

Decrease in Ovalbumin Specific IgE of Mice Serum after Oral Uptake of Lactic Acid Bacteria

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Different kinds of lactobacilli and Bifidobacteria fermented milk were fed to ovalbumin-specific IgE-elevated mice for 3 days, and after the final administration, changes in the ovalbumin-specific IgE values for each sample were compared to the value for non-fermented milk. Seven of the *Lactobacillus*-fermented milks caused a significant decrease in the serum ovalbumin-specific IgE levels. Above all, *Lactobacillus acidophilus* L92, *Lactobacillus acidophilus* CP1613, and *Lactobacillus fermentum* CP34 fermented milk had the most significant effects of decreasing the serum ovalbumin-specific IgE levels compared to a control group. The *L. acidophilus* L92 and *L. fermentum* CP34 cells also showed significant ovalbumin-specific IgE lowering activities. From these results, an active component seems to exist in the cells of *L. acidophilus* L92 and *L. fermentum* CP34 strains. Recovery of the radiolabeled *L. acidophilus* L92 and *L. fermentum* CP34 cells from the small intestine and the large intestine of the mouse 13 h after oral administration were higher than the recovery of any other strain.

Key words: antigen-specific IgE; *Lactobacillus acidophilus*; *Lactobacillus fermentum*; gastrointestinal tract; re-isolation

Many of the probiotic benefits of lactic acid bacteria (LAB) such as the protective effects against intestinal infections,¹⁾ the immunostimulating effect,²⁾ and a decrease in serum cholesterol³⁾ have been reported and reviewed previously.^{4–6)} Generally, probiotic LAB have a strong affinity for the epithelial cells of the gastrointestinal (GI) tract, and adherence of LAB to intestinal epithelial cells is the first step of colonization.^{5,7)} The colonization of LAB in the GI tract of the host seems to be important for the expression of the host response in the probiotic effect.

Of the many of probiotic effects of LAB, the immunomodulating effects of lactobacilli have been extensively studied.^{8,9)} Most of the immunostimulating effects of lactobacilli were evaluated by measuring increases in serum and intestinal IgA and IgG,²⁾

the increase in certain cytokines¹⁰⁾ and the activation of natural killer cells.¹¹⁾ On the other hand, oral feeding of heat-killed *Lactobacillus casei* (strain Shirota) was effective in inhibiting IgE production induced by ovalbumin in mouse serum.¹²⁾ Moreover, intraperitoneal injection of heat-killed *Lactobacillus plantarum* L-137 was also effective in repressing IgE production in response to a casein food allergy in mice.¹³⁾ However, little is known about the influence of various LAB on serum IgE, which is one of the risk factors for the allergic response.

Atopic diseases such as pollen allergy and atopic dermatitis are becoming serious social problems in Japan and other developed countries. Preventative trials involving ingestion of various food materials have been carried out. Recently, *Lactobacillus rhamnosus* GG was effective in prevention of early atopic disease in children at high risk.¹⁴⁾ However, effective food materials that prevent the risk of allergies are limited. In this study, we tested the potential of probiotic lactobacilli in the prevention of allergies by oral administration of various LAB strains. The antigen-specific serum IgE level is a risk factor in allergic inflammation reactions, such as the release of histamine and leukotriene from mast cells. Evaluation of changes in serum IgE after an oral administration seemed to be important for the analysis. The objective of this study is to find whether bioactive lactobacilli could reduce antigen-specific serum IgE in mice as one of the risk factors in allergy. We report here the first evidence of the antigen-specific serum IgE lowering effects of various lactobacilli after oral administration.

Materials and Methods

LAB and media. Various lactobacilli listed in Table 1 were used from our stock culture collection. All lactobacillus strains were cultured in Man Rogosa Sharpe (MRS) medium (Difco, Detroit, MI) and the bifidobacterial strain was cultured in GAM medium (Nissui, Tokyo, Japan).

