

ORIGINAL PAPER

Probiotics in the treatment of Japanese cedar pollinosis: a double-blind placebo-controlled trial

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Clinical and Experimental Allergy

Summary

Background Probiotic bacteria may be effective in the treatment of allergic inflammation and food allergy, but efficacy and underlying mechanisms remain unclear.

Objective The present study investigated the effects of probiotic strain *Bifidobacterium longum* BB536 in the treatment of Japanese cedar pollinosis (JCPsis).

Methods In a randomized, double-blind, placebo-controlled trial, 44 JCPsis subjects received BB536 or placebo for 13 weeks during the pollen season. Subjective symptoms and self-care measures were recorded daily and blood samples were taken before and during intervention to measure blood levels of parameters related to JCPsis.

Results BB536 intake was associated with a significant reduction in number of subjects prematurely terminated due to severe symptoms and pollinosis medication ($P = 0.0057$ vs. placebo group). Comparison of subjective symptom scores indicated significant decreases in rhinorrhea, nasal blockage and composite scores in the BB536 group compared with the placebo group. Comparison of medical scores showed marked improvements in all symptoms on BB536 intake. A T-helper type 2 (Th2)-skewed immune response occurring along with pollen dispersion was observed. BB536 significantly suppressed increases in plasma thymus- and activation-regulated chemokine and tended to suppress elevations of Japanese cedar pollen (JCP)-specific IgE.

Conclusion These results suggest the efficacy of BB536 in relieving JCPsis symptoms, probably through the modulation of Th2-skewed immune response.

Keywords allergy, *Bifidobacterium longum*, cytokine, eosinophil, Japanese cedar pollinosis, probiotic, thymus and activation-regulated chemokine (TARC)

Submitted 31 March 2006; revised 2 July 2006; accepted 14 August 2006

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Introduction

Japanese cedar pollinosis (JCPsis) is an IgE-mediated type I allergy caused by exposure to Japanese cedar (*Cryptomeria japonica*) pollen (JCP). JCPsis is one of the most common allergic diseases in Japan, representing a public health issue affecting >16% of the Japanese population, with increasing prevalence over recent decades [1].

Association of allergy development with intestinal microflora has been suggested from several studies. Studies on composition of the intestinal microflora between allergic and non-allergic 2-year-old children have indicated that the prevalence of bifidobacteria is lower in

allergic infants, whereas counts of *Staphylococcus aureus* and enterobacteria are higher [2]. In comparison with healthy infants, babies with allergies less often display colonization with enterococci during the first month of life and with bifidobacteria during the first year of life [3]. It has been reported in numerous studies that the intake of probiotics [4], such as lactobacilli and bifidobacteria, is associated with the prevention of several allergic diseases from *in vitro* or animal studies [5–11] and human trials [12–17]. These reports indicate that specific probiotics have the potential to modulate and regulate the immune response, but for other strains also conflicting results have been reported [18]. Thus, the property appears to be

strain-specific and further study is needed for each of specific strains.

In 2004, we performed a human trial to investigate the effects of yogurt supplemented with *Bifidobacterium longum* BB536 in the treatment of JCPsis during the pollen season. We found that some of the subjective symptoms were alleviated following BB536 intake, while decreased IFN- γ levels were suppressed particularly at the early stage of pollen season [19]. Furthermore, fecal microflora significantly fluctuated during the pollen season and BB536 intake was found to suppress the changes of microflora, such as the increases in *Bacteroides fragilis* during the pollen season (unpublished results).

As 2004 was a mild season for JCP dispersion and the effects of BB536 were tested using yogurt containing other ordinary lactic acid bacteria, the efficacy of BB536 alone in a normal or heavy pollen season needed to be investigated. We therefore performed a trial using BB536 lyophilized powder in the JCP season of 2005, which happened to be a heavy season, to confirm the efficacy of BB536 in the treatment of JCPsis. Herein we report the effects of BB536 intake on clinical symptoms and blood markers from a double-blind, placebo-controlled trial using BB536 or placebo powder. Analyses of fecal microbiota will be reported in another paper.

Materials and methods

Participants

A total of 44 adult volunteers (26 men, 18 women) with a clinical history of JCPsis were recruited from the area around Kanagawa Prefecture in this intervention. Participants were recruited on the basis of a >2-year clinical history of JCPsis and the presence of serum JCP-specific IgE. No subject had received preventive JCPsis medication for the study season before the start of the intervention. Historical clinical severity of JCPsis for each participant was self-evaluated on a four-point scale (1, mild, endurable without prescribed medication; 2, moderate, taking prescribed medication from time to time; 3, severe, taking prescribed medication almost daily; and 4, extremely severe, unendurable without daily prescribed medication). As use of prescribed pollinosis medication was set as a dropout criterion, participants with a self-reported score of four were excluded from this study to avoid unnecessary dropouts.

