J. Microbiol. Biotechnol. (2008), **18**(8), 1393–1400

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Effect of Oral Probiotics (*Bifidobacterium lactis* AD011 and *Lactobacillus acidophilus* AD031) Administration on Ovalbumin-Induced Food Allergy Mouse Model

Kim, Ji Yeun[1], Young Ok Choi[1], and Geun Eog Ji[1,2]*

[1]*Department of Food and Nutrition, Research Institute of Human Ecology, College of Human Ecology, Seoul National University, Seoul 151-742, Korea*
[2]*Research Institute, Bifido Inc., Hongchon 250-804, Korea*

Received: December 16, 2007 / Accepted: March 7, 2008

Recent study has demonstrated an increasing prevalence of food allergy in Korean children. Specific probiotic bacteria may promote potentially anti-allergenic processes through induction of Th1-type immunity and enhance the regulatory lymphocyte. This study investigated whether orally administrated probiotics could suppress allergic responses in an ovalbumin (OVA)-induced allergy mouse model. Thus, female C3H/HeJ mice were orally sensitized with OVA and cholera toxin for 4 weeks. *Lactobacillus acidophilus* AD031, *Bifidobacterium lactis* AD011, and *L. acidophilus* AD031 plus *B. lactis* AD011 were fed to mice from 2 weeks before the sensitization. The OVA-induced mice that were not treated with probiotics had significantly increased serum levels of OVA-specific IgE and IgG1, and OVA-specific IgA in feces. However, the mice treated with probiotics suppressed production of the OVA-specific IgE, IgG1, and IgA. The level of IL-4 was significantly lower, and the levels of INF-γ and IL-10 were significantly higher in the mice treated with probiotics than that in the non-treated mice. The groups treated with probiotics had decreased levels of degranulated mast cells, eosinophil granules, and tail scabs. These results indicate that *L. acidophilus* AD031 and *B. lactis* AD011 might be useful for the prevention of allergy.

Keywords: Probiotics, food allergy, *Bifidobacterium*, *Lactobacillus*

Probiotics, which are defined as live microorganisms that improve the balance of the intestinal microflora [8], have been reported to benefit several aspects of host physiological responses including immune function [23]. Furthermore, the effects of probiotics have been studied with repect to prevention or treatment of allergic diseases such as atopic dermatitis, allergic rhinitis, and food allergy [19]. Food allergy, which is an adverse immunological hypersensitivity response to food and can be IgE dependent or not [25], may affect children's final height and weight [5]. Food allergies have reached epidemic proportions, and 12.6% of Korean children are affected [17]. The increasing prevalence of allergic diseases has been suggested to be associated with so-called "hygiene hypothesis", in which lack of infections at an early age leads to Th2-dominant immune status later for the development of allergic diseases [3, 6].

There have been several studies to show that oral administration of *Bifidobacterium* or *Lactobacillus* could alleviate the food allergy. Kim *et al.* [12] showed that oral administration of *B. bifidum* BGN4 inhibited serum IgE and IgG1 production in a food allergy mouse model, and *L. casei* Shirota suppressed antigen-specific IgE by stimulating secretion of IL-12 [26]. Spleen cell of the mice administrated with *L. casei* Shirota produced high levels of Th1-associated cytokines, such as IFN-γ and IL-2, but low levels of Th2-associated cytokines such as IL-4, IL-5, IL-6 [15]. Specific probiotic bacteria may potentially promote anti-allergenic processes through maintenance of balance on Th1 and Th2 cytokines [2, 16] and enhancement of regulatory lymphocytes [13, 21]. To support this hypothesis, Torii *et al.* [29] showed that oral administration of *L. acidophilus* L-92 suppressed ovalbumin (OVA)-specific IgE production by regulating Th1 and Th2 cytokines and inducing Treg associated TGF-β production. Additionally, the specific IgE suppressive effect of *B. bifidum* G9-1 was suggested to be mediated by Treg cells independent of IFN-γ production [19].

Previously, most of the studies used intraperitoneal (IP) injection of antigen to induce food allergy. There is a discrepancy between the mechanism of real food allergy

*Corresponding author
Phone: 82-2-880-8749; Fax: 82-2-884-0305;
E-mail: geji@snu.ac.kr

development and IP injection-induced food allergy. IP-injected antigens do not pass through the gastrointestinal tract. Additionally, it is not consistent whether supplementation of probiotics strain is effective for inhibition of allergic sensitization. Supplementation with *L. acidophilus* (LAVRI-A1) resulted in an increased allergic sensitization rate [28]. This implies that different probiotics strains need to be specifically assessd. This study investigated whether orally administrated *Lactobacillus acidophilus* AD031 (*L. acidophilus*), *Bifidobacterium lactis* AD011 (*B. lactis*), and the mixture of these two probiotic bacteria (LB mixture) could suppress allergic symptoms in an orally induced OVA allergy mouse model. Additionally, we investigated the mechanisms related to the immunological effect of suppression on the OVA-induced allergy responses.

Materials and Methods

Mice

Four-week-old female C3H/HeJ mice weighing 12–17 g were purchased from Japan SLC (Hamamatsu, Japan) and maintained on OVA-free chow. Mice were sensitized at 6 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. This experiment was approved by the Institutional Animal Care and Use Committee in Seoul National University.

Microorganisms

B. lactis AD011 was obtained from Research Institute Bifido Co. Ltd. (Hongchungun, Kangwondo, Korea). *B. lactis* AD011 and *L. acidophilus* AD031 were anaerobically cultured in Lactobacilli-MRS broth (Difco, Detroit, MI, U.S.A.), containing 0.05% L-cysteine (Sigma, St. Louis, MO, U.S.A.), at 37°C for 18 h. To prepare mouse diets, bacterial cells were collected by Combi-514R centrifuge (Han il, Seoul, Korea) at 4,000 ×*g* for 30 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 AIN-93G diet.