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Abbreviations: LAB, lactic acid bacteria; OVA, ovalbumin; OVA-IgE, ovalbumin-specific IgE; GI, gastrointestinal

Preparation of fermented milk and LAB. All lactobacilli were first cultured in MRS medium, and the bifidobacterial strain was cultured in GAM medium at 37°C for 24 h. Then, 3% (vol/vol) of each culture was added to 9% (wt/vol) of skim milk medium containing 0.1% yeast extract, and fermented at 37°C for 1 day. After cultivation, the pH, lactic acidity, and the number of cells in the fermented milk were measured. The number of cells in the fermented milk was counted under a microscope after neutralization of the fermented milk by addition of 2.5 N NaOH. These various fermented milk samples were used for oral administration to mice. Cell suspensions of each *Lactobacillus* strain were prepared after culturing at 37°C for 24 h in MRS medium. Cells were harvested by centrifugation at $2000 \times g$ for 10 min and the pellet was washed with 0.9% (wt/vol) of NaCl (saline). The washed cells were suspended in 9% (wt/vol) skim milk at a concentration shown in Table 2.

Preparation of antigen-specific IgE elevated mice. Six-week-old male BALB/c mice were obtained from Charles River Japan (Kanagawa, Japan). The mice were fed a standard diet (CE-2; CLEA, Tokyo, Japan) and were allowed free access to water throughout the experimental period. Before sensitization to antigen, blood samples were obtained from the ophthalmic veins of mice. The mice were injected intraperitoneally with 10 µg of ovalbumin (OVA, Sigma Grade V; Sigma Chemical Co., St. Louis, MO) mixed with 2 mg of Al(OH)₃ in a total volume of 0.3 ml saline on days 0 and 4 (the first immunization). On days 10, 11, 12, 13, 14, 15 and 16, the mice were exposed to OVA solution intranasally (25 mg OVA/ml of saline) 3 times in the morning and 3 times in the afternoon (3 seconds/time) (second immunization). For the measurement of serum-ovalbumin-specific IgE (OVA-IgE), total-IgE, and total IgG levels in model mice during sensitizations, on days 17, 22, and 27, blood samples were obtained from the ophthalmic veins of the mice.

Oral administration to mice. To test the effects of each strain on the mouse serum OVA-IgE levels, the mice with OVA-IgE levels from the mean – SD to mean + SD were selected after analysis of the serum on day 17. On day 18, the mice with high titers of serum OVA-IgE were selected and divided into several groups (10 mice/group) with same averages of serum OVA-IgE titers. Eleven kinds of fermented milk samples were orally administered to OVA-IgE elevated mice on days 19, 20, and 21 in dosages of 1 ml per day. Non-fermented milk was also administered instead of the fermented milk as a control. Cells cultured in MRS medium were suspended in 9% (wt/vol) skim milk at different concentrations, and the suspensions were orally administered on the

above schedule to study the dose-dependent effects of the cells using 9% (wt/vol) skim milk as a control sample.

Blood samples. Blood samples from mice after the administration of test samples were obtained from the ophthalmic veins before the 1st sensitization and on day 17, and from the jugular vein on day 22. Serum samples were prepared by centrifugation of the blood samples at $900 \times g$ for 15 min after incubating them on ice for 3 h. Serum samples were stored at – 80°C until used. Serum samples with high titers of OVA-IgE were prepared in advance by the procedure described above and used as a positive standard for quantitative analysis of OVA-IgE in mice.

