



Efficacy of Oral Administration of a Heat-Killed *Lactobacillus gasseri* OLL2809 on Patients of Japanese Cedar Pollinosis with High Japanese-Cedar Pollen-Specific IgE

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A randomized, double-blind, placebo-controlled clinical trial was conducted to determine whether oral administration of heat-killed *Lactobacillus gasseri* OLL2809 would affect the immune response and reduce the symptoms of Japanese cedar pollinosis (JCP) in subjects with JCP. Following a 1-week pre-observation period, the subjects were randomly divided into two groups and were orally administered a placebo or tablets containing 100 mg of *L. gasseri* OLL2809 per d for 8 weeks during the pollen season in 2007. The results showed no obvious differences between the groups. Supplementary subgroup analysis revealed that the OLL2809 subgroups with CAP-RAST scores of 4 or 5 exhibited improvement in nasal symptoms scores and serum allergy-related items, including Japanese cedar pollen-specific IgE levels. *L. gasseri* OLL2809 was found to be effective in reducing symptoms in subjects with a high predisposition to allergies by modulating systemic immune systems.

Key words: Japanese cedar pollen; Japanese cedar pollinosis; *Lactobacillus gasseri*; probiotics; Th1/Th2

In the past few decades, the incidence of Japanese cedar pollinosis (JCP) has been increasing, and a current paper suggests that at least one-sixth of the Japanese population is affected by this allergic disease.¹⁾ Thus far, several medicines, including antihistamines, leukotriene inhibitors, anti-inflammatory cytokines, and corticosteroids, have been developed that greatly contribute to reduction of the symptoms of allergic disease.²⁻⁷⁾ However, because allergic diseases are chronic, continuous treatment is required, and the cost spent on treatment is enormous. In addition, most of the available treatments are symptomatic.⁸⁾ Therefore, in view of the high prevalence of JCP and the adverse effects that accompany long-term treatment,^{4,9)} more effective treatment methods are required.

The increase in the prevalence of allergic diseases has been explained by the hygiene hypothesis proposed by

Strachan.¹⁰⁾ Strachan suggested that limited exposure to bacterial and viral pathogens during early childhood leads to insufficient stimulation of the Th1 direction of the immune system and primes an overactive Th2 reaction, leading to allergic disease. The mechanisms underlying this phenomenon are considered to include defective maturation or an absence of regulatory T cells and an inappropriate Th1/Th2 balance.¹¹⁾ A number of reports suggest correlations between the incidence of allergic diseases and intestinal microbiota, which might serve as stimuli to develop appropriate immune systems.^{12,13)}

Lactobacilli are gram-positive anaerobic bacteria commensal to humans and animals.¹⁴⁾ For many years, they have been consumed worldwide through foods such as fermented milk. Consequently, they are known to be very safe microorganisms. Moreover, a recent double-blind placebo-controlled clinical study revealed that *Lactobacillus rhamnosus* GG administration suppressed the incidence of atopic diseases in high-risk children by approximately 50%.¹⁵⁾ Hence, the use of lactobacilli might be an easy and effective way to prevent or treating allergies without any side effects.

L. gasseri OLL2809, which was isolated from a human subject, has been selected from approximately 300 *Lactobacillus* strains on the basis of its immunoregulatory effect.¹⁶⁾ We have found using mouse experimental allergy models that when orally administered, heat-killed *L. gasseri* OLL2809 exhibit suppressive effects on antigen-specific IgE and eosinophilia via modulation of the Th1/Th2 balance.¹⁶⁻¹⁸⁾ This suggests that heat-killed *L. gasseri* OLL2809 can reduce the clinical symptoms of JCP. In this study, we examined the clinical efficacy of heat-killed *L. gasseri* OLL2809 as to JCP.

Methods

Subjects. Subjects ($n = 107$) aged 20 to 50 years were enrolled in the clinical study. Subjects with JCP who fulfilled the following criteria were enrolled: (i) subjects who experienced JCP symptoms for

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Abbreviations: JCP, Japanese cedar pollinosis; QOL, quality of life; SMS, symptom medication score; IFN, interferon; IL, interleukin

over 2 years, (ii) subjects with serum cedar pollen-specific IgE levels at scores 2 to 5 by the CAP-radioallergosorbent test (CAP-RAST score), (iii) subjects with a moderate total symptom score (comprising symptoms of sneezing, runny nose, nasal congestion, itchy and watery eyes, scored for JCP in the past 2 years) according to the "Practical Guidelines for the Management of Allergic Rhinitis in Japan," 5th edition.¹⁹⁾ Subjects who lived or worked in the suburbs of Tokyo were preferred as study subjects.

The following subjects were excluded from the study: (i) those whose symptoms had developed before the cedar pollen season, (ii) those with nasal disease that might have affected efficacy evaluation in this trial (perennial allergic rhinitis, acute and chronic rhinitis, *etc.*), (iii) those who planned to travel to Hokkaido, Okinawa, or abroad, and (iv) others whom the physician in charge judged unfit as study subjects.

Prior to participation, written consent was obtained from all the subjects after the physician in charge had explained the study to the group. In addition, the study received the approval of the Ethics Committee of the Division of Research and Development of Meiji Dairies Corporation, and it was performed in accordance with the Declaration of Helsinki.