Intragastric Antigen Sensitization and Treatment

Mice were deprived of diet for 3 h prior to the oral sensitization. Sensitization was performed by intragastric (ig) administration of 50 mg of OVA (Sigma, St. Louis, MO, U.S.A.) with 10 μg of cholera toxin (CT) on days 15, 16, 17, 22, 29, and 36, using a stainless steel blunt feeding needle. CT was purchased from Sigma (St. Louis, MO, U.S.A.). Five groups of mice were used in this study. Mice fed *L. acidophilus*, *B. lactis*, LB mixture, and sham groups were gavaged with 0.2 ml of phosphate-buffer saline solution (PBS, pH 7.2) containing OVA and CT for OVA sensitization. Mice in the naive group were gavaged with PBS without OVA and CT as a negative control. Then, mice in the *L. acidophilus*, *B. lactis*, and LB mixture groups were administered with bacterial powder. Mice in the sham group received OVA and CT but no bacteria, as a control. Bacteria-treated mice were fed 0.2% of lyophilized *L. acidophilus* AD031 (1.5×10^{10} CFU/g), *B. lactis* AD011 (1.0×10^{10} CFU/g), or both of the two bacteria (0.2%, each) *via* a diet pellet. These dosages were based on our preliminary dose-experiment study. 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 just before the initial sensitization. Sera were stored at −80°C.

Measurement of Serum OVA-Specific IgE, IgG1, IgG2a, Spleen IL-4, IL-10, and IFN-γ 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, Immuno-Maxisorp plates (Nunc, Roskilde, Denmark) were coated with 5 μg/ml of OVA in coating buffer, pH 9.6 (1.59 g/l Na_2CO_3, 2.93 g/l $NaHCO_3$) 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) and horseradish peroxidase (HRP)-conjugated streptavidin 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 2 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.

Spleen cells were collected just after the experimental mice were sacrificed. The spleen cells were incubated alone or treated with conA (concanavalinA; Sigma; 10 μg/ml) for 2 days in RPMI1640 (Gibco Life Technologies, U.K.), supplemented with 10% fetal bovine serum (Invitrogen, Paisley, U.K.) and 1% penicillin/streptomycin (Invitrogen), at 37°C in a humidified atmosphere of 5% CO_2. IL-4, IL-10, and IFN-γ levels from spleen cell cultured supernatants were detected using ELISA as described above. All of the antibodies used in this study were purchased from Pharmingen (San Diego, CA, U.S.A.).

Measurement of Fecal Total and OVA-Specific IgA

Extracts of fecal pellets were prepared as described by Marinaro *et al.* [14]. 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 (4,000 ×*g*, 20 min), supernatants were collected and stored at −70°C. For the assays, plates were coated with 2 μg/ml of rat anti-mouse IgA capture antibodies or 5 μg/ml of OVA in coating buffer. After washing and blocking, 100 μl of 1:100 diluted (total IgA) or undiluted (OVA-specific IgA) fecal extracts were added to individual wells and incubated overnight at 4°C. The procedures for the detection of IgA were as described above.

Histology

The mice were sacrificed by cervical dislocation, and the ear and small intestine were taken for histological analysis. Tissues were fixed in 10% formalin in PBS, embedded in paraffin, and cut into 3–5-μm thick sections. The sections were stained with hematoxylin-eosin for general analysis, with toluidine blue for mast cell evaluation, or with Luna's method for eosinophil granule identification. Histological

scores were counted in a double-blind manner; observers unaware of sample identities counted the degranulated mast cells or eosinophil granules in sections using light microscopy. Four fields of toluidine blue stained ear sections were randomly chosen at 400× (871.392 μm^2/field) for mast cell counting. Ten fields were chosen randomly at 400× (μm^2/field) from toluidine blue and Luna's method stained intestine sections in order to count the number of eosinophils and mast cells.

Assessment of Hypersensitivity Reactions

Allergic symptoms were evaluated after mice sacrifice, utilizing a scoring system: 0, no symptoms; 1, puffiness of the tail; 2, 1–3 scabs on the tail; 3, 4–6 scabs on the tail; 4, 7–9 scabs on the tail; 5, more than 9 scabs on the tail; 6, 1–2 blood stains and more than 9 scabs on the tail; 7, more than 2 blood stains and more than 9 scabs on the tail. Scoring of symptoms was performed in a blind manner; scores were evaluated by 10 individuals unaware of sample identities.