ELISA of OVA-specific IgE. For measurement of the OVA-IgE titers in mice serum, a sheep polyclonal antibody to mouse IgE (AAM11, Serotec Ltd., Oxford, UK) was diluted with PBS (137 mM sodium chloride, 8.1 mM disodium hydrogen phosphate, 2.7 mM potassium chloride, and 1.5 mM potassium dihydrogen phosphate, pH 7.4) to 10 µg/ml. Then, 100 µl of diluted antibody was soaked into each well of a microplate (Corning, NY), and the microplate was stored overnight at 4°C. The microplate was washed 3 times with PBS, and was coated with 0.5% casein-PBS for 3 h at room temperature. After the microplate was washed with PBS, 100-µl portions of diluted mouse serum and standard positive serum were added to each well, and the plate was incubated overnight at 4°C. After the plate was washing 4 times with PBS, OVA that had been biotinylated using a Biotinylation kit (American Qualex International Inc., CA) was diluted with 0.5%-casein PBS (10 µg/ml), and 100 µl was added to each well. The plate was then incubated for 2 h at room temperature. After the washing of the plate 5 times with PBS, 100 µl of streptavidin-peroxidase (Sigma Chemical Co., St. Louis, MO) diluted to 0.5 µg/ml with 0.5% casein-PBS was added to each well and incubated for 1 h at room temperature. After the plate was washed 5 times with 0.1%-Tween 20 in PBS, 100 µl of ABTS (Boehringer Mannheim, Germany; 600 µg/ml ABTS, 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid, 0.006% H₂O₂ in 0.2 M citric acid buffer, pH 4.7) was added to the wells. After incubation for 3 h at 37°C in the dark, absorbances at 405 and 492 nm were measured using a microplate reader (Microplate Reader MTP-32, Corona Electric, Ibaraki, Japan). The value of (OD₄₀₅–OD₄₉₂) was used for the specific absorbance from ABTS.

ELISA of IgE and IgG. To measure the concentrations of total IgE and total IgG in mice sera, goat polyclonal anti-mouse IgG (Zymed Laboratories, Inc., San Francisco, CA) and sheep polyclonal anti-mouse IgE (AAM11; Serotec Ltd., Oxford, UK)

antibodies were diluted to 10 $\mu\text{g}/\text{ml}$ with PBS. Then, 50 μl of each diluted antibody was soaked into each well of a microplate. After overnight incubation at 4°C, the microplate was washed 3 times with PBS, and was blocked with 0.5% casein-PBS for 3 h at room temperature. After the plate was washed with PBS, 50- μl portions of the serum samples were added to each well of the IgG plate and the IgE plate, and both plates were incubated overnight at 4°C. The plates were washed 4 times with PBS. Peroxidase-labeled goat anti-mouse IgG (γ) (ICN Pharmaceuticals, Inc., Costa Mesa, CA) and biotin-labeled rat monoclonal anti-mouse IgE (Serotec Ltd., Oxford, UK) were diluted 500-fold with 0.5% casein-PBS. Diluted goat anti-mouse IgG (50 μl) was added to the IgG plate, and 50 μl of diluted rat anti-mouse IgE was added to the IgE plate. Each plate was incubated for 2 h at room temperature. After the IgE plate was washed 4 times with 0.1% Tween 20-PBS, 50 μl of streptavidin-peroxidase diluted with 0.5% casein-PBS was added and incubated for 1 h at room temperature. The absorbance of each well was measured after addition of ABTS solution as described previously.

Adhesion of LAB to the GI tract. Eight kinds of lactobacillus strains, listed in Table 1, were grown in 10 ml of MRS medium with and without 0.2 μl of [$\alpha^{32}\text{P}$]-thymidine-5'-triphosphate (3000 Ci/mmol, Muromachi Pharmaceutical, Tokyo, Japan) per ml for 12 h at 37°C. After growth, cells were harvested by centrifugation at 3000 $\times g$ for 10 min, and the pellet was washed once with PBS. Cells were resuspended in PBS ($1 \times 10^9/\text{ml}$). The radioactivity of the cell suspension was measured using a liquid scintillation counter LSC-900 (ALOKA, Tokyo, Japan), and the number of cells was measured. The mice ($n=2$) that had been given the radiolabeled lactobacillus cells were killed at 13 h after administration, and the small and large intestines were isolated. The radioactivities bound to small intestine and large intestine were counted using a liquid scintillation counter after cutting the organ into several pieces.