In our previous study, as a pilot study, 40 subjects were enrolled to evaluate the effects of yogurt supplemented with BB536 compared with placebo yogurt, which contained ordinary lactic acid bacteria [19]. BB536 yogurt alleviated some of the subjective symptoms and suppressed blood eosinophil rate increases and IFN- γ decreases compared with placebo yogurt. In the present study, lyophilized powder was employed to study the

effects of BB536 in the treatment of JCPsis compared with an inactive control (placebo powder composed of mainly dextrin). As a more distinct comparison was expected in the present study, the sample size was expected to have more power than the previous yogurt trial.

All participants provided written informed consent. All study protocols were approved and controlled by the Local Ethics Committee of NPO (non-profit organization) Japan Health Promotion Supporting Network, Wakayama, Japan, and the Local Ethics Committee of Morinaga Milk Industry Co. Ltd. (Tokyo, Japan).

Sample intake

A randomized, double-blind, placebo-controlled trial was designed. After a 2-week run-in period, participants were randomized into two groups according to computer-generated permuted-block randomization. Each group was then randomized to receive placebo powder (only internal matrix, mainly dextrin) or BB536 powder (approximately 5×10^{10} colony-forming units (CFUs)/2 g) twice daily. The dose used in the present study was approximately 2–10-folds higher than those reported in the literatures [12–17]. BB536 and placebo powders looked and tasted identical. To improve the survivability of probiotics in delivery to the intestinal tract, participants were instructed to ingest samples together with ultra-high temperature pasteurized milk by suspending the powder in 100 mL of milk before ingestion. Strain BB536 was initially isolated from a healthy baby in 1969 [20]. Many physiological effects have been reported [21] and it has been used commercially for various food applications in several countries.

In 2005, the legitimate onset of the pollen season of Japanese cedar was recorded in the middle of February in the study region of Kanagawa Prefecture. However, a small amount of pollen scattering was observed from the beginning of January (pre-season). The pollen season lasted to the end of April, with a peak season at the middle of March (Fig. 1). Total amount of pollen scattered in the 2005 season was about 3- to 4-fold higher than the average of the previous 10 years. Sample intake started on 20 January 2005, approximately 4 weeks before the legitimate onset of the pollen season, and continued for 13 weeks, until 21 April 2005 (Fig. 1).

Participant monitoring

During the study, participants were instructed to complete a questionnaire on compliance, subjective symptoms, self-care measures, medication and health conditions. Nasal blockage and nasal itching, eye symptoms (itching, redness and watering) and throat symptoms (redness and pain) were evaluated on a scale from 0 to 4 in accordance with the guidelines of the Nasal Allergy Clinic 2002,

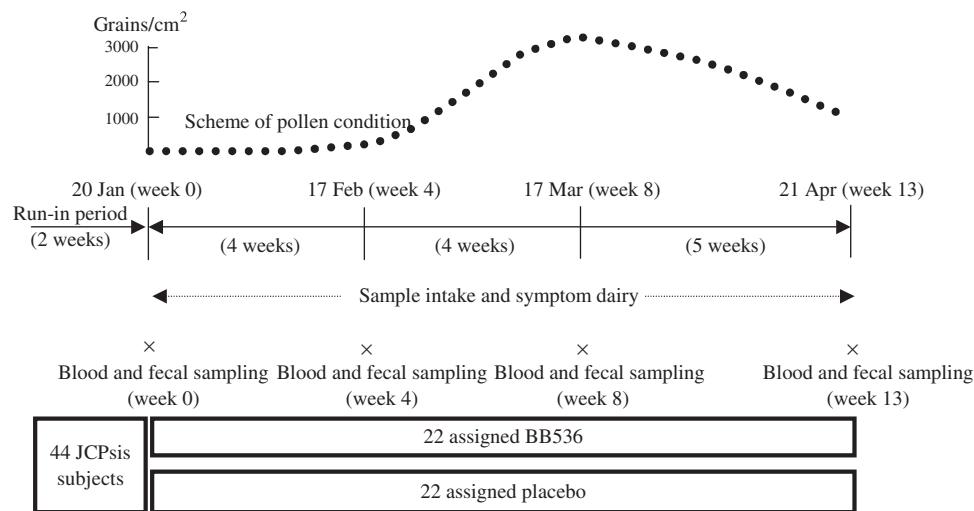


Fig 1. Study protocol and diagrammatic profile of pollen dispersion during the intervention.