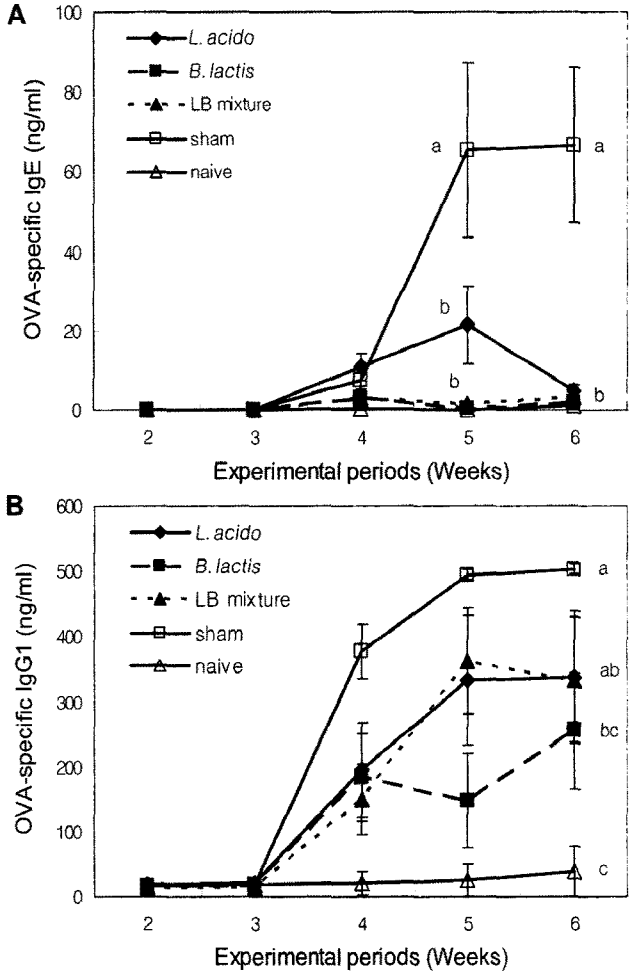

Fig. 1. Effect of probiotics administration on production of OVA-specific IgE and IgG1 in serum from ovalbumin-sensitized mice.

Sera from mice were obtained weekly following initial ovalbumin sensitization. OVA-specific IgE and IgG1 levels were determined by ELISAs. 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).

Statistical Analysis

Data are presented as means±standard error of mean (SEM), indicated by bars in the figures. All statistical analyses were performed using SPSS 12.0 K for Windows (SPSS Inc., Chicago, IL, U.S.A.). Differences between immunoglobulin and cytokine levels in the groups were analyzed by ANOVA followed by Duncan's multiple range test. p Values <0.05 were considered significant.

RESULTS

OVA-Specific IgE and IgG1 Production in Sera

To monitor the effects of supplementation of *L. acidophilus*, *B. lactis*, and LB mixture, sera were obtained from each group of mice every week following OVA sensitization. The OVA-specific IgE levels in sera from each group are presented in Fig. 1A. The OVA-specific IgE levels in the sham group were remarkably increased at the 5th and 6th weeks, whereas those in all of probiotic bacteria treated groups were significantly lower than the sham group (*L. acidophilus*, 4.8±1.7 ng/ml;

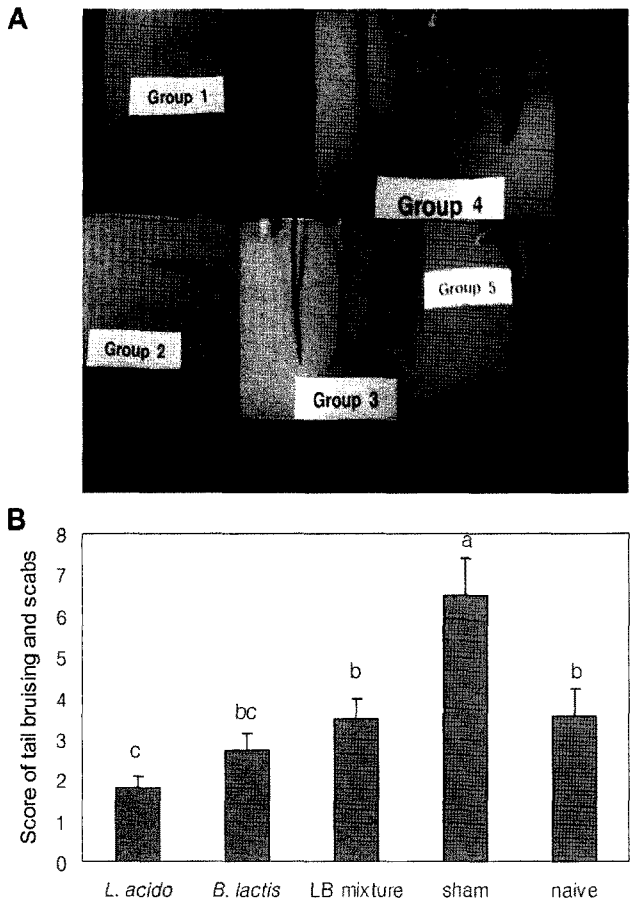

Fig. 2. Severity of allergy symptoms on the tail from OVA-sensitized mice fed *L. acidopilus*, *B. lactis*, or LB mixture.

Tail bruising and scabs were assessed. **A.** Pictures of the tail from experimental mice. Group 1, *L. acidophilus*; Group 2, *B. lactis*; Group 3, LB mixture; Group 4, sham; Group 5, naïve. **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).

B. lactis, 1.9±1.9 ng/ml; LB mixture, 3.3± 2.0 ng/ml; sham, 66.6±19.3 ng/ml; naive, 1.2±1.2 ng/ml at 6th week).

The OVA-specific IgG1 levels in sera were increased from the 4th week in all groups except the naive group (Fig. 1B). The OVA-specific IgG1 levels in the sham group were the highest at the 4th, 5th, and 6th weeks, and those in all of the probiotics groups were significantly lower than in the sham group at the 4th week. The OVA-specific IgG1 in the *B. lactis* group was significantly lower than that in the sham group at the 5th and 6th weeks; however, those in the *L. acidophilus* and LB mixture groups were not significantly lower than the sham group at the 5th and 6th weeks (*L. acidophilus*, 337.7±101.95 ng/ml; *B. lactis*, 255.2±88.9 ng/ml; LB mixture, 503.5±7.6 ng/ml; sham, 38.0±38.0 ng/ml; naive, 1.2±1.2 ng/ml at 6th week).