Results

Preparation of antigen-specific IgE elevated mice

To select active lactobacilli that can reduce serum IgE, mice with elevated OVA-IgE levels in the serum were generated by intraperitoneal injections of OVA (the 1st immunization) followed by nasal immunizations (the 2nd immunization) following the schedule shown in Fig. 1(A). The OVA-IgE and non-specific serum IgE (total-IgE) increased in parallel during the 1st and 2nd immunizations (Fig. 1(B)). The OVA-IgE and the total-IgE levels on day 17 significantly increased over 10- and 6-fold compared to the values on day 0. On the other hand, the increase in the total

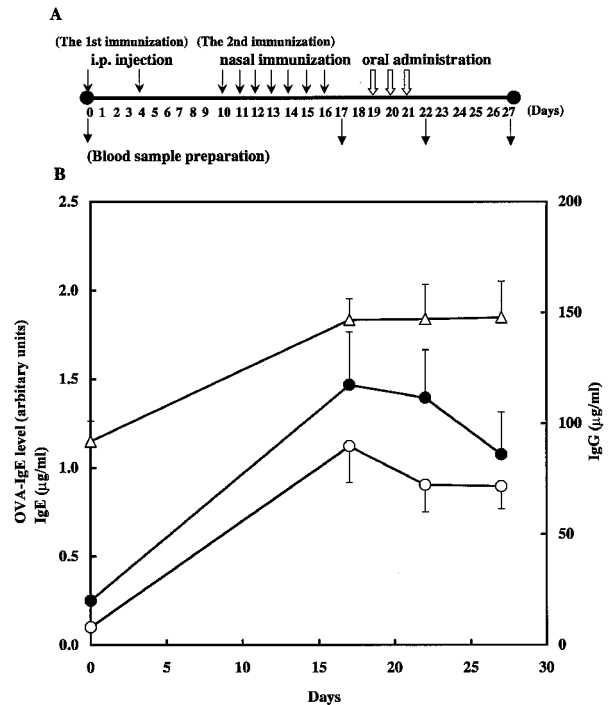


Fig. 1. Protocol for the Preparation of Ovalbumin Specific IgE (OVA-IgE) Elevated Mice and the Results for the Changes in Serum IgE, IgG, and OVA-IgE in the Mice.

(A) Schedule of immunization of mice, oral administrations of the test sample, and collection of the blood samples. (B) Changes in serum OVA-IgE (○), total IgE (●), and total IgG (△) levels during the immunizations. The concentration of OVA-IgE, total IgE, and total IgG were measured by the ELISA method as described in Materials and Methods. The OVA-IgE value was calculated by comparisons of optical density of the test sample to a standard serum, and shown in arbitrary units. The values of the OVA-IgE and the total IgE on day 0 are shown as detection limits, respectively. The averages and error bars representing the standard error were obtained from the data of 9 mice.

IgG level was about 1.5-fold, which is much lower than the increase in OVA-IgE and total-IgE levels. These results indicate that the OVA-IgE levels in the mice were specifically increased by the intraperitoneal and nasal antigen stimulation and suggest that these mice could provide an appropriate model to evaluate the effects of various lactobacilli on antigen-specific IgE levels in the serum.

Effects of various LAB fermented milk on serum IgE levels

Various lactobacilli and a Bifidobacterium strain were used to prepare eleven kinds of fermented milk (Table 1). The effects of these fermented milk samples on serum OVA-IgE levels in mice and were investigated in two sets of experiments. One ml of each fermented milk sample was fed to the OVA-IgE elevated mice on days 19, 20, and 21, and the OVA-IgE, on day 22 was compared to a standard serum. Total IgE and total IgG levels in the serum were