Study design. The study was a randomized, double-blind, placebo-controlled clinical trial performed at a single institution in Tokyo between January 10, 2007 and April 6, 2007. The study protocol is summarized in Fig. 1. After obtaining informed consent from the subjects, they were screened to confirm compliance with the inclusion and exclusion criteria and to examine the physical condition of each individual. Subject background, clinical laboratory analysis (hematology, blood biochemistry, and serology), and physical examination were included in the screening.

Following a 1-week pre-observation period, the subjects were randomly divided into two groups: one group of subjects who were orally administered tablets containing 100 mg (approximately 1×10^{10} cells) of heat-killed (75°C for 60 min) *L. gasseri* OLL2809 per d and the other, who received placebo tablets. The placebo tablets contained dextrin instead of heat-killed *L. gasseri* OLL2809, and were identical in color and taste to the OLL2809 tablets. Each subject received tablets for an 8-week course (from February 5 to April 6).

On dividing the groups, a controller who was not directly involved in the study was responsible for group allocation. The subjects were divided randomly into the active (OLL2809) and placebo groups according to the total symptom scores for JCP in the past 2 years, the Japanese-cedar-pollen specific IgE not to be different between the groups. A group allocation number was given to each subject. To prevent leakage of information, this number was closely guarded jointly by the controller and a member of the ethical committee who was not directly involved in the study, until accessed with the key after completion of the study.

The physician in charge examined each subject a total of 3 times: during the pre-observation period, and 4 and 8 weeks after treatment. The subjects were asked to fill out the Japanese Allergic Rhinitis QOL standard Questionnaire (JRQLQ) during the pre-observation period, and 4 and 8 weeks after treatment. They were asked to record pollinosis symptoms (sneezing, runny nose, nasal congestion, itchy eyes, watery eyes, and interference with daily life) and compliance with the administration schedule in the subject diary.

Evaluation items.

Nasal cavity findings. Nasal examinations were conducted by rhinoscopy during the pre-observation period and after 4 and 8 weeks of treatment. Mucosal swelling of the inferior turbinate and the amounts of watery rhinorrhea were scored on a 4-point scale of severity (0 = none to 3 = severe).¹⁹⁾

Allergy diary. Allergy diaries were kept by the subjects to self-assess items from a list, including sneezing (number of attacks per d), runny nose (number of incidences of nose blowing per d), nasal congestion, and itchy and watery eyes. These symptoms were scored subjectively on a 5-point severity scale, where 0 indicated no symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; and 4, very severe symptoms.¹⁹⁾ The total scores for sneezing, runny nose, and nasal congestion were counted as the nasal symptom score, while the total scores for itchy and watery eyes were counted as the ocular symptom score. The medication score was

recorded as described elsewhere.¹⁹⁾ Severity during the season was scored daily as nasal or ocular symptom medication scores (SMS), and the mean SMS each week was compared between the placebo and OLL2809 groups.

Japanese allergic rhinitis QOL standard questionnaire (JRQLQ). JRQLQ was used for evaluation of the subjects' QOL during the pollen season. The questionnaire is composed of three parts: nasal and eye symptoms (JRQLQ-I), 17 questions regarding the QOL (JRQLQ-II), and a comprehensive evaluation (face scale).²⁰⁾

Nasal and ocular symptoms included the following six categories: runny nose, sneezing, nasal congestion, itchy nose, and itchy and watery eyes. The symptoms of each subject were evaluated on a 5-point scale, 0 denoting no symptoms; 1, mild; 2, moderate to severe; 3, severe; and 4, very severe.

The QOL-related questionnaire included 17 items concerning (i) reduced productivity at work/home; (ii) poor mental concentration; (iii) reduced thinking power; (iv) impaired reading book/newspaper; (v) reduced memory; (vi) limitation of outdoor life (*e.g.*, sports, picnic); (vii) limitation of going out; (viii) hesitation visiting friend or relatives; (ix) reduced contact with friends or others by telephone or conversation; (x) not an easy person to be around; (xi) impaired sleeping; (xii) tiredness; (xiii) fatigue; (xiv) frustration; (xv) irritability; (xvi) depression; and (xvii) unhappiness. Each item was evaluated on a 5-point scale, 0 denoting no significant problem; 1, a mild problem; 2, moderately severe; 3, severe; and 4, very severe.

Blood examination. Blood samples were collected 3 times: at the pre-observation period and after 4 and 8 weeks of treatment. The samples were used to determine the concentrations of total and Japanese cedar pollen-specific IgE levels and number of eosinophils. The ratio of Th1 to Th2 cells (Th1/Th2 ratio) was determined 2 times: in the pre-observation period and after 8 weeks of treatment. The blood examinations described above were performed at SRL (Tokyo).

Assessment of outcomes. The primary efficacy outcome was the difference in the nasal and the ocular symptom medication scores over the 8-week administration period between the OLL2809 and placebo groups. The secondary assessments were based on the QOL-related scores on JRQLQ at 4 and 8 weeks after treatment.

Statistical analysis. A blind data review was performed before decoding, and decisions concerning the handling of drop-outs were made on the basis of blinded results. The assessment of efficacy was based on all the subjects who completed the study. Subgroup analyses based on CAP-RAST scores were done additionally after decoding.

