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Oral probiotic bacterial administration suppressed allergic responses in an ovalbumin-induced allergy mouse model

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Abstract

This study investigated whether orally administered probiotic bacteria (*Bifidobacterium bifidum* and *Lactobacillus casei*) and a gram-negative bacterium (*Escherichia coli*) function as allergic immune modulators to prevent food allergy, according to the hygiene hypothesis. C3H/HeJ mice were sensitized with ovalbumin (OVA) and cholera toxin for 5 weeks. After sensitization, the OVA-induced mice that were not treated with bacteria had significantly increased levels of OVA-specific IgE, total IgE, and IgG1 in sera, as well as scab-covered tails. In comparison, groups treated with *B. bifidum* BGN4 (BGN4), *L. casei* 911 (*L. casei*), or *Escherichia coli* MC4100 (*E. coli*) had decreased levels of OVA-specific IgE, total IgE, and IgG1, and decreased levels of mast cell degranulation and tail scabs. OVA-specific IgA levels were decreased in BGN4- and *L. casei*-treated groups. In conclusion, administration of *E. coli*, BGN4, or *L. casei* decreased the OVA-induced allergy response. However, a normal increase in body weight was inhibited in the *E. coli*-treated mice and in the montreated mice groups during allergy sensitization. Thus, BGN4 and *L. casei* appear to be useful probiotic bacteria for the prevention of allergy.

Keywords: Allergy; *Bifidobacterium*; *Escherichia coli*; *Lactobacillus casei*; Immunoglobulin E

1. Introduction

The prevalence of atopic disease has increased rapidly around the world during recent decades [1,2]. Food allergy is not entirely separate from other allergic reactions such as eczema, asthma, and rhinoconjunctivitis. An incidence of 90.5% of food allergy in patients with atopic dermatitis and an increased risk of clinical sensitivity to food in asthmatic patients were reported [3,4].

The so-called hygiene hypothesis attributes the increasing prevalence of atopic diseases [5,6] to disease reduction resulting from vaccinations and improved hygiene in industrialized countries. Specifically, the hygiene hypothesis suggests that modern methods of hygiene and sanitation have decreased children's exposure to certain microbes and negative bacteria. The neonatal and early childhood periods are believed to be critical periods for the establishment of the Th1–Th2 balance. Early infections during these stages are believed to result in a Th1-biased immunity and to prevent the induction of the pro-allergic Th2 system [7]. The intestinal microflora established during infancy may be a source for the induction of immune deviation, and the flora composition may determine whether allergy disorders develop [8]. Children who developed allergy during

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the first two years of life were less often colonized with enterococci, bifidobacteria, and bacteroides, and had higher stool counts of *Staphylococcus aureus* and *Clostridium* in comparison with healthy infants [9].

In an attempt to reverse these possible effects of reduced microbial exposure in early life, probiotics have been administered to infants. Probiotics are traditionally defined as live microbial food supplements that improve intestinal microbial balance [10]. The frequency of atopic eczema in a *Lactobacillus* GG-treated group was half that in a placebo-treated group, suggesting that *Lactobacillus* GG effectively prevented early atopic disease in children at high risk [7]. Another study showed that *Lactobacillus casei* might inhibit antigen-induced IgE production by inducing interleukin-12 (IL-12) secretion by macrophages [11]. Specific microbes in the gastrointestinal of the host may promote potentially anti-allergenic processes through induction of Th1 type immunity [12,13] and/or enhance the production of transforming growth factor-β [14,15], which plays an essential role in the suppression of T-helper 2 cell-induced allergy [10,16]. The splenic production of Th1 cell-associated cytokines, such as interferon-γ and interleukin-2, was higher in mice fed the *L. casei* Shirota strain than in the control group [11]. Natural killer (NK) cells [17] and cytotoxic T-cells [18] were also stimulated by *L. casei*. These results suggest that *L. casei* enhances cellular immunity, in which Th1 cells may play an important role. However, the precise mechanisms by which probiotics inhibit allergy remain unknown.

To compare the utility of probiotic and non-probiotic bacteria for allergy prevention, we investigated the effects of the *Bifidobacterium* strain *Bifidobacterium bifidum* BGN4, the *Lactobacillus* strain *L. casei* 911, and the *Escherichia coli* strain *E. coli* MC4100 in an ovalbumin (OVA)-induced allergy mouse model. Additionally, we investigated mechanisms relating to the immunological effect of suppression on the OVA-induced allergy response. To our knowledge, this is the first report to assess the effect of probiotics in an orally sensitized food allergy animal model. Thus, the method described in the present study is probably better adapted to studying the effect of probiotics on food allergies than previous experimental studies in which antigens were intraperitoneally injected.