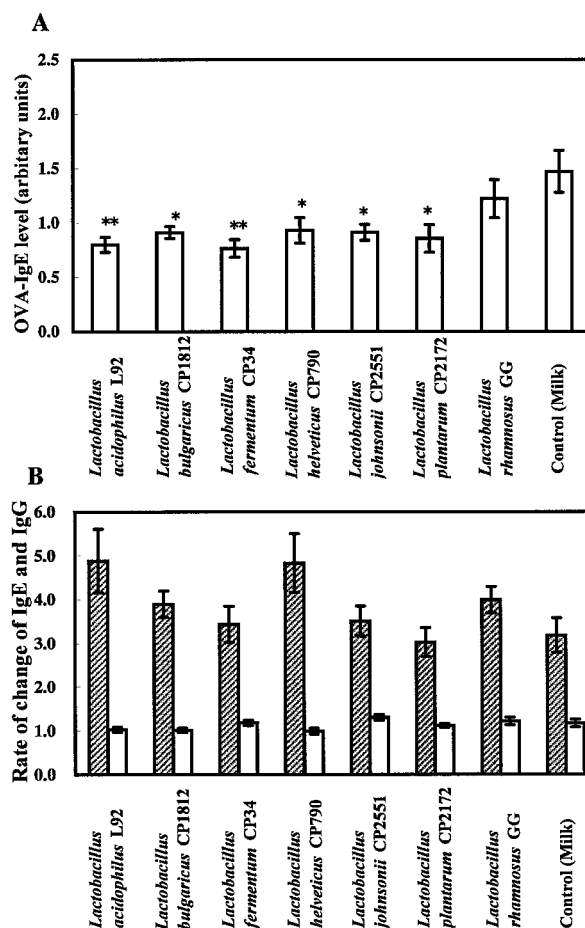
Table 1. Final pH, Acidity, and the Number of Cells of Each Fermented Milk

Fermented milk	Final pH	Acidity (%)	Number of cells (cells/ml)
(experiment 1)			
<i>Lactobacillus acidophilus</i> L92	3.8	1.5	1.9×10^8
<i>Lactobacillus bulgaricus</i> CP1812	3.6	1.8	1.5×10^8
<i>Lactobacillus fermentum</i> CP34	4.4	0.9	5.3×10^8
<i>Lactobacillus helveticus</i> CP790	3.5	2.2	2.4×10^8
<i>Lactobacillus johnsonii</i> CP2551	3.8	1.4	2.7×10^8
<i>Lactobacillus plantarum</i> CP2172	4.4	0.8	5.9×10^8
<i>Lactobacillus rhamnosus</i> CP963	5.9	0.2	1.0×10^8
Control (Milk)	6.3	—	—
(experiment 2)			
<i>Lactobacillus acidophilus</i> CP1613	3.6	1.9	4.4×10^8
<i>Lactobacillus gasseri</i> CP2209	3.9	1.1	4.3×10^8
<i>Lactobacillus reuteri</i> CP729	4.9	0.5	9.6×10^8
<i>Bifidobacterium breve</i> CP2425	4.7	0.8	1.3×10^8
Control (Milk)	6.3	—	—
Cyclophosphamide (750 μ g/ml)	6.3	—	—

measured on day 22 and compared to the values on day 17. The results showed that the OVA-IgE levels in mice were different depending on the bacterial strain used to ferment the milk (Fig. 2(A) and 3(A)). In the first experiment, some of the fermented milk samples, including those generated with *Lactobacillus acidophilus* L92, *Lactobacillus bulgaricus* CP1812, *Lactobacillus fermentum* CP34, *Lactobacillus helveticus* CP790, *Lactobacillus johnsonii* CP2551, and *Lactobacillus plantarum* CP2172 significantly reduced the OVA-IgE levels compared to the control sample ($P < 0.01$, 0.05, 0.01, 0.05, 0.05, and 0.05, respectively) (Fig. 2(A)). Above all, *L. acidophilus* L92 and *L. fermentum* CP34 fermented milk had the most significant effects in lowering the OVA-IgE levels than the other fermented milk samples. In the second experiment, a different *L. acidophilus* strain, *L. acidophilus* CP1613, also showed a significant effect in the OVA-IgE lowering activity ($P < 0.01$) (Fig. 3(A)). A group fed 0.75 mg of cyclophosphamide, an immunosuppressant,¹⁵⁾ also showed a significant effect in this test. However, other tested milk samples that had been fermented by *Lactobacillus rhamnosus* GG, *Lactobacillus gasseri* CP2209, *Lactobacillus reuteri* CP729, and *Bifidobacterium breve* CP2425 had no OVA-IgE lowering activities. There was no correlation between the OVA-IgE lowering effect and the pH, acidity, or the number of cells in the fermented milk. No significant differences in total IgG or total IgE levels among all tested samples were observed (Fig. 2(B) and 3(B)). *L. acidophilus* L92 and *L. fermentum* CP34, strains that were the most effective in the above test were selected for more a detailed study.