Data were expressed as mean $\pm$ SE. Statistical differences between the placebo and OLL2809 groups and subgroups were analyzed by Student's *t* test or Mann-Whitney's *U* test. The differences between pre-observation and 4 and 8 weeks after treatment were analyzed in each group and subgroup by Student's paired-*t* test with Bonferroni's correction. Differences were considered significant when the *p*-value was less than 0.05.

Results

Background characteristics of subjects and cedar pollen count

Among 107 subjects (placebo *n* = 54, OLL2809 *n* = 53) enrolled, seven subjects withdrew from the study citing personal reasons and 100 (placebo *n* = 53, OLL2809 *n* = 47) successfully completed it. The baseline characteristics of the subjects were similar in the placebo and OLL2809 groups in terms of age, sex, duration of JCP, nasal cavity findings, nasal and ocular symptom medication scores in the allergy diary, and a blood examination (data not shown).

The Japanese cedar pollen season started on January 31 and continued to the end of April in 2007. The total pollen count during the study period was 1,263 grains/cm² in Chiyoda-ku, central Tokyo, slightly higher than the pollen count for 2006 (874 grains/cm²)

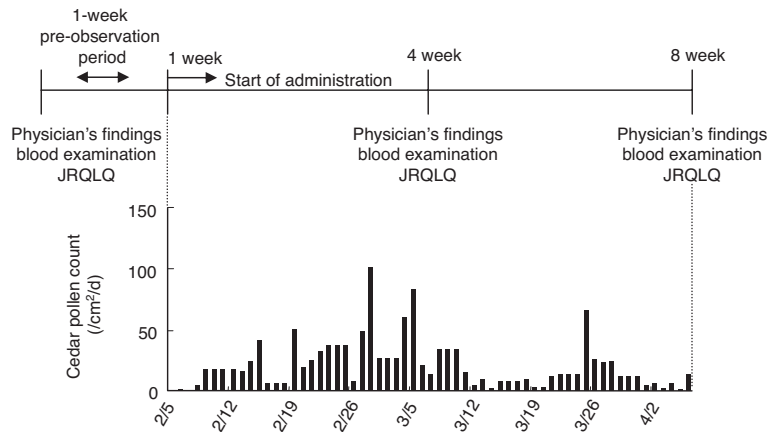


Fig. 1. Study Protocol and Diagrammatic Profile of Japanese Cedar Pollen Dispersion during the Clinical Trial in Tokyo, in 2007.

These data were obtained from the Bureau of Social Welfare and Public Health, Tokyo Metropolitan Government, by determining the pollen collected in a Durham pollen catcher on the roof of a building in Chiyoda Ward.

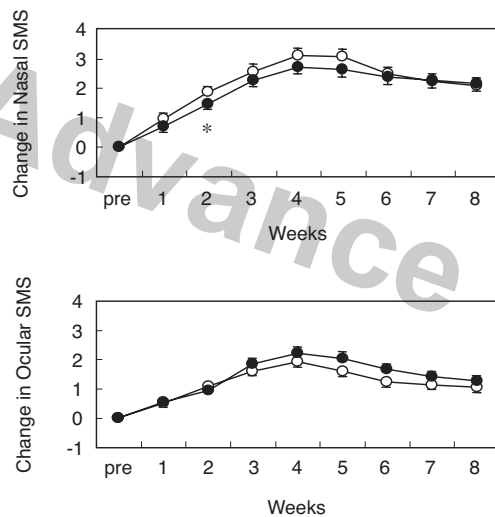


Fig. 2. Time-Course Change in Nasal and Ocular Symptom Medication Scores (SMS).

The scores are differences from those in the pre-observation period. Placebo group (○), $n = 53$; OLL2809 group (●), $n = 47$. * $p < 0.05$ (Mann-Whitney's U test).

(Fig. 1), according to a survey conducted by the Bureau of Social Welfare and Public Health of the Tokyo Metropolitan Government.

Analysis of subjects

The nasal cavity findings, blood examinations, and JRQLQ results revealed no significant differences between the placebo and OLL2809 groups that were administered *L. gasseri* OLL2809. Examination of the allergy diaries revealed that although ocular SMS did not differ between the groups, and nasal SMS at 2 weeks was significantly low ($p = 0.0409$) in the OLL2809 group as compared with the placebo group (Fig. 2). Overall, no efficacy of *L. gasseri* OLL2809 was observed in this study.

Subgroup analysis: analysis of high CAP-RAST score group

Type I allergic diseases are characterized by an elevation in serum antigen-specific IgE levels.²¹⁾ The CAP-RAST score of the subjects enrolled in this study

was 3.22 ± 0.80 (placebo group, 3.28 ± 0.77 , $n = 53$; OLL2809 group, 3.15 ± 0.83 , $n = 47$; $p = 0.3371$). Hence, each group was divided into two subgroups. The first subgroup consisted of subjects with CAP-RAST scores of 2 or 3, and the second group of subjects with CAP-RAST scores of 4 or 5. Subsequently, these subgroups were analyzed again to investigate the efficacy of *L. gasseri* OLL2809 on them. Although there were no significant differences between the placebo and OLL2809 subgroups with CAP-RAST scores of 2 or 3 (data not shown), *L. gasseri* OLL2809 was found to be effective, particularly in subgroups of subjects with CAP-RAST scores of 4 or 5, as described below.