Allergic Symptoms on the Tails

After administration of OVA and CT, the mice started to scratch their tail and pluck their tail hairs, resulting in injuries. The sham mice had severe injuries and bleeding, whereas the mice fed probiotic bacteria and the naive group had mild symptoms (Fig. 2A). The severity of OVA-induced allergic symptoms in mice was scored (Fig. 2B). The severity of tail injuries was significantly reduced in the *L. acidophilus*, *B. lactis*, and LB mixture treated groups compared with the sham group (*L. acidophilus*, 1.8±0.3; *B. lactis*, 2.7±0.4; LB mixture, 3.5±0.5; sham, 6.5±0.9; naive, 3.6±0.7).

Histological Features of Ears and Small Intestines

Pathological changes were marked by mast cell hyperplasia (Figs. 3A and 3B). The numbers of mast cells of ears and small intestines in the sham group were significantly greater than in the naive group. In the small intestine, the numbers of mast cells from the mice fed *B. lactis* and LB mixture were significantly fewer than in the sham group; however, that from the mice fed *L. acidophilus* was not statistically significant (*L. acidophilus*, 4.0±0.5; *B. lactis*, 3.2±1.2; LB mixture, 3.4±1.0; sham, 6.4±0.26; naive, 2.4±0.7; Fig. 4A). In the ear tissues, mast cell numbers of the *L. acidophilus* treated group were significantly reduced compared with the cells of the sham group. Mast cells of *B. lactis* and LB mixture treated groups were fewer than that of the sham group, but not statistically significant (*L. acidophilus*, 10.3±0.5; *B. lactis*, 13.7±1.1; LB mixture, 12.9±1.3; sham, 15.9±0.6; naive, 10.3±1.4; Fig. 4B).

Eosinophils are evidence of inflammatory cell infiltration, and the picture of eosinophils is presented in Fig. 3C. The number of eosinophils was significantly increased in the small intestine of the sham group compared with the naive group, whereas the eosinophils in the small intestine of probiotic bacteria treated groups were not increased (*L. acidophilus*, 17.1±1.8; *B. lactis*, 22.4±1.8; LB mixture, 22.9±2.9; sham, 41.7±4.4; naive, 20.1±1.8; Fig. 4C).

OVA-Specific Mucosal IgA Production

To evaluate mucosal IgA levels, the fecal samples were collected at the 7th week. The OVA-specific IgA level in the sham group was significantly higher than that in the probiotics groups (*L. acidophilus*, 2.2±0.2 ng/ml; *B. lactis*, 2.6±0.7 ng/ml; LB mixture, 2.6±0.7 ng/ml; sham, 6.5±2.0 ng/ml; naive,

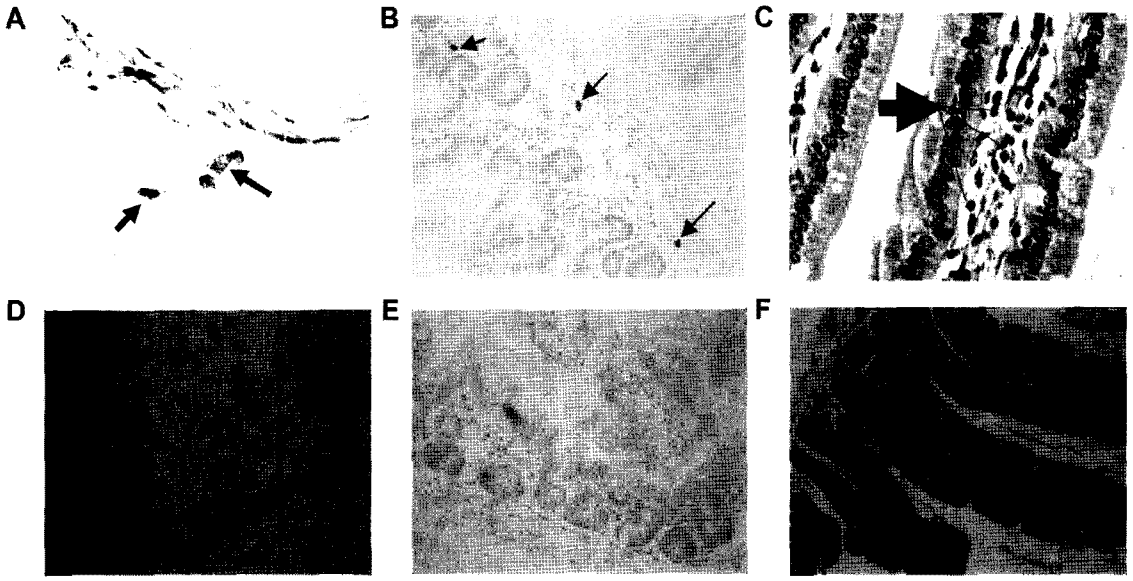

Fig. 3. Pictures of the representative histological section.
The ear sections from sensitized mouse (**A**) and naive mouse (**D**) were stained with toluidine blue. The medium arrows in (**B**) indicate mast cells. The proximal small intestines from sensitized mouse (**B**) and naive mouse (**E**) were stained with toluidine blue. The small arrows in (**A**) or (**D**) indicate mast cells. The proximal small intestine from sensitized mouse (**C**) and naive mouse (**F**) were stained with hematoxylin and eosin. The large arrows in (**C**) and (**F**) indicate eosinophils.