Japan, as follows: 0, no sensation; 1, mild; 2, moderate; 3, severe; and 4, extremely severe. Episodes of sneezing and nose blowing (rhinorrhea) per day were rated from 0 to 4 as follows: 0, none; 1, 1–5 episodes; 2, 6–10 episodes; 3, 11–20 episodes; and 4, >21 episodes).

Participants were allowed to try self-care measures such as wearing a mask, eye drops or local nasal sprays when they felt the need, and were asked to record use of these items daily. Mask wearing was recorded on a 'yes or no' basis, and eye drops and local nasal sprays were reported as number of used per day. Use of prescribed medicines for internal use, which was set as a dropout criterion, was permitted when the subject felt the necessity, but further symptom evaluation and blood sampling was terminated and these participants were treated as prematurely terminated. The use of other medications and the presence of a fever or cold were also recorded daily on a 'yes or no' basis.

Blood sampling

Physical examination and blood sampling were performed before and during (weeks 4, 8 and 13) intervention. Two participants in the placebo group did not attend blood sampling due to personal reasons. Blood samples were drawn from an antecubital vein after overnight fasting. Analysis of collected blood samples was performed by Mitsubishi BCL Co. (Tokyo, Japan). Serum total IgE antibodies and JPC-specific IgE antibodies were analysed using the UniCap system (Diagnostic, Uppsala, Sweden). Plasma cytokine and chemokine levels were quantified using commercially available ELISA kits [human IFN- γ ELISA: Bender Medsystems, Vienna, Austria; human IL-10 UltraSensitive: BioSource International, Camarillo, CA, USA; human thymus- and activation-regulated chemokine (TARC) ELISA: R&D Systems, Minneapolis, MN, USA]. IFN- γ and IL-10 were measured

as T-helper type 1 (Th1)-type and regulatory cytokines, respectively. TARC was measured as Th2-type marker as Th2-type cytokines such as IL-4 and IL-5 were below detection limits. Peripheral blood cell counts were performed using an autoanalyser.

Statistical analysis

All statistical analyses were performed using SAS statistical software version 9.1.3 (SAS Institute, Cary, NC, USA). This study was conducted in a double-blinded manner whereby subjects and investigators assessing outcomes were unaware of subject randomization. The treatment code was opened after data had been collected and edited. Primary outcome measures for assessment of BB536 efficacy were the scores of subjective symptoms. Subjective symptom scores were recorded daily and expressed as weekly totals (scale, 0–28 each). Weekly total scores for sneezing, rhinorrhea, nasal blockage, nasal itching, eye symptoms and throat symptoms were totaled as composite scores (scale, 0–168).

The distribution of times to events of the premature termination in subjects due to severe symptom and taking pollinosis medicines was estimated by the Kaplan–Meier method and the log-rank test was used to evaluate the difference of the distribution between groups. For the composite score and each of the subjective symptoms, efficacy data were analysed in two ways.

First, each weekly individual symptom score and the composite score between weeks 5 and 13 (corresponding to the pollen season) were analysed by constructing mixed models for repeated-measures analysis of covariance, including treatment effect, time effect, and week 1 symptom score as baseline data. The data were analysed as continuous variables and log-transformed data was used for the analysis to alleviate the deviation from the normal

distribution. The covariance structure employed in the present study was the first-order ante-dependence structure, which allows for unequal variances over time and the correlations between pairs of observations are the product of the correlations between adjacent times between observations. The covariance structure was chosen based on Akaike Information Criteria. For prematurely terminated subjects, scores up to premature termination were evaluated by the mixed model and scores after termination were treated as lost data.

Second, to compare scores over the entire treatment phase, medical scores were calculated for prematurely terminated subjects by substituting scores after termination with the most severe scores recorded by continuously participating subjects during the same stage. As the distribution of medical scores was highly skewed, mixed models for repeated measurements analysis of covariance was inapplicable in this case. Alternatively, an area-under-the curve (AUC) analysis was performed for each medical score. Symptom scores were averaged within each week for each subject and changes in score compared with week 1 were calculated. AUCs were then calculated for each subject using changes in score for weeks 5–13 (pollen season). AUCs were then analysed as non-parametric data using Mann–Whitney *U*-test.

Blood data were expressed as geometric means (95% confidence interval) for measured values. Differences from baseline were calculated as ratios relative to week 0 and were expressed as geometric means (95% confidence interval). Blood data after termination of prematurely terminated subjects were treated as lost data. As distributions of some blood markers were skewed, comparisons were made after logarithmic transformation. As the sample size is relatively small especially at the late stage of the intervention due to early termination, normal distribution was not obtained on some data even after logarithmic transformation, the Mann–Whitney *U*-test and Wilcoxon test were used to analyse between-group and within-group differences, respectively. Limit values were used in analyses of blood parameters for data under detection limits. Values of $P < 0.05$ were considered statistically significant.