Effects of the Precipitate of the Fermented Product

To study the active component in the fermented

**Fig. 2.** Effects of Seven Kinds of Lactobacilli Fermented Milk and Non-fermented Milk (control) on the OVA-IgE, Total IgE, and Total IgG in the OVA-IgE Elevated Mice after Nasal Immunization (experiment 1).

(A) the OVA-IgE values on day 22 calculated to a standard serum (an arbitrary unit), (B) the rate of the IgE (shaded box) and IgG (open box) on day 22 to the values on day 17. Student's *t*-test was used for statistical analysis using the milk sample as a control. The error bar represents the standard error with 10 samples. *: $P < 0.05$ and **: $P < 0.01$.

milk, *L. acidophilus* L92 and *L. fermentum* CP34 cells that had been cultured in MRS medium were washed and tested for OVA-IgE lowering activity. A dosage of 1×10^8 cells of each strain suspended in 9% (wt/vol) skim milk had a significant effect compared to the control sample (9% skim milk) ($P < 0.05$) (Table 2). Moreover, at dosage of 10^6 cells, the *L. acidophilus* L92 and the *L. fermentum* CP34 strains also showed significant effects in the OVA-IgE lowering assay. These results suggest that the cell components of both strains could cause the decrease in OVA-IgE levels in mouse serum. However, no clear dose-dependent effect with either *L. acidophilus* L92 or *L. fermentum* CP34 was detected within a range from 10^6 to 10^8 cells.

Radiolabeled Cells in the GI Tract

The reason for the differences in the OVA-IgE

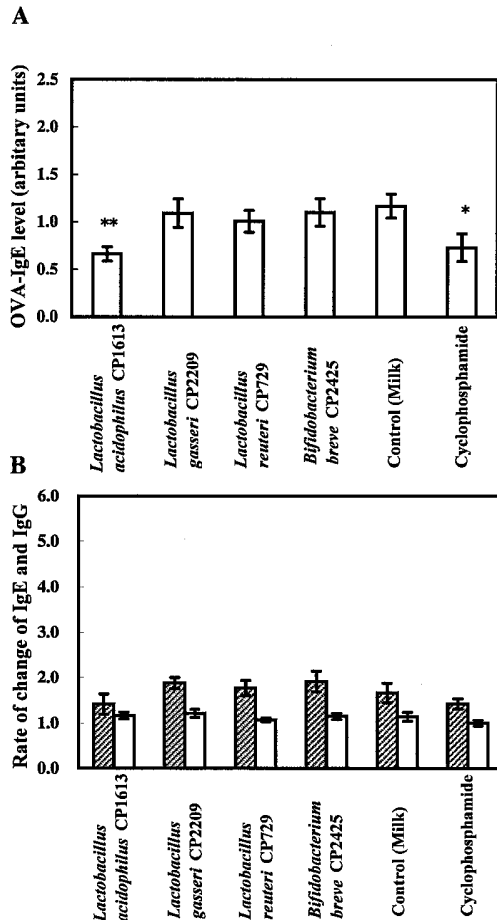


Fig. 3. Effects of Three Kinds of Lactobacilli, and Bifidobacteria-fermented Milk, Non-fermented Milk (control), and Cyclophosphamide on the OVA-IgE, Total IgE, and Total IgG in the OVA-IgE Elevated Mice (experiment 2).