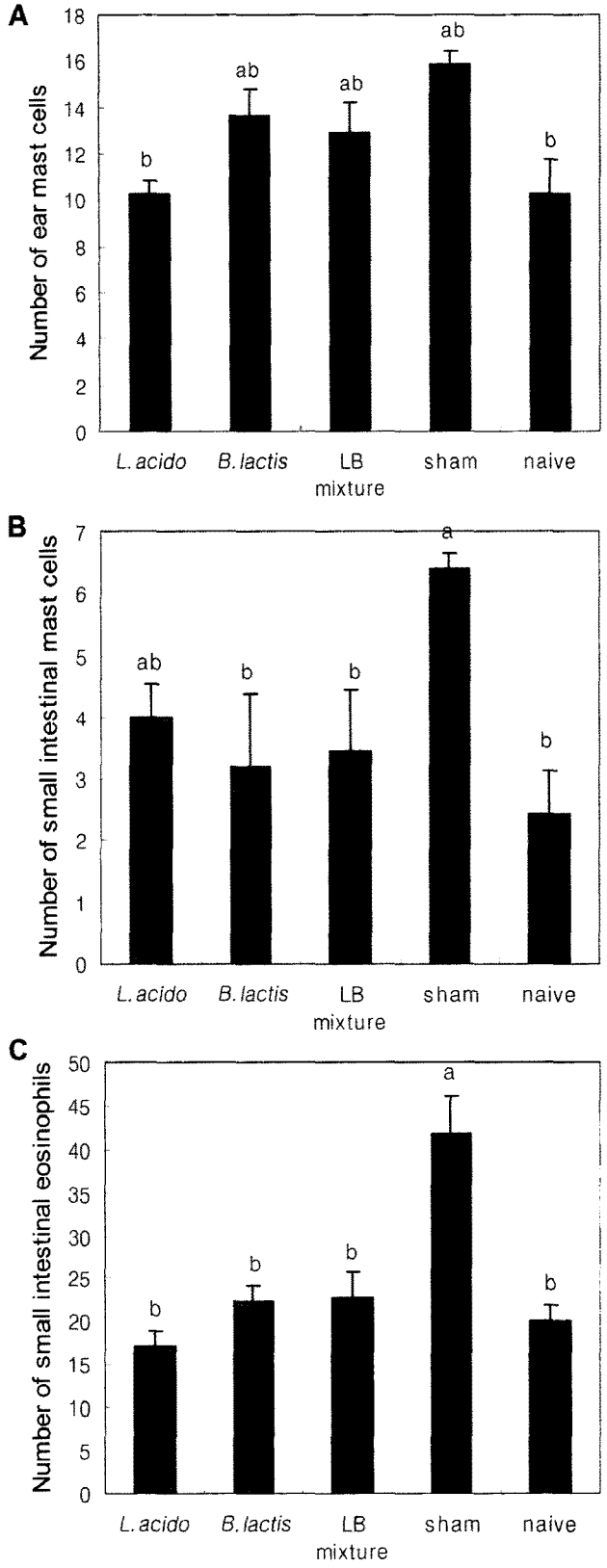

Fig. 4. Effect of probiotics on ear and small intestine in the ovalbumin-sensitized mouse model.

The number of mast cells in small intestinal sections (**A**), in ear sections (**B**), and the number of eosinophils in proximal small intestinal sections (**C**). Data shown are means±SEM of six mice per group. Different letters indicate significant differences ($p<0.05$).

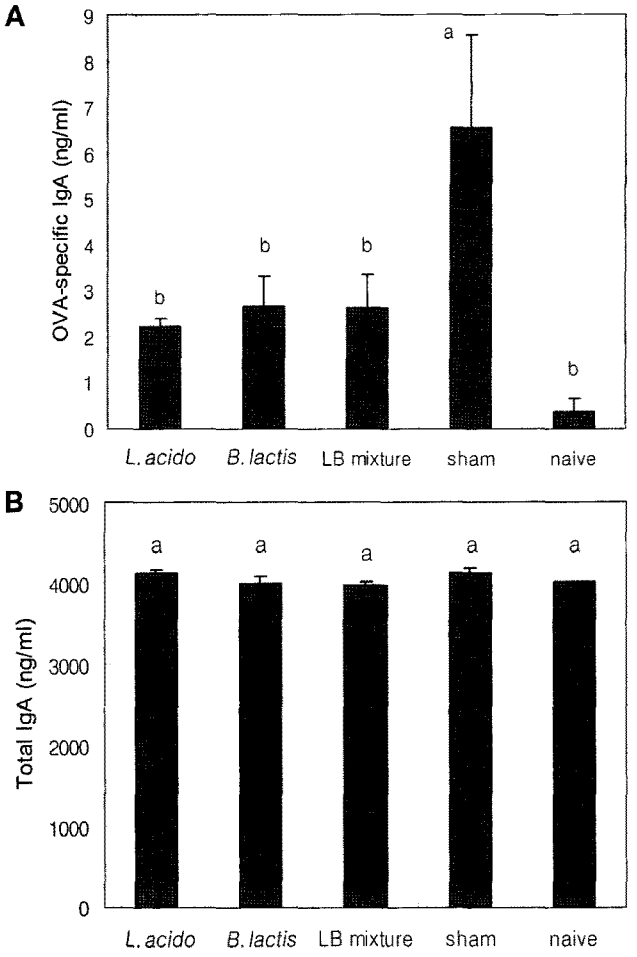

Fig. 5. Effect of probiotics on the production of ovalbumin-specific IgA (**A**) and total IgA (**B**) in fresh fecal samples from ovalbumin-sensitized mice.

The IgA levels were detected by ELISAs. Data shown are means±SEM of six mice per group. Different letters indicate significant differences ($p<0.05$).

0.4±0.3 ng/ml; Fig. 5A). However, total mucosal IgA levels did not differ significantly among groups (Fig. 5B).