Results

Baseline characteristics of participants and continued participation

Baseline characteristics of JCPsis subjects were similar in placebo and BB536 groups (Table 1). Symptom scores at week 1 were quite balanced for the two groups for each subjective symptoms and composite score (Table 1).

Owing to the heavy pollen dispersion, nine of 22 subjects in the placebo group (including one not participated in blood sampling) and two of 22 subjects in the

Table 1. Subject characteristics

	Placebo group	BB536 group
Number of participants	22	22
Age (mean $\pm$ SD) (years)	36.0 $\pm$ 7.3	36.5 $\pm$ 8.1
Range (years)	(22–48)	(26–57)
Gender		
Male	$n = 13$	$n = 13$
Female	$n = 9$	$n = 9$
Clinical history of JCPsis		
Years (mean $\pm$ SD)	11.9 $\pm$ 5.9	11.2 $\pm$ 8.1
Range (years)	(3–20)	(2–30)
Severity (mean $\pm$ SD)	2.1 $\pm$ 0.8	1.9 $\pm$ 0.9

JCPsis, Japanese cedar pollinosis.

BB536 group took prescribed medicines for internal use and were thus prematurely terminated from the intervention (Fig. 2). Compared with the placebo group, BB536 intake significantly reduced the number of prematurely terminated subjects ($P = 0.0056$, log-rank test). No participants left the trial prematurely due to adverse effects or other factors (such as poor compliance or moving away) or because they failed to finish the questionnaire.

Changes in subjective symptoms along with pollen dispersion and clinical effects of *Bifidobacterium longum* BB536 intake

Figure 3 shows mean scores for subjective symptoms in each group when scores after termination of prematurely terminated subjects were treated as lost data. Along with pollen prevalence, subjective symptom scores increased markedly from the middle of February and peaked from the middle of March to the middle of April. Subjective scores for rhinorrhea ($P = 0.0167$), nasal blockage ($P = 0.0118$) progressed at significantly lower levels in the BB536 group

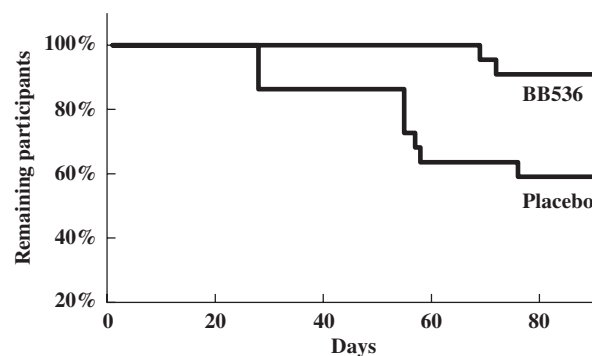


Fig 2. Occurrence of prematurely terminated subjects during the pollen season. Participants were permitted to take prescribed medicines when this was considered necessary due to severe symptoms, but further symptom evaluation and blood sampling were then terminated and these participants were treated as prematurely terminated. Log-rank testing showed a significant difference between BB536 and placebo groups ($P = 0.0056$).