Background characteristics of subjects with CAP-RAST scores of 4 or 5

The baseline characteristics of the subjects were similar in the placebo and OLL2809 subgroups, with CAP-RAST scores of 4 or 5, as well as those of the all subjects, in terms of age, sex, duration of JCP, nasal cavity findings, nasal and ocular symptom medication scores taken in allergy diaries and blood exams (Table 1).

Nasal cavity findings

Both the nasal cavity scores for mucosal swelling of the inferior turbinate and the amount of watery rhinorrhea significantly ($p < 0.01$) increased at 4 weeks in both the placebo and OLL2809 subgroups. However, no differences were observed between these subgroups. When the scores were summed and total scores were compared, the results tended to be low ($p = 0.0991$) in the OLL2809 subgroup at 4 weeks (Table 2). The scores decreased at 8 weeks as compared to those at 4 weeks, and no difference was observed between the subgroups.

Nasal and ocular SMS

Both nasal and ocular SMS increased and symptoms were exacerbated, with a peak at 4 to 5 weeks during the experimental period. While no differences were observed in the ocular SMS between the subgroups, the nasal SMS exhibited significantly ($p < 0.05$) lower values in the OLL2809 subgroup than in the placebo subgroup at 1, 5, 6, 7 and 8 weeks (Fig. 3).

JRQLQ

The scores for all the QOL items in the JRQLQ-I increased at 4 weeks, and then slightly decreased at 8

Table 1. Background Factors of Subjects with CAP-RAST Scores of 4 or 5

	Placebo (<i>n</i> = 19)	OLL2809 (<i>n</i> = 12)	<i>p</i> -value
Age (year)	30.3 ± 1.6	30.7 ± 2.1	0.8766 ^a
Sex (male:female)	8:11	6:6	0.9524 ^b
Duration of JCP (year)	10.3 ± 1.6	9.9 ± 1.4	0.8503 ^a
Mucosal swelling of the inferior turbinate	0.474 ± 0.177	0.750 ± 0.250	0.3273 ^c
Amount of watery rhinorrhea	0.211 ± 0.096	0.167 ± 0.112	0.7671 ^c
Total nasal finding scores	0.684 ± 0.242	0.971 ± 0.336	0.5015 ^c
Ocular symptom medication score	0.233 ± 0.085	0.036 ± 0.020	0.1066 ^c
Nasal symptom medication score	1.02 ± 0.27	1.17 ± 0.31	0.6245 ^c
Total-IgE (IU/ml)	281 ± 60	296 ± 87	0.8845 ^a
Japanese cedar pollen-specific IgE (UA/ml)	38.7 ± 4.3	42.6 ± 6.0	0.5887 ^a
Eosinophils (%)	2.58 ± 0.44	2.58 ± 0.34	0.9937 ^a
Th1/Th2 ratio	8.16 ± 0.60	8.77 ± 1.36	0.6486 ^a

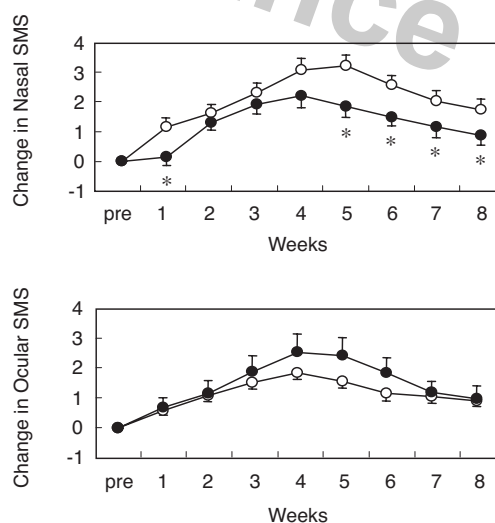
^{a,b,c} Analyzed by Student's *t*-test, Chi-square test, and Mann-Whitney's *U* test respectively.

Th1/Th2 ratio represents the proportion of IFN- γ ⁺CD4⁺ and IL-4⁺CD4⁺ peripheral mononuclear cells.

Table 2. Mean Change in Scores for Nasal Cavity Findings for Subjects with CAP-RAST Scores of 4 or 5 after 4 and 8 Weeks of Treatment

	4 weeks			8 weeks		
	Placebo	OLL2809	<i>p</i> -value	Placebo	OLL2809	<i>p</i> -value
Mucosal swelling of the inferior turbinate	0.842 ± 0.206	0.500 ± 0.230	0.3837	0.474 ± 0.160	0.417 ± 0.149	0.8018
Amount of watery rhinorrhea	0.895 ± 0.130	0.667 ± 0.142	0.2766	0.211 ± 0.123	0.500 ± 0.195	0.1411
Total nasal finding scores	1.737 ± 0.295	1.167 ± 0.271	0.0991	0.684 ± 0.242	0.917 ± 0.260	0.2478

The scores are differences from those in the pre-observation period. Total nasal finding scores represent sums of the scores for swelling of the inferior turbinate and amount of watery rhinorrhea. Placebo subgroup, *n* = 19; OLL2809 subgroup, *n* = 12.

**Fig. 3.** Time-Course Change in Nasal and Ocular Symptom Medication Scores (SMS) of Subgroups with CAP-RAST Scores of 4 or 5.