Cytokine Secretions of Spleen Cells

IL-4 levels of non-treated spleen cells in all groups were not detected (data not shown). The level of IL-4 by conA-treated spleen cells in sham mice was more than 3-fold higher than in probiotic bacteria-fed mice, as shown in Fig. 6A (*L. acidophilus*, 74.2±7.0 pg/ml; *B. lactis*, 37.4± 6.0 pg/ml; LB mixture, 71.5±9.9 pg/ml; sham, 233.8± 39.2 pg/ml; naive, 72.4±8.8 pg/ml). The levels of IFN-γ of spleen cells in the *L. acidophilus*, *B. lactis*, and LB mixture treated groups were significantly higher than those in naive groups, and those in the sham group was markedly lower than those in the other groups (*L. acidophilus*, 1,328±27 pg/ml; *B. lactis*, 1,170±70 pg/ml; LB mixture, 776±64 pg/ml; sham, 136±19 pg/ml; naive, 721±185 pg/ml, Fig. 6B). The levels of IL-10 of spleen cells in the *B. lactis* and LB mixture

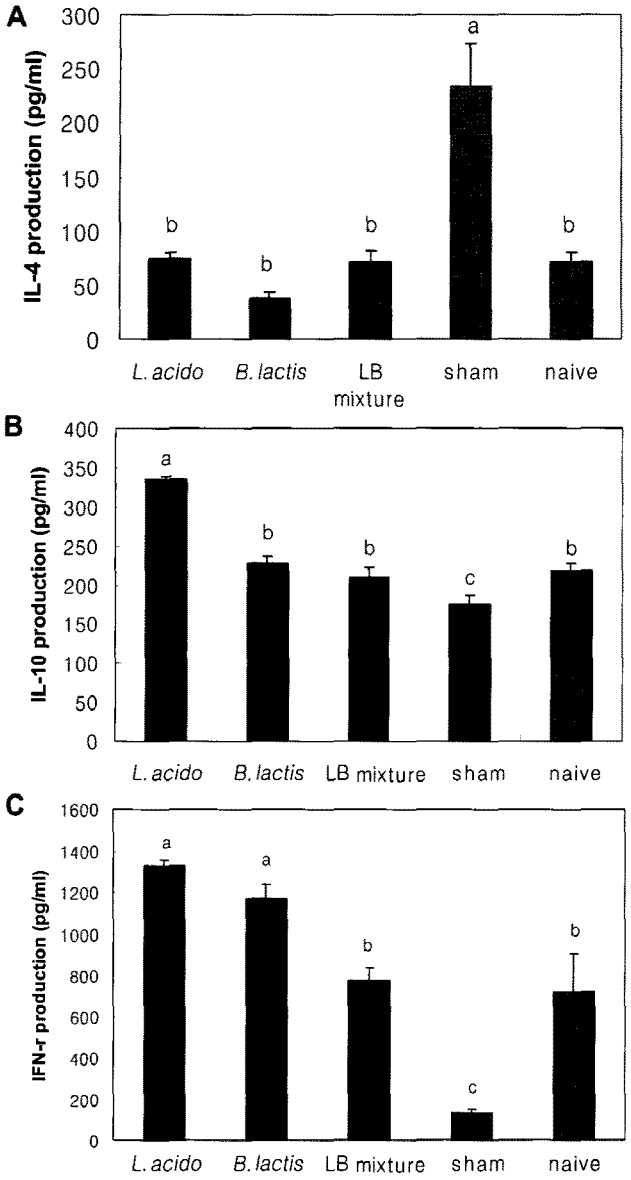

Fig. 6. Effect of probiotics on the production of interleukin (IL)-4 (**A**), IL-10 (**B**), and interferon (IFN)-γ (**C**) of spleen cells from experimental mice.
IL-4 levels of conA-treated spleen cells, and IL-10 and IFN-γ levels of conA non-treated spleen cells were detected by ELISAs. Data shown are means±SEM of six mice per group. Different letters indicate significant differences ($p<0.05$).

treated groups were similar to those in the naive group and significantly higher than in the sham group. The IL-10 level of spleen cells in the *L. acidophilus* group was the highest (*L. acidophilus*, 335±4 pg/ml; *B. lactis*, 228±9 pg/ml; LB mixture, 211±11 pg/ml; sham, 175±11 pg/ml; naive, 219±8 pg/ml, Fig. 6C).

Changes of Body Weights

Mean body weights at the beginning of the experiment did not differ between the five experimental groups (Fig. 7). After the OVA sensitization, the sham and *L. acidophilus*

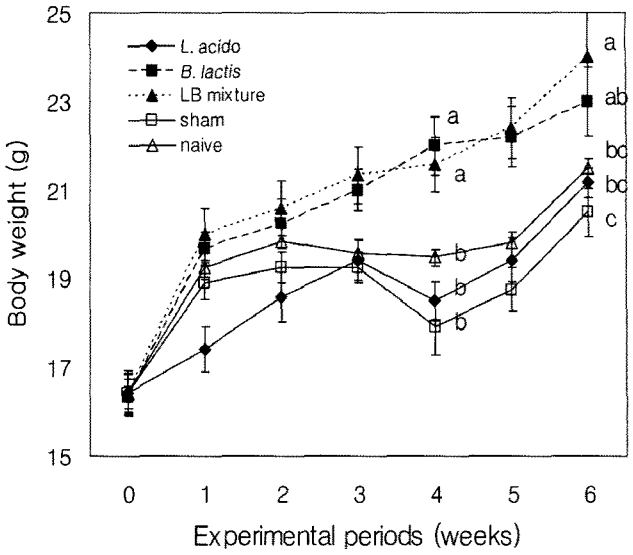

Fig. 7. Body weight of mice fed a diet containing 0.2% of *L.acidophilus*, *B. lactis*, or LB mixture up to 6 weeks.
Data are reported as means±SEM in grams for 6 mice in each group. Different letters indicate significant differences ($p<0.05$).

groups had a weight loss at the 4th week, and the naive group had a retarded growth of the body weight, whereas the body weights of the *B. lactis* and LB mixture groups increased continuously up to 6 weeks.