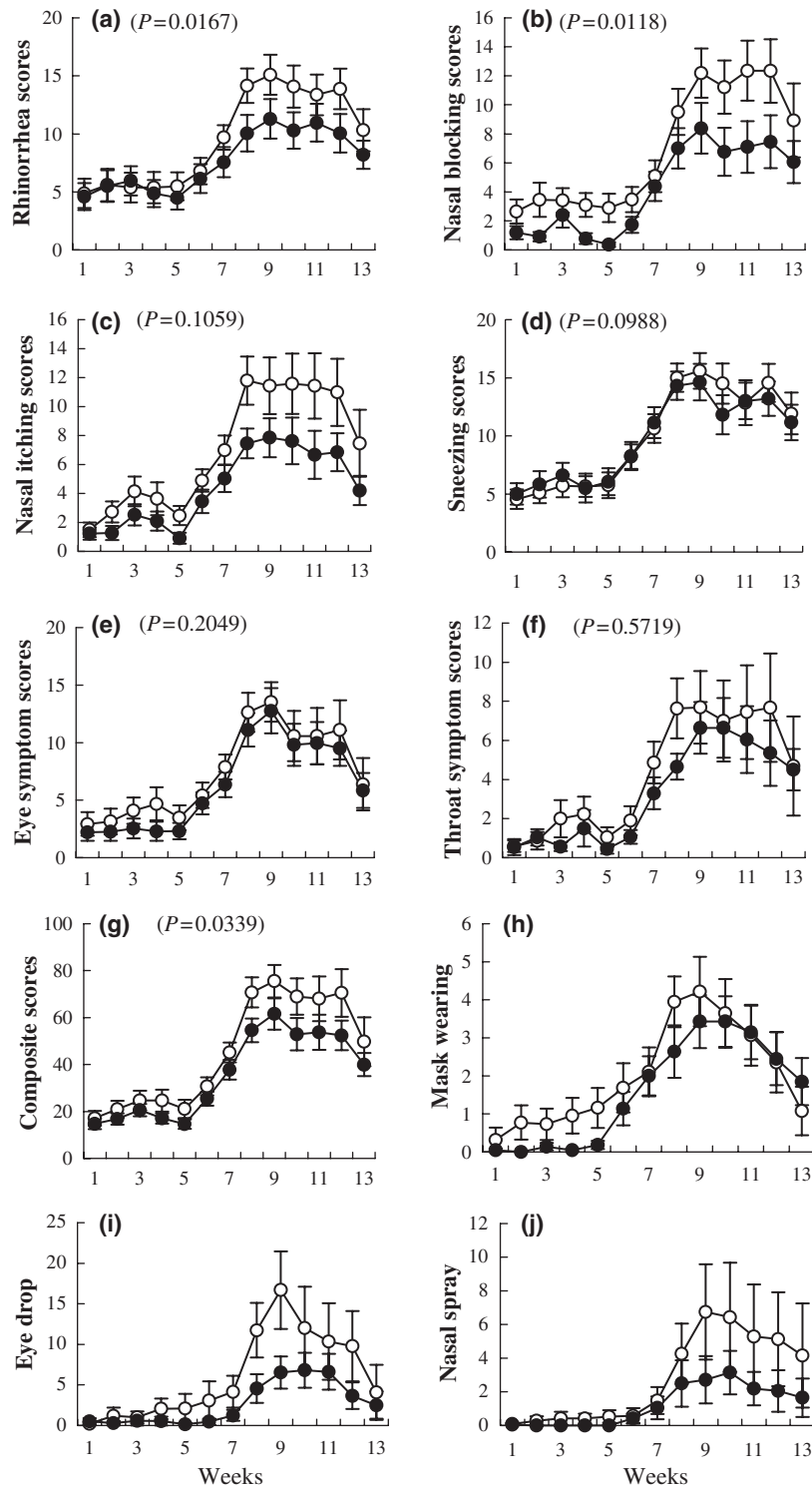


Fig 3. Mean weekly totals for subjective symptoms and self-care measures in the pollen season for BB536 group (●) and placebo group (○). Subjective symptom scores were recorded daily and expressed as weekly totals (scale, 0–28 each). Weekly total scores of sneezing, rhinorrhea, nasal blockage, nasal itching, eye symptoms and throat symptoms were totaled as composite scores (scale, 0–168). Daily counts for eye drops and nasal sprays were totaled weekly. The weekly count of mask wearing was expressed as the number of days with measures (scale, 0–7 each). (a) Rhinorrhea; (b) nasal blockage; (c) nasal itching; (d) sneezing; (e) eye symptoms; (f) throat symptoms; (g) composite scores; (h) mask wearing; (i) eye drops; and (j) nasal sprays. *P* values of treatment effect (placebo vs. BB536) were obtained from analysing by mixed models for repeated-measures analysis of covariance as described in the Materials and methods.

during the pollen season compared with the placebo group. Mean scores for sneezing ($P=0.0988$) and nasal itching ($P=0.1059$) were lower in the BB536 group than in the placebo group, but no significant differences were identified. No marked differences were observed in scores for eye ($P=0.2049$) and throat symptoms ($P=0.5719$) between JCPsis groups. As a total of these subjective symptom scores, composite scores were significantly lower in the BB536 group compared with the placebo group ($P=0.0339$, Fig. 3g).

No marked differences were apparent in frequencies of other medication use and fever or cold, between JCPsis groups (data not shown). However, lower frequencies appeared to be seen for self-care measures of mask wearing, eye drops and nasal sprays in the BB536 group compared with the placebo group (Figs 3h–j).

Evaluations of medical scores as defined in the Materials and methods indicated marked differences between BB536 and placebo groups for each subjective symptom and for composite score (Fig. 4). Comparisons of AUCs calculated from medical scores during the pollen dispersion phase revealed significantly lower levels of rhinorrhea, nasal blockage, nasal itchy, sneezing, throat symptoms and composite score, and a strong tendency towards reduced eye symptoms in the BB536 group compared with placebo (Table 2).