2. Materials and methods

2.1. Mice

Three-week-old female C3H/HeJ mice weighing 11–13 g were purchased from Japan SLC (Hamamatsu, Japan) and maintained on OVA-free chow. Mice were sensitized at 5 weeks of age and each group included six mice. Mice were kept in plastic cages, allowed free access to water, and maintained on a 12:12 h light:dark cycle in an environmentally controlled animal chamber. The temperature and humidity were controlled at 23 ± 1 °C and 55 ± 10%, respectively. The animal experimentation guidelines of Seoul National University were followed.

2.2. Microorganisms

B. bifidum BGN4 and *L. casei* 911 were used as they were previously suggested to be promising probiotic strains with regard to anti-cancerogenic activity [19,20] and to have the ability to attach to human epithelial cell lines [21]. *E. coli* MC4100 was used as gram-negative bacterium. *Bifidobacterium* and *Lactobacillus* were anaerobically cultured in Lactobacilli-MRS broth (Difco, Detroit, MI, USA) containing 0.05% L-cysteine (Sigma, St. Louis, MO, USA) at 37 °C for 24 h. The *E. coli* MC4100 strain was cultured aerobically in LB broth (Criterion, CA, USA) at 37 °C for 24 h. To prepare the mouse diets, bacterial cells were collected by Mega 21R centrifuge (Hanil, Seoul, Korea) at 4000*g* for 40 min at 4 °C, and washed twice with sterile phosphate buffer saline. Then the pellets were dried by the FD5508 lyophilizer (Ilshin, Seoul, Korea) and mixed with the mouse diet.

2.3. Intragastric antigen sensitization and treatment

Mice were deprived of diet for 2 h preceding the oral sensitization. Sensitization was performed by intragastric (ig) administration of 50 μg OVA with 10 μg of cholera toxin (CT) on days 0, 1, 2, 7, 21, and 35 using a stainless steel blunt feeding needle. OVA (Sigma, St. Louis, MO, USA) was used as the antigen. CT and concanavalin A (Con A) were purchased from Sigma (St. Louis, MO, USA). Five groups of mice were used in this study (Fig. 1). Mice in groups 2–5 were gavaged with 0.2 ml phosphate-buffer saline solution (PBS, pH 7.2) containing OVA and CT. Mice in the naïve group (group 1) were gavaged with PBS without OVA and CT as a negative control. Mice in groups 2–5 were subjected to the same OVA sensitization. Then mice in groups 3–5 were administered bacterial powder. Mice in group 2 received OVA and CT but no bacteria as a sham control. Bacteria-treated mice were fed 0.2% of lyophilized *B. bifidum* BGN4 (BGN4), *L. casei* 911, or *E. coli* MC4100 via a diet pellet. The concentration of bacteria was tested in a preliminary study, which was not published. Mice were fed the experimental bacterial powders for 7 weeks, starting 2 weeks before the initial sensitization, until they were finally sacrificed. To determine serum antibody responses, tail vein blood was collected weekly after the initial sensitization. Sera were stored at −80 °C.

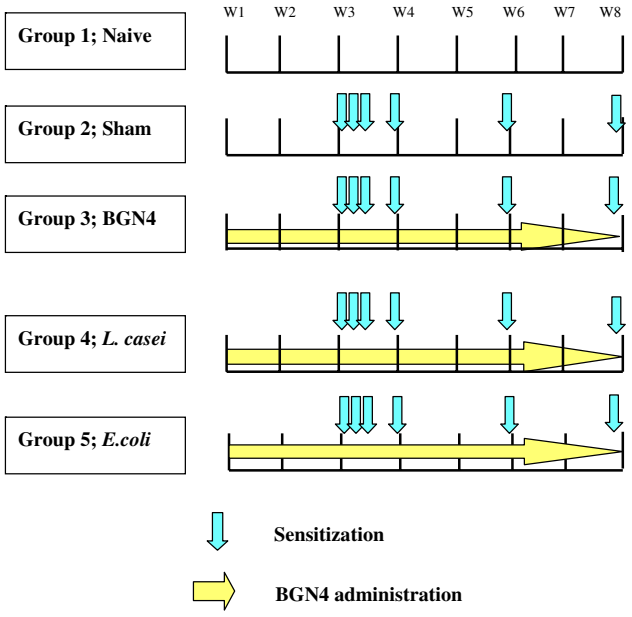

Fig. 1. Experimental protocol: intragastric ovalbumin sensitization and bacteria administration. Mice were sensitized on weeks 3, 4, 6, and 8 with ovalbumin and choleratoxin. Mice in group 3, group 4, and group 5 were fed 0.2% lyophillzed BGN-4, *L. casei*, or *E. coli* in diet pellet for 8 weeks starting 2 weeks before initial sensitization until sacrifice, respectively. The naïve mice in group 1 served as a negative control. Mice in group 2 received PBS buffer instead of ovalbumin and choleratoxin as a sham treatment.