Discussion

Our preliminary study demonstrated that some commercial probiotics strains and different *L. acidophilus* strains have the potential to induce differential cytokine profiles [20]. We have selected *B. lactis* AD011 and *L. acidophilus* AD031, since they were able to induce IFN-γ and IL-10 production when cultured with C3H/HeJ mouse splenocytes in *in-vitro* culture system (data not shown). In the present study, we assessed the *in vivo* response of whether oral administration of *B. lactis* AD011, *L. acidophilus* AD031, and LB mixture could prevent food allergy in an OVA-induced mouse model.

Food allergy is a hypersensitivity response to orally administered food antigens, and oral tolerance is obtained on the mucosal system of the gastrointestinal tract. Consequently, a food allergy model is appropriately performed by administrating allergens through the oral route. Our allergy model, using the OVA-induced oral sensitization, closely simulated human food allergy development. In the previous OVA-induced mouse model experiment, pre-treatment of the probiotics before the sensitization was more effective than the post-treatment [11]. Therefore, we administrated the experimental probiotics prior to OVA sensitization.

Saldanha *et al.* [22] showed that mice sensitized to OVA showed high levels of IgE and IgG1 production and mast

cell hyperplasia. In the present study, the OVA-specific IgE and IgG1 levels in the sham group were remarkably increased, and mast cell numbers of ear and small intestine were elevated. This suggests that the induction of food allergy was successful in our study.

It was of a great interest to observe that administration of *L. acidophilus*, *B. lactis*, and LB mixture prior to OVA sensitization markedly reduced OVA-specific IgE levels and inhibited OVA-specific IgG1 levels compared with sham mice. Additionally, the ears and small intestine tissues of all groups fed probiotics reduced mast cell hyperplasia compared with the sham group.

IgE-dependent food allergy reactions usually affect one or more target organs such as skin, respiratory tract, gastrointestinal tract, and cardiovascular systems [27]. Moreover, chronic food allergic disorders typically affect the gastointestinal tract with different degrees of eosinophilic inflammation [24]. All the mice in the present study had tail wounds because sera were collected *via* the tail vein. The wounds in sensitized OVA and non-treated groups were worsened, accompanied with a bleeding, whereas they were gradually resolved in probiotics-treated groups. The increase of secretory IgA was associated with stimulation of eosinophil degranulation [1]. Activated eosinophils may induce gastrointestinal damage by releasing cytotoxic mediators, major basic protein, eosinophilic cationic protein, and eosinophil-derived neurotoxin [10, 22]. In the present study, infiltration of the eosinophils in the small intestine was suppressed by the administration of the experimental probiotics. Mice without eosinophil infiltration did not show gastrointestinal pathogenic disorder, including gastromegaly and cachexia [9]. Accordingly, *L. acidophilus* and *B. lactis* treatments prevented the OVA-induced allergic symptoms on the skin and gastrointestinal tract.

To clarify the mechanisms involved in the inhibition of serum IgE production, we examined the cytokine production of spleen cells. The sham mice showed Th2-dominant immune status with lower production of IFN-γ and Th1 cytokine, and higher production of IL-4 and Th2 cytokine, in the spleen cells than the naive mice. The observed normalizing effect of the experimental probiotics on the productions of IL-4 and IFN-γ suggested that administration of *L. acidophilus* and *B. lactis* helped maintain the balance of Th1 and Th2 cells, which might regulate IgE production. The production of IL-10 by spleen cells from mice fed experimental probiotics was higher than that by spleen cells from sham mice. IL-10 secretion might have partially contributed to the induction of oral tolerance by activating regulatory T cells [4], and suppressed food-induced anaphylaxis and IgE-associated sensitization [7].

The OVA-treated positive control mice (sham group) and the OVA-treated and *L. acidophilus* administrated mice showed a weight loss at the 4th week, whereas the negative control mice (naive group) had no decrease in body weight. The allergic response in the gastrointestinal tract might have induced a temporary weight loss. However, the mice fed *B. lactis* or LB mixture showed excellent growth in terms of weight. In our repeated previous experiments, immature mice administrated with *B. lactis* showed better growth than control mice, suggesting that further studies are needed to explore whether supplementation of *B. lactis* could affect the growth of mice in their early age.

Taken together, the present results clearly demonstrate that oral administration of *L. acidophilus* AD031 or *B. lactis* AD011 prevents IgE-mediated OVA hypersensitivity, and suppresses traditional allergy symptoms. However, the synergistic effect between *L. acidophilus* AD031 and *B. lactis* AD011 on the prevention of allergy was not noted under our experimental conditions.

Acknowledgment

This work was supported by a grant (A060546) from the Ministry of Health and Welfare, Republic of Korea.