Effect of BB536 on blood parameters. Before treatment, no significant inter-group differences in total IgE, JCP-specific IgE, IFN- γ , IL-10, TARC or eosinophil rate were identified (Table 3). Serum levels of total IgE and JCP-specific IgE increased along with pollen dispersion within each group. Significant elevations in both total and JCP-specific IgE were found at week 13, in the late stage of pollen dispersion. Significant decreases from baseline IFN- γ levels were noted (placebo group, $P < 0.01$ at weeks 4, 8 and 13; BB536 group, $P < 0.01$ at weeks 8 and 13). Significant increases from baseline TARC levels were found in the placebo group at weeks 4 ($P < 0.05$) and 8 ($P < 0.01$), but not in the BB536 group. Significant increases from baseline were seen in IL-10 levels at week 4 in the placebo group ($P < 0.05$), but significant decreases were seen at weeks 8 and 13 in the BB536 group ($P < 0.01$). Furthermore, significant increases from baseline were noted in eosinophil rates with pollen dispersion (placebo group, $P < 0.05$ at weeks 8 and 13; BB536 group, $P < 0.05$ at week 8). No marked changes were observed in rates of basophils, neutrophils, monocytes, lymphocytes or other items from peripheral blood cell counts (data not shown).

No significant differences were noted in changes of total IgE between BB536 and placebo groups, although the difference from baseline of JCP-specific IgE tended to be reduced at week 13 in the BB536 group compared with

the placebo group ($P=0.086$; Table 4). Increase of eosinophil rates and decreases of IFN- γ appeared to be lower in the BB536 group compared with the placebo group, but no significant differences were identified (Table 4). Significant differences were found in changes from baseline of TARC at weeks 4 ($P=0.038$) and 8 ($P=0.031$) in the BB536 group compared with the placebo group (Table 4). No significant differences were found in changes to IL-10 levels from baseline between BB536 and placebo groups.

Discussion

In contrast to 2004, 2005 happened to be a heavy JCP dispersion season, with the total amount of pollen scattered about 30-fold higher than the previous year and 3–4-fold higher than the average of the previous 10 years. No subjects dropped out in 2004, but approximately 25% of JCPsis subjects terminated intervention prematurely due to severe symptoms and use of prescribed pollinosis medication in 2005. The present study found that BB536 intake increased the number of JCPsis subjects going without the taking prescribed pollinosis medicines for the pollen season, with significantly fewer subjects prematurely terminated in the BB536 group compared with the placebo group. Nasal and eye symptoms are known as the typical symptoms of JCPsis, and greatly affect quality of life. Wearing a mask, using eye drops or nasal sprays and taking pollinosis medicines are known as typical precautions or self-care or medication actions for pollinosis. Drugs such as antihistamines and local steroids are commonly used, but at least some of them cause adverse effects such as sleepiness and thirst. Decreasing the use of such agents is thus a worthwhile goal.

Direct comparison of scores without considering lost data from prematurely terminated subjects found that BB536 intake significantly improved symptom scores for rhinorrhea ($P=0.0167$), nasal blockage ($P=0.0118$) and composite score ($P=0.0339$). Scores for sneezing and nasal itching tended to be suppressed in the BB536 group compared with the placebo group, but no significant differences were identified. However, no marked difference in scores for eye and throat symptoms was noted between groups. Several factors might have contributed to these results. First, analysis of primary and secondary symptoms causing premature termination found that seven of 11 subjects (including six of nine subjects in the placebo group) recorded high scores in eye symptoms on termination, which might have diminished the difference in eye symptoms due to unbalanced dismissal between groups. Second, the higher frequency of mask-wearing and eye-drop use that occurred in the placebo group may have had some influence on scores of sneezing, eye and throat symptoms.

As a large imbalance existed in numbers of prematurely terminated subjects in the BB536 and placebo groups,