2.4. Measurement of serum OVA-specific IgE, IgG1, IgG2a, total IgE, IgG1, IgG2a, spleen IL-5, IL-13, IgG1, and IgG2a levels

Tail vein blood was obtained weekly following initial sensitization. Sera were collected and stored at −80 °C. Levels of OVA-specific-IgE, IgG1, and IgG2a were measured using an enzyme-linked immunosorbent assay (ELISA). Briefly, Nunc-Immuno-Maxisorp plates (Nunc, Roskilde, Denmark) were coated with 5 μg ml^{-1} of OVA in coating buffer, pH 9.6 (Sigma, St. Louis, MO, USA) overnight at 4 °C. Plates were blocked and washed. Samples were added to the plates and incubated overnight at 4 °C. Plates were washed, and biotinylated rat anti-mouse IgE, IgG1, or IgG2a monoclonal antibodies (2 μg ml^{-1}) were added to the plates for detection of OVA-specific IgE, IgG1, and IgG2a, respectively, for 1 h at room temperature. The reactions were developed with the 3,3′,5,5′-tetramethylbenzidine (TMB) substrate (Fluka, Neu-Ulm, Switzerland) for 30 min at room temperature. The color reactions were stopped with 6 N H_2SO_4 and read at 450 nm. Equivalent levels of IgE, IgG1, or IgG2a were calculated by comparison with a reference curve generated with standards of total mouse IgE, IgG1, or IgG2a, respectively.

Total IgE, total IgG1, and total IgG2a from serum were determined using ELISAs. To detect these antibodies, plates were coated with 2 μg ml^{-1} of rat monoclonal anti-mouse IgE, IgG1, or IgG2a, respectively. Serial dilutions of serum were added and then followed by addition of 100 μl of a biotinylated rat monoclonal anti-mouse IgE, IgG1, or IgG2a. The houseradish peroxidase (HRP)-conjugated streptavidin, described above, was used for detection, and plates were developed with the TMB substrate. IL-5 and IL-13 levels from spleen culture supernatants were detected using ELISA as described above. All of the antibodies used in this study were purchased from Pharmingen (San Diego, CA, USA).

2.5. Measurement of OVA-specific and total fecal IgA

Extracts of fecal pellets were prepared as described by Marinaro et al. [22]. In brief, 100 mg of pellet was mixed with 1 ml of PBS containing 0.1% NaN_3 and incubated at 4 °C for 2 h. Then the pellet was vortexed for 10 min. After centrifugation (4000*g*, 20 min), supernatants were collected and stored at −70 °C. For the assays, plates were coated with 5 μg ml^{-1} of OVA in coating buffer. After washing and blocking, 100 μl of fecal extracts were added to individual wells and incubated overnight at 4 °C. Plates were then washed, and biotinylated rat anti-mouse IgA monoclonal antibodies (2 μg ml^{-1}) were added to the plates and incubated for an additional hour at room temperature. After washing, avidin-peroxidase was added for 1 h at room temperature. The reactions were developed with TMB (Fluka, Neu-Ulm, Switzerland) for 30 min at room temperature. The color reactions were stopped with 6 N H_2SO_4 and read at 450 nm. Equivalent levels of IgA were calculated by comparison with a reference curve generated with a mouse total IgA standard.

For measurement of total IgA, plates were coated with rat anti-mouse IgA capture antibodies (2 μg ml^{-1}) in coating buffer. Plates were then blocked and washed in the manner described above. Fecal extracts (1:50 dilutions) were added to the plates and incubated overnight at 4 °C. Plates were washed and then 100 μl of biotinylated rat anti-mouse IgA were added to each well. Subsequent steps were as described above. IgA levels were calculated from a reference curve generated with a mouse total IgA standard.

2.6. Histology

Mast cell degranulation during food allergy response was assessed by examination of ear and tongue samples collected immediately after sacrifice. Tissues were fixed in 10% neutral buffered formalin, and paraffin sections were stained with toluidine blue (Sigma, St. Louis, MO, USA). Histologic scores were counted in a double-blind manner; observers unaware of sample identities counted the degranulated mast cells in sections

from mouse ears and tongues using light microscopy (100×). Degranulated mast cells were defined as toluidine blue positive cells with five or more distinctly stained granules completely outside of the cells. Each ear sample contained 200–900 mast cells.