The scores are differences from those in the pre-observation period. Placebo subgroup (○), *n* = 19; OLL2809 subgroup (●), *n* = 12. **p* < 0.05 (Mann-Whitney's *U* test).

weeks. Although there was no significant difference in the scores between the subgroups at 4 weeks (data not shown), the scores for nasal congestion and itchy nose were significantly lower (*p* = 0.009 and 0.0156 respectively), and the frequency of the subjects who exhibited no symptoms was higher in the OLL2809 subgroup at 8 weeks (Fig. 4).

Of the 17 items in JRQLQ-II, the scores for reduced memory, reduced contact with friends or others by telephone or conversation at 4 weeks and for tiredness at 8 weeks tended to be low (*p* = 0.0666, 0.0934, and 0.0848 respectively) in the OLL2809 subgroup

(Table 3). Furthermore, of the 17 items, scores at lower values were observed in 15 items in the OLL2809 subgroup at 8 weeks. Although the subjects responded to the questionnaires at 4 and 8 weeks, they were queried regarding the severity of their symptoms during the 1–2 weeks preceding the time of response. Therefore, these scores at 8 weeks represent symptoms occurring at 6 to 7 weeks, and they correspond well with the nasal SMS recorded in the allergy diaries.

Blood examination

At 4 weeks, all the blood examination items, including the total IgE and Japanese cedar pollen-specific IgE levels, numbers of eosinophils, and the Th1/Th2 ratio, increased, with a peak at 4 weeks, but no differences were observed between the placebo and OLL2809 subgroups. When we analyzed the relative values where the mean values in each subgroup at the pre-observation period were expressed as 1.0, the Japanese cedar pollen-specific IgE levels in the OLL2809 subgroup tended to be lower than in the placebo subgroup at 4 weeks (*p* = 0.0525, Fig. 5). While there were no intra-subgroup differences even in the relative values of eosinophils, intra-period analysis in each subgroup revealed that although this value significantly (*p* < 0.05) increased in the placebo subgroup at 8 weeks as compared with the pre-observation period, this increase was not significant in the OLL2809 subgroup (*p* = 0.2104). Further, the Th1/Th2 ratio tended to increase in the OLL2809 subgroup but not in the placebo subgroup as compared with the respective values for the pre-observation period (*p* = 0.0687). Total IgE did not significantly change between the subgroups during the study period.

Safety

No adverse effects were observed throughout the study. No significant changes in blood or biochemistry

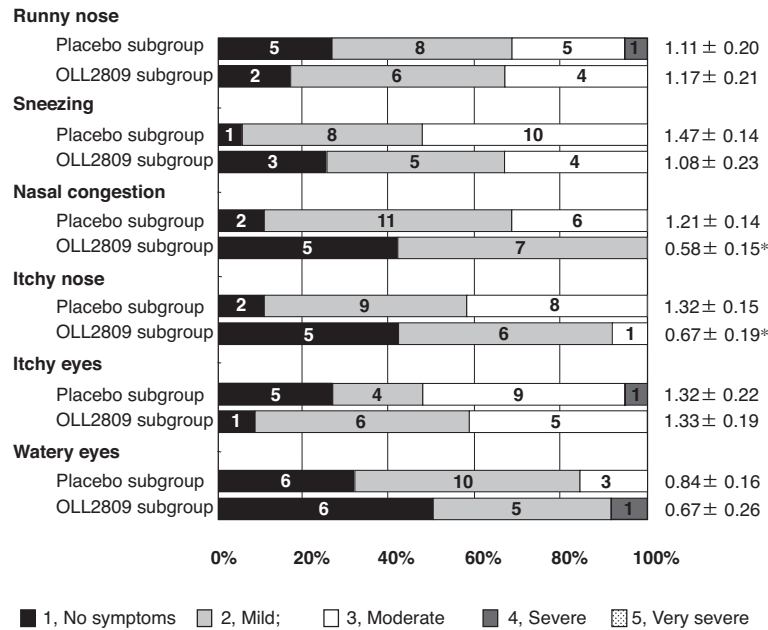


Fig. 4. Japanese Allergic Rhinitis Standard Quality of Life Questionnaire for Subgroups with CAP-RAST Scores of 4 or 5 after 8 Weeks of Treatment.

The numbers in the histogram represent the numbers of subjects, and the mean scores ± SE are presented on the right. Placebo subgroup, $n = 19$; OLL2809 subgroup, $n = 12$. * $p < 0.05$ (Mann-Whitney's U test).

Table 3. Mean Change in JRQLQ-II Scores of Subgroups with CAP-RAST Scores of 4 or 5 after 4 and 8 Weeks of Treatment