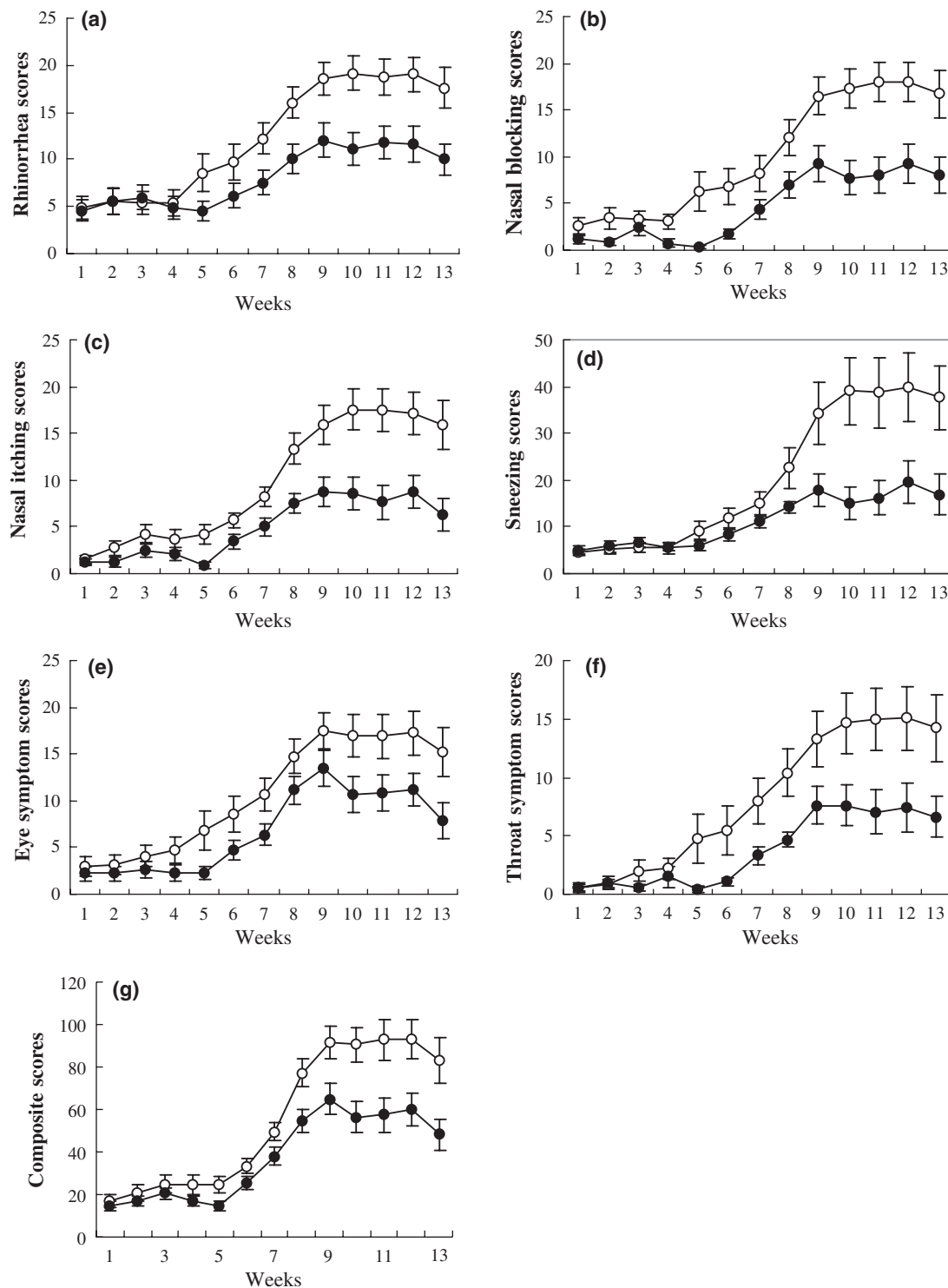


Fig 4. Mean of weekly total for medical subjective symptoms in the pollen season for BB536 group (●) and placebo group (○). Medical scores were calculated when scores after termination for prematurely terminated subjects were substituted using the most severe scores recorded by remaining subjects during the same stage as described in Method. Weekly total scores of sneezing, rhinorrhea, nasal blockage, nasal itching, eye symptoms and throat symptoms were totaled as composite scores (scale, 0–168). (a) Rhinorrhea; (b) nasal blockage; (c) nasal itching; (d) sneezing; (e) eye symptoms; (f) throat symptoms; (g) composite scores.

direct comparison of subjective symptom scores between groups was difficult. Prematurely terminated subjects could be considered to comprise those subjects who

experienced the most severe symptoms and were then dismissed due to taking medication, so average scores might be underestimated in each group, but particularly in

the placebo group. Attempts were made to compare overall clinical efficacy of BB536 on subjective scores. We substituted lost data for prematurely terminated subjects with the most severe scores recorded by continuously participating subjects during the same stage, on the supposition that prematurely terminated subjects would have experienced severe scores if they had continued in the study. As a result, mean scores and AUCs calculated from medical scores during the pollen dispersion phase were significantly lower for most subjective symptoms and for composite scores.