2.7. Assessment of hypersensitivity reactions

Allergic symptoms were evaluated after sacrifice utilizing a scoring system: 0, no symptoms; 1, puffiness of the tail; 2, 1–2 scabs on the tail; 3, 3–4 scabs on the tail; 4, 5–6 scabs on the tail; 5, more than 7 scabs on the tail. Scoring of symptoms was performed in a blind manner; scores were evaluated by 10 individuals unaware of sample identities.

2.8. Statistical analysis

All data are presented as the means ± standard error of mean (SEM), indicated by bars in the figures. Data were analyzed using SAS (Release 8.01, O, USA). Differences between immunoglobulin and cytokine levels in the groups were analyzed by ANOVA followed by Duncan's multiple range test for multiple comparisons. p values <0.05 were considered significant.

3. Results

3.1. Effect of BGN4, L. casei, and E. coli on IgE production

To monitor the effects of the three bacterial treatments, sera were obtained from each group of mice every week following OVA sensitization. The OVA-specific IgE levels in sera from each group at week 7, measured by ELISA, are presented in Fig. 2B. All three groups administered 0.2% of BGN4, *L. casei*, or *E. coli* had significantly lower OVA-specific IgE levels than the sham group.

Total serum IgE concentrations were dramatically increased in the sham group at weeks 6 and 7 (Fig. 2A). However, total IgE levels in all three bacterial-treated groups were not increased, and were significantly lower than the sham group at weeks 6 and 7. The total serum IgE levels in the three treated groups were not significantly different at week 7 from the levels in naïve mice (naïve, 295 ± 25 ng ml^{-1}; BGN4, 389 ± 31 ng ml^{-1}; *L. casei*, 333 ± 69 ng ml^{-1}; *E. coli*, 314 ± 78 ng ml^{-1}).

3.2. Allergic symptoms in the tail

Mice sensitized with OVA and CT had numerous tail injuries. After administration of OVA and CT, sham-treated mice started to scratch their tails, resulting in severe injuries and bleeding (Fig. 3A). The severity of OVA-induced allergic reactions in mice was scored (Fig. 3B). The severity of tail injuries was significantly reduced in the BGN4-, *L. casei*-, and *E. coli*-treated groups compared with the sham group. BGN4 and *L. casei* administration, but not *E. coli* administration, reduced the severity of symptoms in ear tissues.

3.3. Mast cell degranulation

Histologic analysis of ear and tongue tissues revealed a significant increase in the number of degranulated mast cells in OVA-immunized mice compared with naïve

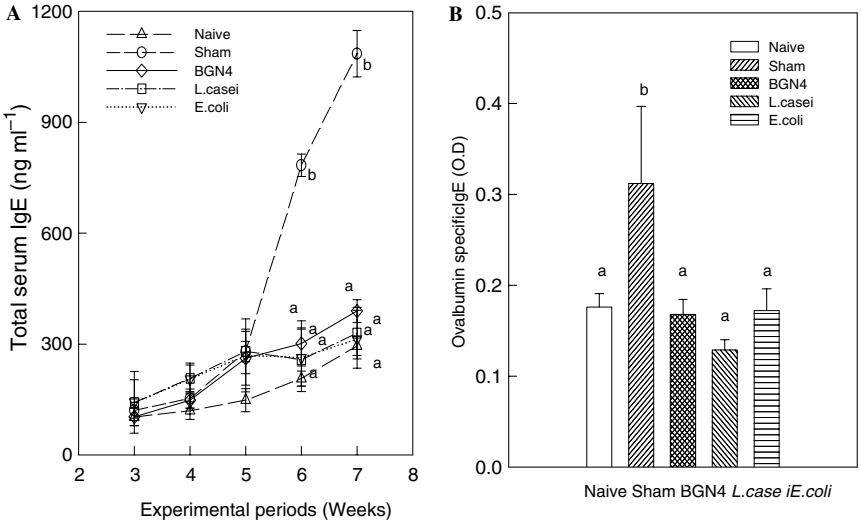

Fig. 2. Effect of bacteria administration on production of total IgE and ovalbumin-specific IgE in serum from ovalbumin-sensitized mice. Sera from all groups of mice were obtained weekly following initial ovalbumin sensitization. IgE levels were determined by ELISAs. Ovalbumin-specific IgE levels were determined at week 7. Data are shown as means ± SEM of six mice per group. Different letters indicate significant differences determined by Duncan's multiple range test ($p < 0.05$).