(A) the OVA-IgE values on day 22 calculated to a standard serum (an arbitrary unit), (B) the rate of the IgE (shaded box) and IgG (open box) on day 22 to the values on day 17. Student's *t*-test was used for statistical analysis using the milk sample as a control. The error bar represents the standard error with 10 samples. *: $P < 0.05$ and **: $P < 0.01$.

lowering effects among tested lactobacilli and Bifidobacteria (Fig. 2(A) and 3(A)) was investigated. To differentiate the orally administered strains from other intestinal bacteria, most of the strains listed in Table 1 were metabolically labeled with ^{32}P and used for each recovery test. The radioactivity present in the feces of mice was measured using a scintillation counter after the administration of each radiolabeled strain. The results showed that most of the fed LAB was excreted within 5.5 h, and no good correlation between the total recovery and the OVA-IgE lowering effect of the strain was not detected in this study (data not shown). So, for more direct analysis of affinity of various strains for the GI tract of mice ($n=2$), some of the mice fed with radiolabeled cells were killed at 13 h after administration, and the radioactivity of the small and large intestines was

Table 2. Effects of the *Lactobacillus acidophilus* L92 and *Lactobacillus fermentum* CP34 on Mouse Serum OVA-IgE with Different Dosages

Strain	Dosage of the cells (n = 10)	Serum OVA-IgE level (n = 10)
<i>Lactobacillus acidophilus</i> L92	1×10^6	$0.40 \pm 0.19^{**}$
	1×10^7	$0.54 \pm 0.18^*$
	1×10^8	$0.56 \pm 0.24^*$
<i>Lactobacillus fermentum</i> CP34	1×10^6	$0.50 \pm 0.29^*$
	1×10^7	0.57 ± 0.25
	1×10^8	$0.57 \pm 0.13^*$
Control (Milk)	—	0.78 ± 0.21

* $P < 0.05$.

** $P < 0.01$.

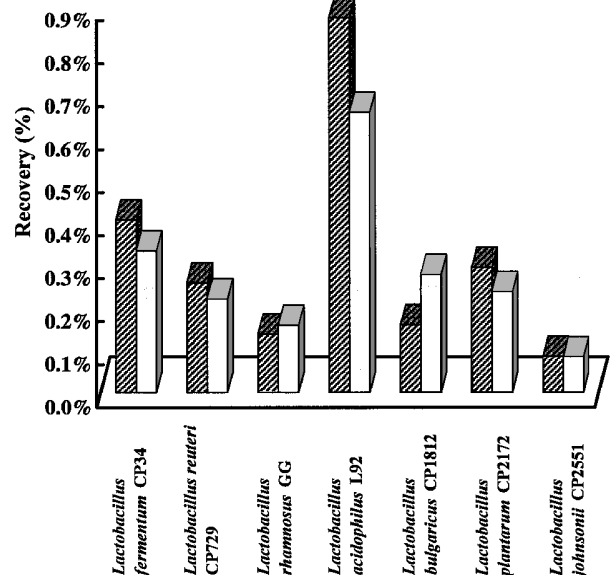


Fig. 4. Recovery of Various ^{32}P -Radiolabeled *Lactobacillus* Strains from the Small and Large Intestines 13 h after a Single Oral Administration of the Cells.

Each mouse was killed 13 h after the oral administration and each small (shaded box) and large (open box) intestine was isolated and the radioactivity was measured. Recovery was calculated as a ratio (%) of the counts obtained to the total ingested counts.

counted. During the test period, no difference was observed in the fecal exhaust rates of mice after the oral administration of lactobacilli. Radioactivities in the small intestine and the large intestine from each mouse were changed in parallel, but were different depend on the fed lactobacillus strains. Among them, the strains that showed lower recoveries in the feces, *L. acidophilus* L92 and *L. fermentum* CP34, were more efficient in the recoveries of radioactivity in the GI tract than the other strains (Fig. 4). From these results, it seems that a direct interaction of the *Lactobacillus* cells with the mouse GI cells may play an

important role for the immunological response of the host to occur.