	4 weeks			8 weeks		
	Placebo	OLL2809	<i>p</i> -value	Placebo	OLL2809	<i>p</i> -value
1. Reduced productivity at work/home	1.158 ± 0.220	0.667 ± 0.225	0.2142	0.632 ± 0.191	0.333 ± 0.188	0.3555
2. Poor mental concentration	1.158 ± 0.206	0.833 ± 0.271	0.4170	0.632 ± 0.157	0.583 ± 0.229	>0.999
3. Reduced thinking power	1.053 ± 0.223	0.833 ± 0.271	0.6399	0.632 ± 0.175	0.417 ± 0.229	0.5180
4. Impaired reading book/newspaper	0.842 ± 0.206	0.417 ± 0.193	0.5083	0.526 ± 0.160	0.333 ± 0.142	0.5083
5. Reduced memory	0.789 ± 0.224	0.167 ± 0.167	0.0666	0.316 ± 0.134	0.083 ± 0.149	0.3137
6. Limitation of outdoor life	0.842 ± 0.175	0.417 ± 0.228	0.1014	0.579 ± 0.221	0.333 ± 0.225	0.6341
7. Limitation of going out	1.053 ± 0.235	0.750 ± 0.279	0.4114	0.579 ± 0.176	0.417 ± 0.193	0.5745
8. Hesitation visiting friend or relatives	0.789 ± 0.196	0.417 ± 0.260	0.4558	0.421 ± 0.139	0.250 ± 0.131	0.4558
9. Reduced contact with friends or others by telephone or conversation	0.632 ± 0.175	0.250 ± 0.131	0.0934	0.316 ± 0.134	0.250 ± 0.131	0.8731
10. Not an easy person to be around	0.632 ± 0.191	0.250 ± 0.131	0.8672	0.263 ± 0.129	0.250 ± 0.131	0.8672
11. Impaired sleeping	0.421 ± 0.221	0.750 ± 0.372	0.4932	0.316 ± 0.134	0.333 ± 0.142	0.7584
12. Tiredness	1.000 ± 0.229	1.000 ± 0.348	0.8301	0.737 ± 0.185	0.250 ± 0.131	0.0848
13. Fatigue	0.789 ± 0.211	1.083 ± 0.358	0.5735	0.579 ± 0.221	0.333 ± 0.142	0.5817
14. Frustration	0.789 ± 0.224	0.917 ± 0.358	0.9828	0.316 ± 0.154	0.417 ± 0.149	0.5548
15. Irritability	0.842 ± 0.191	1.000 ± 0.326	0.8251	0.368 ± 0.114	0.250 ± 0.131	0.4991
16. Depression	0.737 ± 0.200	0.833 ± 0.366	0.8419	0.263 ± 0.129	0.250 ± 0.131	0.8814
17. Unhappiness	0.759 ± 0.192	0.833 ± 0.366	0.8423	0.211 ± 0.164	0.333 ± 0.142	0.7151

The scores are differences from those in the pre-observation period.
Placebo subgroup, $n = 19$; OLL2809 subgroup, $n = 12$.

results were observed during the study period in any of the subjects.

Discussion

In recent years, a number of clinical trials have evaluated the efficacy of probiotics and of heat-killed lactobacilli in allergic diseases. The results imply that some clinical effects on pollinosis,^{22–25)} atopic dermatitis,^{26,27)} perennial allergic rhinitis induced by house-dust mites,²⁸⁾ and food allergy²⁹⁾ occurred. On the other hand, Brouwer *et al.* and Grüber *et al.* reported that there were no clinical or immunological effects of probiotic

L. rhamnosus GG, which was used in infants with atopic dermatitis.^{30,31)} Such diverse results were due to differences in study design, and perhaps were due to heterogeneity of exposure to the allergens, the allergic backgrounds of the subjects, and the efficacy of the microorganisms used. Hence, further studies are required to determine the efficacy of probiotics and of lactic acid bacteria with immunoregulatory activity.

Although our clinical study had a substantial sample size, no obvious efficacy of *L. gasseri* OLL2809 was observed. Hence, we performed subgroup analyses based on the CAP-RAST scores. Because serum antigen-specific IgE levels play a crucial role in the

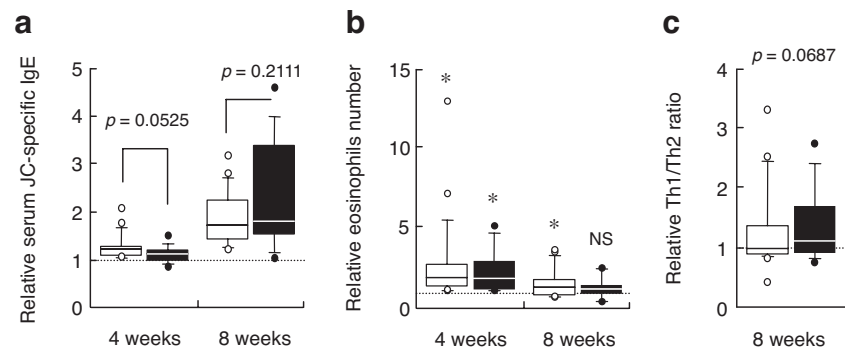


Fig. 5. Relative Changes in Serum Japanese Cedar Pollen-Specific IgE (a), Number of Peripheral Blood Eosinophils (b), and the Th1/Th2 Ratio (c) in Subgroups of Subjects with CAP-RAST Scores of 4 or 5.