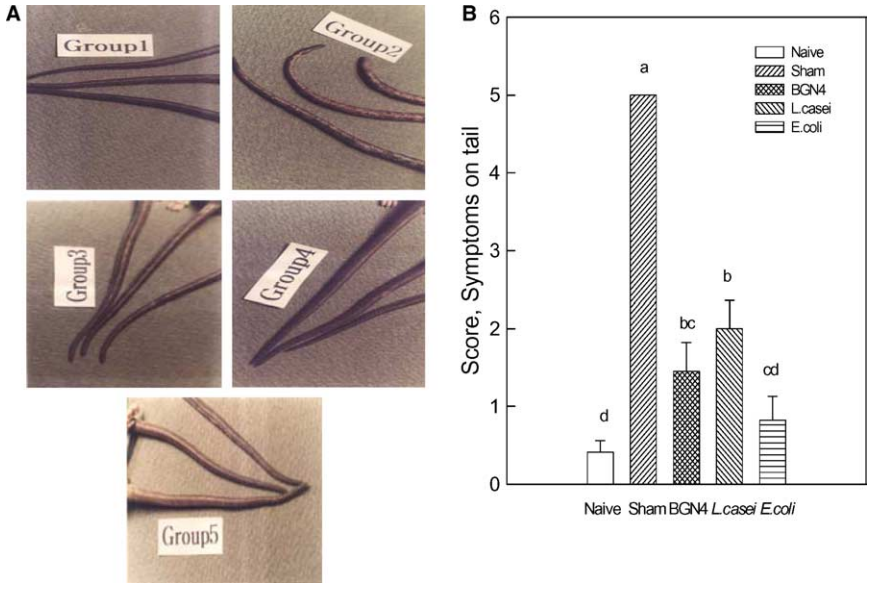

Fig. 3. Severity of allergy symptoms on the tail from ovalbumin-sensitized mice treated with BGN4, *L. casei*, or *E. coli*. Immunized mice showed marked tail bruising and scabs. (A) Pictures of the tail from ovalbumin-sensitized mice treated with bacteria. Group 1, naïve; Group 2, sham; Group 3, BGN4-treated; Group 4, *L. casei*-treated; Group 5, *E. coli*-treated. (B) Severity of allergy symptoms on tail of ovalbumin-sensitized mice was evaluated utilizing a scoring system, and scoring was performed in a blind manner ($p < 0.05$).

mice (Fig. 4). Consistent with the elevated OVA-specific IgE levels in sham-treated mice, the percentage of degranulated mast cells in the sham group was much greater than the percentage in the naïve and bacteria-treated groups. In the ear tissues, BGN4 or *L. casei* administration, but not *E. coli*-administration, reduced the severity of symptoms.

3.4. OVA-specific mucosal IgA

OVA-specific IgA levels in sham-treated mice were more than threefold higher than in BGN4- or *L. casei*-treated animals (Fig. 5). OVA-specific IgA in *E. coli*-treated animals was higher than in BGN4- or *L. casei*-treated animals (naïve, 101 ± 15 μg ml^{-1}; sham,

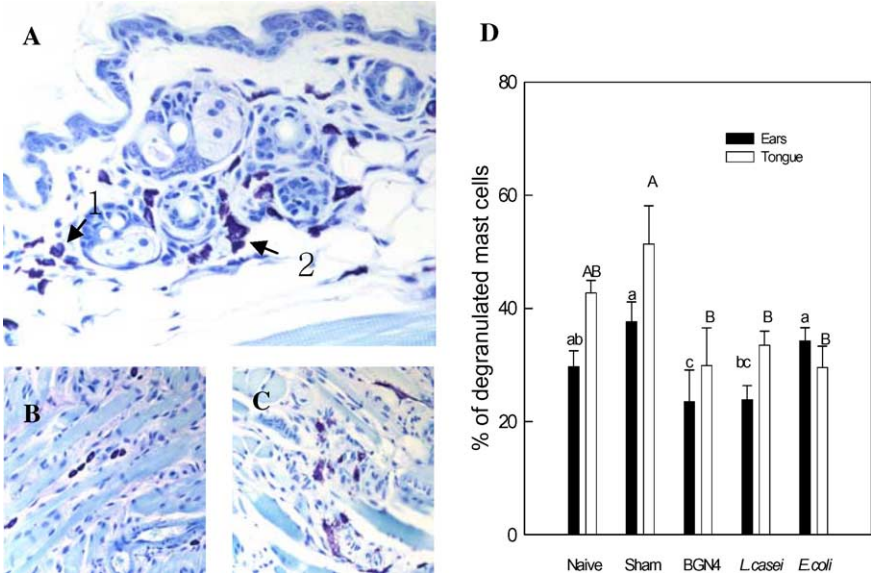

Fig. 4. Effect of bacteria on percentage of mast cell degranulation in ovalbumin-sensitized mouse model. (A) Non-degranulated (arrow 1) and degranulated mast cells (arrow 2) in ear sample. (B) Non-degranulated mast cells in tongue sample. (C) Degranulated mast cells in tongue sample from ovalbumin-induced mice. (D) Percentage of degranulated mast cells in ear and tongue samples. Two to 900 mast cells infiltrated in the tissues were counted, and mast cells with more than five released granules were defined as degranulated cells. Data shown are means ± SEM of six mice per group. Different letters indicate significant differences ($p < 0.05$, in ear and tongue sample).