Discussion

Many immunomodulating activities of LAB have been reported.^{9,11} Little is known, however, about the immunorepressive effects of LAB on antigen-specific serum IgE. In this study, we first characterized the influence of various *Lactobacillus* and *Bifidobacterium* strains on the antigen-specific IgE levels in serum by oral administration. After oral administration of various *Lactobacillus* and *Bifidobacterium* fermented milks to the OVA-IgE elevated mice, some of the *Lactobacillus* fermented milk showed good repressive effects on the serum OVA-IgE levels. Interestingly, significant differences were observed in only 3 days of administrations of the active fermented milk after serum OVA-IgE levels were elevated. Among them, *L. acidophilus* L92, *L. acidophilus* CP1613, and *L. fermentum* CP34 showed the most of significant OVA-IgE lowering effects in this test.

By the study of the IgE lowering effect of washed cells cultured in MRS medium, it is clear that the active component exists in the cells of active strains (Table 2). However, there was no dose-dependent effect of both strains in OVA-IgE lowering activities. A dosage of 1×10^6 cells of *L. acidophilus* L92 or *L. fermentum* CP34 strain seemed to be an excess for causing the OVA-IgE lowering effect in this study. So, the dosage of 1×10^9 cells used in the recovery tests for various strains was thought to be high for the binding capacity of GI tract. However, there were big differences in the recoveries of radiolabelled cells from mice GI tracts. *L. acidophilus* L92 and *L. fermentum* CP34 showed higher recoveries than that of the other strains in that comparative study. For more detailed study of recoveries for highly recovered strains, oral administrations of these strains with low dosage would be appropriate in future study. Results of the recovery study suggest that a direct interaction of *L. acidophilus* and *L. fermentum* strains with the mouse GI tract may be an important process in exerting immunomodulating effects in the host. However, identification and characterization of the major adhesion factor that may exist in the active strain would be necessary for more understanding of the molecular events occurring in the cell attachment to the GI tract.

Surface layer protein,⁷ lipoteichoic acid,¹¹ and other proteins¹⁶ were reported as adhesion factors to epithelial cells in some *Lactobacillus* strains. However, the active components that commonly exist in these active strains in this study, such as adhesion factors to the mouse GI tract have not yet been identified. The *L. acidophilus* group was classified into 6 subgroups, and of these, 4 groups were catego-

rized into group A and 2 groups were into group B.¹⁷ In this study, the two active *L. acidophilus* strains were members of group A. A more detailed comparative study of group A strain and group B strain of *L. acidophilus* may be helpful to understand differences among the tested lactobacilli and identify the active component in the cells. For a more extensive study for the active component, cell surface proteins and peptidoglycan that have affinity for the GI cells need to be identified.

Many of the immunomodulating effects of LAB, including regulation of antigen-specific serum IgE production, would be affected by IL4 and IL5, which are produced by T helper 2 (Th2) cells.¹² On the other hand, LAB induction of interferon (IFN)- γ production by Th1 cells has been reported.¹² The production of certain cytokines by Th1 and Th2 cells in response to the oral administration of *L. acidophilus* L92, *L. acidophilus* CP1613 and *L. fermentum* CP34 needs to be analyzed in more detail. Moreover, the results of the Th1/Th2 balance would be helpful to determine the potential of the active *Lactobacillus* strains for use in clinical studies.

Recently, a preventive effect of *L. rhamnosus* GG for early atopic disease was reported under a double-blind placebo controlled study by feeding of the strain for 6 months.¹⁴ However, no change in serum IgE was reported in that study. The preventive effect of the *L. rhamnosus* GG for atopic disease seemed to occur without serum IgE change. In our study, some of LAB strains that showed IgE lowering activities in this mouse study could be expected to express immunomodulating effect in human studies. From the recovery test and the adhesive activity of the radiolabeled lactobacilli, the importance of direct interaction of the LAB with the GI tract of mice was suggested in this study. In our previous study, *L. acidophilus* L92 strain showed long survival in a human volunteer study by feeding the fermented milk,¹⁸ and healthy volunteers showed improvements in gastrointestinal disorders.¹⁹ Therefore, the *L. acidophilus* L92 may have the potential to prevent allergic responses in human clinical trials as a probiotic strain.

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