REFERENCES

1. Abu-Ghazaleh, R. I., T. Fujisawa, J. Mestecky, R. A. Kyle, and G. J. Gleich. 1989. IgA-induced eosinophil degranulation. *J. Immunol.* **142:** 2393–2400.
2. Benner, R. H. and F. J. Savelkoul. 1991. Regulation of IgE production in mice. *Eur. Respir. J.* **13(Suppl):** 97–104.
3. Bufford, J. D. and J. E. Gern. 2005. The hygiene hypothesis revisited. *Immunol. Allergy Clin. North Am.* **25:** 247–262.
4. Chehade, M. and L. Mayer. 2005. Oral tolerance and its relation to food hypersensitivities. *J. Allergy Clin. Immunol.* **115:** 3–12.
5. Christie, L., R. J. Hine, J. G. Parker, and W. Burks. 2002. Food allergies in children affect nutrient intake and growth. *J. Am. Diet. Assoc.* **102:** 1648–1651.
6. Eder, W. and E. von Mutius. 2004. Hygiene hypothesis and endotoxin: What is the evidence? *Curr. Opin. Allergy Clin. Immunol.* **4:** 113–117.
7. Frossard, C. P., L. Steidler, and P. A. Eigenmann. 2007. Oral administration of an IL-10-secreting *Lactococcus lactis* strain prevents food-induced IgE sensitization. *J. Allergy Clin. Immunol.* **119:** 952–959.
8. Fuller, R. 1989. Probiotics in man and animals. *J. Appl. Bacteriol.* **66:** 365–378.
9. Hogan, S. P. and A. Mishra. 2001. A pathological function for eotaxin and eosinophils in eosinophilic gastrointestinal inflammation. *Nat. Immunol.* **2:** 353–360.
10. Kelly, K. J. 2000. Eosinophilic gastroenteritis. *J. Pediatr. Gastroenterol. Nutr.* **30(Suppl):** 28–35.
11. Kim, H. Y., S. Y. Lee, and G. E. Ji. 2005. Timing of *Bifidobacterium* influences the development of allergy to ovalbumin in mice. *Biotechnol. Lett.* **227:** 1361–1367.
12. Kim, H., K. Kwack, D. Y. Kim, and G. E. Ji. 2005. Oral probiotic bacterial administration suppressed allergic responses

in an ovalbumin-induced allergy mouse model. *FEMS Immunol. Med. Microbiol.* **45:** 259–267.
13. Kim, N. J. and G. E. Ji. 2006. Modulatory activity of *Bifidobacterium* sp. BGN4 cell fractions on immune cells. *J. Microbiol. Biotechnol.* **16:** 584–589.
14. Marinaro, M., P. N. Boyaka, F. D. Finkelman, H. Kiyono, R. J. Jackson, E. Jirillo, and J. R. McGhee. 1997. Oral but not parenteral interleukin-12 redirects T helper 2-type responses to an oral vaccine without altering mucosal IgA responses. *J. Exp. Med.* **185:** 415–427.
15. Matsuzaki, T., R. Yamazaki, S. Hashimoto, and T. Yokokura. 1998. The effect of oral feeding of *Lactobacillus casei* strain Shirota on immunoglobulin E production in mice. *J. Dairy Sci.* **81:** 48–53.
16. Mosmann, T. R. and R. L. Coffman. 1989. Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv. Immunol.* **46:** 111–147.
17. Oh, J. W., B. Y. Pyun, J. T. Chong, K. M. Ahn, C. H. Kim, S. W. Song, J. A. Son, S. Y. Lee, and S. I. Lee. 2004. Epidemiological change of atopic dermatitis and food allergy in school-aged children in Korea between 1995 and 2000. *J. Korean Med. Sci.* **19:** 716–723.
18. Ohno, H., S. Tsunemine, Y. Isa, M. Shimakawa, and H. Yamamura. 2005. Oral administration of *Bifidobacterium bifidum* G9-1 suppresses total and antigen specific immunoglobulin E production in mice. *Biol. Pharm. Bull.* **28:** 1462–1466.
19. Ouwehand, A. C. 2007. Antiallergic effect of probiotics. *J. Nutr.* **137:** 794S–797S.
20. Park, M. S., M. J. Kim, and G. E. Ji. 2007. Assessment of lipopolysaccharide binding activity of *Bifidobacterium* and its relationship with cell surface hydrophobicity, autoaggregation and inhibition of interleukin-8 production. *J. Microbiol. Biotechnol.* **17:** 1120–1126.
21. Rook, G. A. W. and L. R. Brunet. 2005. Microbes, immunoregulation, and the gut. *Gut* **54:** 317–320.
22. Saldanha, J. C. S., D. L. Gargiulo, S. S. Silva, F. H. Carmo-Pinto, M. C. Andrade, J. I. Alvarez-Leite, M. M. Teixeira, and D. C. Cara. 2004. A model of chronic IgE-mediated food allergy in ovalbumin-sensitized mice. *Braz. J. Med. Biol. Res.* **37:** 809–816.
23. Salminen, S., C. Bouley, M. C. Boutron-Ruault, J. H. Cummings, A. Franck, G. R. Gibson, E Isolauri, M. C. Moreau, M. Roberfroid, and I. Rowland. 1998. Functional food science and gastrointestinal physiology and function. *Br. J. Nutr.* **80:** s147–s171.
24. Sampson, H. A., S. H. Sicherer, and A. H. Birnbaum. 2001. AGA technical review on the evaluation of food allergy in gastrointestinal disorders. *Gastroenterology* **120:** 1026–1040.
25. Samson, H. A. 1999. Food allergy. Part 1: Immunopathogenesis and clinical disorders. *J. Allergy Clin. Immunol.* **103:** 717–728.
26. Shida, K., R. Takahashi, E. Iwadate, K. Takamisawa, H. Yasui, T. Sato, S. Habu, S. Hachimura, and S. Kaminogawa. 2002. *Lactobacillus casei* strain Shirota suppresses serum immunoglobulin E and immunoglobulin G1 responses and systemic anaphylaxis in a food allergy model. *Clin. Exp. Allergy* **32:** 563–570.
27. Sicherer, S. H. 2002. Food allergy. *Lancet* **360:** 701–710.
28. Taylor, A. L., J. A. Dunstan, and S. L. Prescott. 2007. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: A randomized controlled trial. *J. Allergy Clin. Immunol.* **119:** 184–191.
29. Torii, A., S. Torii, S. Fujiwara, H. Tanaka, N. Inagaki, and H. Nagai. 2007. *Lactobacillus acidophilus* strain L-92 regulates the production of Th1 cytokine as well as Th2 cytokines. *Allergol. Int.* **56:** 293–301.