Data are represented by their relative values where the mean values in each subgroup at the pre-observation period are expressed as 1.0 (broken line). The differences in the relative serum Japanese cedar pollen-specific IgE and relative Th1/Th2 ratio between the placebo and OLL2809 subgroups were analyzed by Student's *t*-test. Those in the relative eosinophil numbers between pre-observation and 4 and 8 weeks after treatment were analyzed in each group by Student's paired *t*-test with Bonferroni's correction (**p* < 0.05). NS, not significant. Placebo subgroup (open bars), *n* = 19; OLL2809 subgroup (solid bars), *n* = 12.

onset of symptoms in type I allergic diseases, it is possible that subjects with high CAP-RAST scores have a higher predisposition to allergic diseases. The results of the subgroup analyses revealed that subjects with CAP-RAST scores of 4 or 5 exhibited the efficacy of *L. gasseri* OLL2809: it caused a significant reduction in the nasal SMS throughout the administration period, and the nasal symptoms in JRQLQ at 8 weeks. In addition, the relevant evaluation items such as clinical scores for the nasal cavity at 4 weeks and the serum allergy-related items, including the Japanese cedar pollen-specific IgE levels, eosinophils, and the Th1/Th2 ratio improved, though the differences were not statistically significant. There was a difference in that efficacies were observed for each item, *e.g.*, the nasal cavity findings tended to be low in the OLL2809 subgroup at 4 weeks, but other parameters such as nasal SMS, nasal congestion, and itchy nose in JRQLQ-I were lower in the OLL2809 subgroup in the later period (5–8 weeks). This might have been caused by a difference in objective and subjective evaluation. For instance, even if an objective examination item is improved, subjective symptoms are not necessarily improved, and *vice versa*. This sometimes occurs in clinical examination. Yet, considering these data comprehensively, it was assumed that *L. gasseri* OLL2809 mainly ameliorated the nasal symptoms of the subjects with CAP-RAST scores of 4 or 5, *via* affecting the immune systems.

Similar results have been reported for *L. casei* strain Shirota. Tamura *et al.* reported that supplementation with *L. casei* strain Shirota did not affect the nasal or ocular SMS, but it tended to reduce the nasal SMS in the subgroup of subjects with moderate to severe nasal symptom scores prior to start of ingestion of the test samples.²⁵⁾ Type I allergic diseases such as JCP are associated with elevated serum antigen-specific IgE. Binding of inhaled allergens to IgE on the surfaces of basophils and mast cells, with subsequent cross-linkage of IgE and aggregation of high-affinity receptors for IgE (FcεRI), triggers the release of histamine, leukotrienes, and other inflammatory mediators, followed by the onset of allergic symptoms.³²⁾ Consequently, there must be a correlation between the symptoms and the Japanese cedar pollen-specific IgE levels.

Here, we hypothesize the mechanism by which *L. gasseri* OLL2809 exhibited efficacy in the subjects with CAP-RAST scores of 4 or 5. It has been widely reported that some *Lactobacillus* strains, such as *L. gasseri* OLL2809, stimulate IL-12 (p70) production by immune competent cells, and that this promotes a shift in the Th1/Th2 balance from Th2 toward Th1.^{16–18)} This immunoregulatory effect in the Th1/Th2 balance is observed as certain lactobacilli induce IFN-γ production and reduce IL-4 production by CD4⁺ T cells.^{16,33)} However, this effect occurs when antigen-sensitized CD4⁺ T cells are stimulated by antigens. For instance, when CD4⁺ transgenic T cells expressing ovalbumin-specific T-cell receptors were cultured with *Mycobacterium tuberculosis* in the absence of ovalbumin, they did not produce substantial levels of IFN-γ or IL-4 as compared with those cultured in the presence of both *M. tuberculosis* and ovalbumin.³⁴⁾ Likewise, stimulation of IFN-γ production and suppression of IL-4 production by *L. gasseri* OLL2809 are observed specifically in antigen-sensitized CD4⁺ T cells when the antigen is present in the cell culture, whereas it does not occur in non-sensitized CD4⁺ T cells even in the presence of the antigen (Sashihara *et al.*, unpublished observation). This suggests that the immunostimulatory effect of microbes in shifting the Th1/Th2 balance from Th2 to Th1 can be effective when the host immune cells are highly sensitized and the antigen level is sufficient to stimulate antigen-sensitized CD4⁺ T cells. In the present clinical trial, it is hard to assume that there was a difference in the amount of cedar pollen exposed in the subjects. Therefore, the observation that *L. gasseri* OLL2809 was effective in the subjects with CAP-RAST scores of 4 or 5 suggests that their CD4⁺ T cells were highly sensitized to the antigen, and that consequently the reactivity of the immune cells to *L. gasseri* OLL2809 was higher than those from subjects with CAP-RAST scores of 2 or 3.

In conclusion, although no obvious clinical efficacy of heat-killed *L. gasseri* OLL2809 was observed in subjects with JCP, this strain possesses efficacy to ameliorate symptoms by modulating the systemic immune responses in subjects with a high predisposition to allergic disease.