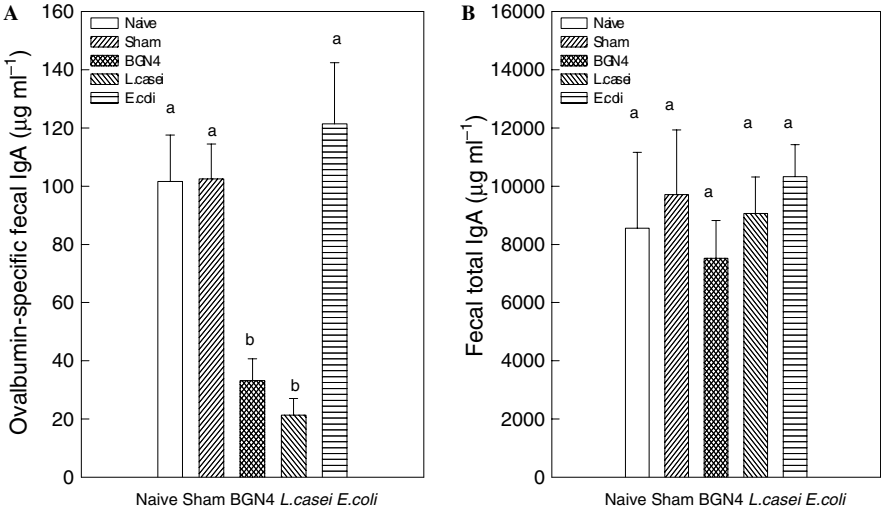

Fig. 5. Effect of bacteria on the production of ovalbumin-specific IgA (A) and total IgA (B) in fecal sample from ovalbumin-sensitized mice and bacteria-treated mice. Fresh fecal pellets from each group were collected. Fecal extracts were prepared and ovalbumin-specific IgA levels were detected by ELISAs. Data shown are means ± SEM of six mice per group. Different letters indicate significant differences ($p < 0.05$).

102 ± 11 μg ml^{-1}; BGN4, 33 ± 7 μg ml^{-1}; *L. casei*, 21 ± 5 μg ml^{-1}; *E. coli*, 121 ± 21 μg ml^{-1}). However, total mucosal IgA levels did not differ significantly among groups.

3.5. Alteration of IgG1 and IgG2a levels in sera

The levels of OVA-specific IgG1 were significantly lower in BGN4- and *L. casei*-treated mice but not in *E. coli*-treated mice compared to sham mice at week 7 (naïve, 1099 ± 495 ng ml^{-1}; sham, 13,183 ± 4223 ng ml^{-1}; BGN4, 5197 ± 1017 ng ml^{-1}; *L. casei*, 3353 ± 517 ng ml^{-1}; *E. coli*, 9531 ± 1811 ng ml^{-1}). The levels of total IgG1 in the BGN4-, *L. casei*-, and *E. coli*-treated groups were significantly lower than in sham mice (naïve, 70 ± 15 μg ml^{-1}; sham, 139 ± 36 μg ml^{-1}; BGN4, 62 ± 5.8 μg ml^{-1}; *L. casei*, 67 ± 15 μg ml^{-1}; *E. coli*, 55 ± 10 μg ml^{-1}). However, OVA-specific IgG2a and total IgG2a levels were not significantly different between the sham and bacteria-treated groups (Fig. 6).

3.6. Effect of BGN4, L. casei, or E. coli on body weight

The initial mean body weights did not differ significantly among the groups (Table 1). Although the body weight of the sham group was greater than that of the naïve group at week 2, the mean body weight of the sham group decreased below that of the naïve group at week 7. On the other hand, the mean body weights of the groups treated with BGN4 or *L. casei* were similar to the mean weights of the naïve group. The mean body weight of the *E. coli*-treated group was consistently lower than that of the naïve group during the experimental period.

