\text{ min}}\) (area under the response curve) for nasal vascular permeability from 0 to 50 min. Data are expressed as the mean ± SEM \( (n = 11) \). An asterisk indicates significant difference from control group \( (**P < 0.01, *P < 0.05) \).

Fig. 2. Effect of Lactobacilli on Nasal Vascular Permeability Test. A, Change in nasal vascular permeability from 0 to 50 min. B, AUC\(_{0-50\text{ min}}\) (area under the response curve) for nasal vascular permeability from 0 to 50 min. Data are expressed as the mean ± SEM \( (n = 11) \). An asterisk indicates significant difference from control group \( (**P < 0.01, *P < 0.05) \).

The immune response associated with allergic rhinitis can be divided into the immediate or early-phase response and the late-phase response\(^{1,2,13}\). Sneezing, increased secretions and nasal blockage are characteristic symptoms during the early phase reaction, mediated by the action of released preformed chemical mediators such as histamine after IgE-dependent activation of nasal mucosal mast cells and other inflammatory cells. As a consequence of the early inflammatory responses involved in allergic rhinitis, enhanced nasal vascular permeability plays a critical role in the development of the early and late symptoms of allergic rhinitis. Therefore, the vascular permeability of nasal mucosa is among the prospective targets in the management of allergic rhinitis.

In the present study, two probiotic strains, *Lactobacilli*...
*Lactobacillus* GG and *L. gasseri* TMC0356, were tested for their regulatory effects on the enhanced nasal vascular permeability associated with allergic rhinitis using an animal model established for allergic rhinitis.\(^{10-12}\) As for the results, oral administration of *Lactobacillus* GG and *L. gasseri* TMC0356 significantly inhibited the increase in nasal vascular permeability in the experimental allergic rhinitis in the rats. To our knowledge, this is the first study to demonstrate the oral activity of lactic acid bacteria in suppressing antigen-stimulated vascular permeability of the nasal mucosa in a model of allergic rhinitis. The results indicate that the alleviation of allergic rhinitis by some specific lactic acid bacteria can be attributed to their characteristic implication in the aberrant nasal vascular permeability in the related inflammation, although the effects of lactic acid bacteria might be different according to the each strain.

*Lactobacillus* GG and *L. gasseri* TMC0356 were selected in view of potent anti-allergy effects tested in animal experiments, cell cultures in mice, and in human tests, as described below. *Lactobacillus* GG is a well-known probiotic strain widely used in the dairy industry. In a four-year follow up clinical study, this probiotic bacterium effectively protected infants with a genetically high risk for allergic diseases from development of atopic disease.\(^5,6\) Such protection by *Lactobacillus* GG may, at least partly, be attributed to enhancement of the production of an anti-inflammatory cytokine IL-10, TGF-\(\beta\).\(^{14,15}\) However, the apparent effect of *Lactobacillus* GG in altering allergic rhinitis was not observed in a clinical study.\(^{16}\) *L. gasseri* TMC0356 was originally isolated from healthy adult intestine.\(^{17}\) This bacterium can induce secretion of pro-inflammatory (IL-12) and anti-inflammatory (IL-10) cytokines from murine mac-

**Fig. 3.** Effect of Lactobacilli on Serum IgE Level in Rats.

A, Total IgE; B, OVA-specific IgE. Data are expressed as the mean \(\pm\) SEM (n = 11). No significant difference was found between the control and the lactobacilli group.
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rophere (J774.1). L. gasseri TMC3056 may be able to modulate the immune response either by preventing or by intensifying inflammation, depending on the particular case. Furthermore, this bacterium effectively inhibited antigen-augmented serum IgE in tested BALB/c mice immunized intraperitoneally with the food antigen OVA (M. Kawase, unpublished results) and in human subjects with perennial allergic rhinitis. In a previous study, chromosomal DNA from both Lactobacillus GG and L. gasseri TMC3056 was found to be a potent stimulator of the immune responses of host animals, although those from Lactobacillus GG had stronger effects. The combination of Lactobacillus GG and L. gasseri TMC3056 may favor Th1-type immunity of regulatory T cells and to induce greater proliferation of regulatory T cells by the tested bacteria-modified DCs in the gut tract is one possible way in which the tested bacteria can alleviate local inflammation and subsequently exhibit a decrease in the vascular permeability of nasal mucosa-associated rhinitis. In fact, Lactobacillus GG was found to enhance the proliferation of CD4+CD25+ and to induce greater production of IL-10 and TGF-β. The development of regulatory T cells is heavily influenced by the type and maturation state of dendritic cells (DCs), antigen-presenting cells (APC) distributed in the epithelium of the gut. DCs are characteristically activated by lactobacilli, which might manipulate the T cells mediated immune responses of the host. Therefore, enhanced proliferation of regulatory T cells by the tested bacteria-modified DCs in the gut tract is one possible way in which the tested bacteria can alleviate local inflammation and subsequently exhibit a decrease in the vascular permeability of nasal mucosa-associated rhinitis. In the present study, the increase in the vascular permeability of nasal mucosa associated with allergic rhinitis in experimental rats was significantly inhibited, but the decrease in serum IgE of the tested rats was not significant. These results indicate that the inhibition of enhanced vascular permeability of the nasal mucosa associated with allergic rhinitis is due not only to a decrease in serum IgE, but to other upper-grade regulatory mechanisms that can also contribute to these inhibitory effects. These results are in good agreement with previous human studies with Lactobacillus GG and other probiotic strains, in which these strains significantly improved the symptoms of allergic diseases but did not greatly alter serum IgE. Suplatast tosilate (IPD-1151T) is an anti-allergic agent that inhibits T-cell synthesis of IL-4 and IL-5 in both human and murine Th2 cells. IPD-1151T also inhibits the growth of mast cells and the release of chemical mediates from mast cells.Lactobacillus GG and L. gasseri TMC3056 inhibited production of IL-4 and IL-5 in both human peripheral blood mononuclear and murine splenocytes co-cultured with antigen (M. Kawase, unpublished results). Therefore, there may be a possibility that the tested bacteria can directly reduce the release of inflammatory agents from activated mast cells. Further studies should be conducted to obtain evidence for this.

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