References

- 1) Okuda M, *Ann. Allergy Asthma Immunol.*, **91**, 288–296 (2003).
- 2) Bousquet J, van Cauwenberge P, and Khaltaev N, Aria Workshop Group, World Health Organization, *J. Allergy Clin. Immunol.*, **108**, S147–S334 (2001).
- 3) Creticos PS, *Drugs*, **63**, 1–20 (2003).
- 4) Okubo K, Gotoh M, Shimada K, Ritsu M, Okuda M, and Crawford B, *Int. Arch. Allergy Immunol.*, **136**, 148–154 (2005).
- 5) Okubo K and Gotoh M, *Allergol. Int.*, **55**, 261–269 (2006).
- 6) Kaliner MA, *Ann. Allergy Asthma Immunol.*, **99**, 383–390 (2007).
- 7) Krouse JH, *Otolaryngol. Clin. North Am.*, **41**, 347–358 (2008).
- 8) Simons FE, *N. Engl. J. Med.*, **351**, 2203–2217 (2004).
- 9) Juniper EF, Ståhl E, Doty RL, Simons FE, Allen DB, and Howarth PH, *J. Allergy Clin. Immunol.*, **115**, S390–S413 (2005).
- 10) Strachan DP, *Br. Med. J.*, **299**, 1259–1260 (1989).
- 11) Rautava S, Ruuskanen O, Ouwehand A, Salminen S, and Isolauri E, *J. Pediatr. Gastroenterol. Nutr.*, **38**, 378–388 (2004).
- 12) Björkstén B, Sepp E, Julge K, Voor T, and Mikelsaar M, *J. Allergy Clin. Immunol.*, **108**, 516–520 (2001).
- 13) Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, and Isolauri E, *J. Allergy Clin. Immunol.*, **107**, 129–134 (2001).
- 14) Felis GE and Dellaglio F, *Curr. Issues Intest. Microbiol.*, **8**, 44–61 (2007).
- 15) Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, and Isolauri E, *Lancet*, **357**, 1076–1079 (2001).
- 16) Sashihara T, Sueki N, and Ikegami S, *J. Dairy Sci.*, **89**, 2846–2855 (2006).
- 17) Sashihara T, Sueki N, Furuichi K, and Ikegami S, *Int. J. Food Microbiol.*, **120**, 274–281 (2007).
- 18) Sashihara T, Ikegami S, Sueki N, Yamaji T, Kino K, Taketomo N, Gotoh M, and Okubo K, *Allergol. Int.*, **57**, 397–403 (2008).
- 19) Committee of practical guideline for management of Allergic rhinitis, “Practical Guideline for the Management of Allergic Rhinitis in Japan” 5th edn. (in Japanese), Life Science, Tokyo, pp. 18–30 (2005).
- 20) Okuda M, Okubo K, Gotoh M, Okamoto Y, Imano A, Baba K, Ogino T, Ishikawa K, Takenaka Y, So N, Imai T, Enomoto M, Mandai T, and Crawford B, *Arerugi* (in Japanese), **52** (Suppl. 1), 21–56 (2003).
- 21) Dreborg S, *Ann. Allergy Asthma Immunol.*, **89**, 83–85 (2002).
- 22) Ishida Y, Nakamura F, Kanzato H, Sawada D, Hirata H, Nishimura A, Kajimoto O, and Fujiwara S, *J. Dairy Sci.*, **88**, 527–533 (2005).
- 23) Xiao JZ, Kondo S, Yanagisawa N, Takahashi N, Odamaki T, Iwabuchi N, Iwatsuki K, Kokubo S, Togashi H, Enomoto K, and Enomoto T, *J. Investig. Allergol. Clin. Immunol.*, **16**, 86–93 (2006).
- 24) Xiao JZ, Kondo S, Yanagisawa N, Takahashi N, Odamaki T, Iwabuchi N, Miyaji K, Iwatsuki K, Togashi H, Enomoto K, and Enomoto T, *Clin. Exp. Allergy*, **36**, 1425–1435 (2006).
- 25) Tamura M, Shikina T, Morihana T, Hayama M, Kajimoto O, Sakamoto A, Kajimoto Y, Watanabe O, Nonaka C, Shida K, and Nanno M, *Int. Arch. Allergy Immunol.*, **143**, 75–82 (2007).
- 26) Rosenfeldt V, Benfeldt E, Nielsen SD, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppensen DL, Valerius NH, and Paerregaard A, *J. Allergy Clin. Immunol.*, **111**, 389–395 (2003).
- 27) Roessler A, Friedrich U, Vogelsang H, Bauer A, Kaatz M, Hipler UC, Schmidt I, and Jahreis G, *Clin. Exp. Allergy*, **38**, 93–102 (2008).
- 28) Peng G-C and Hsu C-H, *Pediatr. Allergy Immunol.*, **16**, 433–438 (2005).
- 29) Majamaa H and Isolauri E, *J. Allergy Clin. Immunol.*, **99**, 179–185 (1997).
- 30) Brouwer ML, Wolt-Plompen SA, Dubois AE, van der Heide S, Jansen DF, Hoijer MA, Kauffman HF, and Duiverman EJ, *Clin. Exp. Allergy*, **36**, 899–906 (2006).
- 31) Grüber C, Wendt M, Sulser C, Lau S, Kulig M, Wahn U, Werfel T, Niggemann B, and Sulser C, *Allergy*, **62**, 1270–1276 (2007).
- 32) Ishizaka T and Ishizaka K, *Prog. Allergy*, **34**, 188–235 (1984).
- 33) Shida K, Makino K, Morishita A, Takamizawa K, Hachimura S, Ametani A, Sato T, Kumagai Y, Habu S, and Kaminogawa S, *Int. Arch. Allergy Immunol.*, **115**, 278–287 (1998).
- 34) Sano K, Haneda K, Tamura G, and Shirato K, *Am. J. Respir. Cell Mol. Biol.*, **20**, 1260–1267 (1999).