4. Discussion

Our modified allergy model was developed from a previously described peanut allergy murine model [23]. The oral sensitization method using OVA as the allergen was employed, since it most closely mimics the route through which human food allergies develop. The normal immune response to dietary proteins is associated with the induction of oral tolerance, and when this active immune suppression is abrogated, adverse reactions to food proteins may arise. Because oral tolerance is obtained on the mucosal site of the gastrointestinal system, studies on food allergy need to be performed by administrating allergens through the intragastric or oral route. Although the immune responses of mouse are not exactly the same as those of human and the mouse model is limited for the scoring of allergic symptoms, murine models provide a useful tool to identify and test new therapeutic strategies, and to expand our knowledge of mechanisms underlying the development of food allergy [24].

Though the precise pathogenic mechanisms involved in food allergy after ingestion of OVA are unknown, the increased OVA-specific IgE production and elevated mast cell degranulation suggest that allergic response was successfully induced, and that activated mast cells might have contributed, at least in part, to the symptoms of food allergy response.

In the present study, BGN4, *L. casei*, and *E. coli*, when administered orally prior to OVA-sensitization, inhibited total IgE production and markedly reduced OVA-specific IgE levels. Additionally, tongue samples from bacteria-treated mice had significantly decreased numbers of degranulated mast cells compared with the sham group. These results demonstrate that bacteria

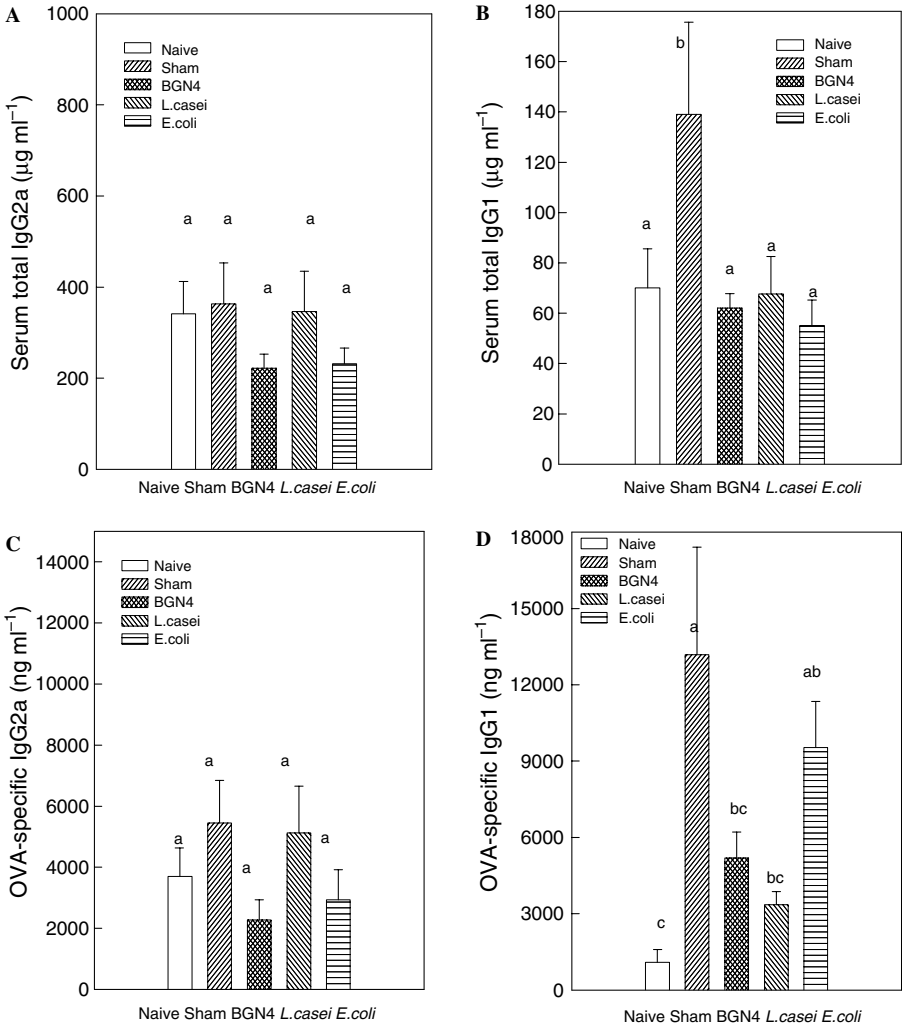

Fig. 6. Effect of bacteria on the production of ovalbumin-specific IgG2a, ovalbumin-specific IgG1, total IgG2a, and total IgG1 in serum from ovalbumin-sensitized mice and bacteria-treated mice. Levels of antibodies were detected by ELISAs. Data shown are means ± SEM of six mice per group. Different letters indicate significant differences ($p < 0.05$).

Table 1
Body weight of mice fed a diet containing (wt/wt) 0.2% of BGN4, *L. casei*, or *E. coli* lyophilized powder per mouse for 7 weeks[A]

Groups	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Naïve	16.4 ± 0.2^{c}	17.8 ± 0.2^{b}	21.6 ± 0.4^{a}	23.1 ± 0.3^{a}	23.3 ± 0.5^{a}	23.8 ± 0.4^{a}
Sham	17.7 ± 0.3^{b}	21.1 ± 0.6^{a}	21.3 ± 0.3^{a}	22.4 ± 0.6^{ab}	21.6 ± 0.4^{ab}	21.6 ± 0.4^{bc}
BGN-4	18.7 ± 0.5^{a}	19.0 ± 0.4^{b}	20.0 ± 0.6^{b}	21.3 ± 0.7^{bc}	22.3 ± 0.9^{a}	22.7 ± 0.9^{ab}
L. casei	18.0 ± 0.3^{ab}	21.0 ± 0.3^{a}	20.5 ± 0.3^{ab}	21.3 ± 0.2^{bc}	21.9 ± 0.6^{ab}	23.2 ± 0.4^{ab}
E. coli	18.0 ± 0.3^{ab}	18.6 ± 0.4^{b}	18.8 ± 0.3^{c}	20.2 ± 0.4^{c}	20.2 ± 0.4^{b}	20.3 ± 1.0^{c}

Values are given as means ± SEM of six mice per group. Different superscripts indicate significant differences ($p < 0.05$).
[A] Experimental diets: naïve, 0.2% cornstarch; sham, 0.2% cornstarch; BGN-4, 0.2% *Bifidobacterium bifidum* BGN-4; *L. casei*, 0.2% *Lactobacillus casei* 911; *E. coli*, 0.2% *Escherichia coli* MC4100.

administration inhibited OVA-specific IgE synthesis and thereby reduced the intensity of allergy symptoms.

Interestingly, tail wounds (clearly induced by oral OVA sensitization in our mouse model) have not been reported in other orally sensitized animal models. Because sera were collected via the tail vein every week, all the mice had tail wounds. Wounds in the naïve group were gradually resolved without treatment, while wounds in the sham group worsened. Just following sensitization with OVA and CT, mice in the sham group, but not the groups treated with BGN4, *L. casei*, or *E. coli*, started scratching their own tails with their teeth, which caused the tail wounds to bleed. Therefore, we concluded that the tail wounds and itching were genuine

OVA-induced allergic responses and that the experimental bacterial treatments prevented these OVA-induced allergic symptoms.

Hessel et al. [25] showed that gram-positive bacteria and gram-negative bacteria produced different cytokines in human peripheral blood cells. The differences in OVA specific fecal IgA levels and OVA specific serum IgG1 levels among the experimental bacteria suggests that these bacteria inhibit allergy responses in different ways.

BGN4 and *L. casei* apparently exerted tight control over Th1 action, and thereby repressed the production of OVA-specific IgE, IgA, and IgG1. Lower levels of IgA in BGN4- and *L. casei*-treated groups might have been partially due to amelioration of CT-induced mucosal stimulation. CT, in combination with IL-4, has been reported to increase the IgG1 response of lipopolysaccharide (LPS)-activated spleen B cells in vitro [26]. Because C3H/HeJ mice are known to be defective in Toll-like-receptor 4 (TLR4) (Toll-like-receptor) and unresponsive to LPS, components other than LPS or mechanisms not directly related to the LPS-TLR4-dependent signaling pathways may play roles in the suppression of IgE and allergy symptoms in the *E. coli*-treated group. Even in experiments that used LPS responsive animal models, the effects of LPS on allergy were not always the same. Park et al. [27] reported that LPS administration prevented development of Th2 responses, pulmonary inflammation, and airway hyperresponsiveness. However, other studies showed that TLR4 might be necessary for the optimal development of Th2 responses rather than Th1 responses [28].

Normal growth in terms of weight was achieved in the mouse groups fed either BGN4 or *L. casei*, but not *E. coli*. Normal absorption of nutrients in the gastro intestinal tract might have been inhibited by the allergy response. The suppression of the allergy response by BGN4 and *L. casei* might have contributed to normal growth, whereas the pathogenic nature of *E. coli* might have inhibited the normal growth of the experimental mice.

In conclusion, the present results demonstrate that oral administration of *Bifidobacterium* or *Lactobacillus* prevents IgE-mediated OVA-hypersensitivity and maintains normal growth of OVA-sensitized mice, and that traditional allergy symptoms were clearly blocked by the bacterial treatments. Additional investigations should help to elucidate the mechanisms involved in OVA-hypersensitivity and identify ways to ameliorate OVA-hypersensitivity.

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