In our previous study, BB536 yogurt alleviated some of the subjective symptoms and suppressed blood eosinophil rate increases and IFN- γ decreases compared with placebo

yogurt [19]. Placebo yogurt, as an active placebo, contained ordinary lactic acid bacteria and thus some effect in the treatment of JCPsis could not be excluded. In the present study, lyophilized powder was employed to study the effects of BB536 in the treatment of JCPsis compared with an inactive control (placebo powder composed of mainly dextrin). Furthermore, total number of BB536 CFUs ingested daily in the present study was several-fold higher than in the yogurt study. These factors may contribute to the result of a more marked effect in the present study compared with the yogurt trial. Taken together, the results of these trials demonstrate the efficacy of BB536 intake in relieving JCPsis symptoms, even during a heavy pollen season.

So far, the efficacy of probiotics in preventing allergic development and treating already established allergic diseases remains controversial and warrants further study. In addition, although much evidence has been shown from *in vitro* and *in vivo* studies that probiotics are fundamentally active in inducing Th1 cytokines and suppressing Th2-skewed immuno-responses [5–11], few human trials have been able to provide evidence in terms of blood parameters, which is considered to be important for evaluating the underlying mechanisms. Viljanen et al. [22] recently found that plasma IL-6 and C-reactive protein levels increased after *Lactobacillus rhamnosus* GG (LGG) treatment among infants with IgE-associated AEDS, and induction of inflammation may thus represent a possible mechanism of probiotic effect in AEDS. However, these results by no means exclude other possible mechanisms involved in probiotic bacteriotherapy. In our previous yogurt trial, we observed a dramatic decline in plasma IFN- γ secretion during the pollen season, and this decrease tended to be suppressed in the BB536 yogurt

Table 2. AUC analysis of medical scores

	AUCs of medical scores [†]		
	Placebo group	BB536 group	P value
Sneezing	207.4 $\pm$ 39.7	83.1 $\pm$ 18.4	0.023*
Rhinorrhea	97.0 $\pm$ 13.1	45.3 $\pm$ 7.6	0.006**
Nasal blockage	96.2 $\pm$ 15.6	45.3 $\pm$ 11.4	0.012*
Nasal itching	103.3 $\pm$ 14.0	48.2 $\pm$ 9.0	0.008**
Eye symptoms	98.8 $\pm$ 14.4	62.5 $\pm$ 10.9	0.082
Throat symptoms	96.0 $\pm$ 16.5	44.7 $\pm$ 8.7	0.028*
Composite scores	484.4 $\pm$ 5.7	291.5 $\pm$ 42.2	0.015*

Values are expressed as mean $\pm$ SEM.

[†]Area-under-the curves (AUCs) for medical scores during the pollen dispersion stage (5–13 weeks) were calculated as described in the Materials and methods.

* $P < 0.05$.

** $P < 0.01$, difference between BB536 and placebo groups, Mann–Whitney *U*-test.

Table 3. Changes in blood IgE, cytokine, chemokine and eosinophil rate levels

	Group	Week 0	Week 4	Week 8	Week 13
Number of subjects	Placebo	20	20	18	12
	BB536	22	22	22	20
Total IgE (IU/mL)	Placebo	57.9 (47.7–175.2)	65.2 (54.2–181.2)	61.1 (46.2–188.8)	110.1 (74.3–313.9)*
	BB536	86.7 (75.0–181.5)	94.7 (84.4–194.4)*	92.6 (92.6–190.6)*	117.0 (99.3–217.0)**
JCP-specific IgE (UA/mL)	Placebo	4.5 (3.9–14.3)	5.1 (4.5–16.3)*	4.1 (2.8–13.6)	19.0 (11.9–54.2)**
	BB536	6.7 (5.6–14.1)	7.5 (6.4–13.8)**	7.4 (6.5–13.8)	22.9 (20.4–42.7)**
IFN- γ (pg/mL)	Placebo	5.0 (–48.3–155.8)	2.8 (–13.9–53.6)**	1.9 (–7.8–30.0)**	2.0 (–13.0–42.1)**
	BB536	6.0 (–12.9–94.9)	4.6 (–8.5–82.5)	2.6 (1.2–35.5)**	3.3 (–1.1–40.4)**
TARC (pg/mL)	Placebo	62.2 (53.7–87.9)	81.2 (62.0–133.6)*	92.0 (73.8–145.9)**	68.9 (55.2–95.0)
	BB536	82.5 (71.6–129.8)	72.9 (64.0–117.7)	88.3 (79.4–127.1)	71.3 (62.0–98.4)
IL-10 (pg/mL)	Placebo	2.4 (1.8–4.5)	3.2 (2.6–4.7)*	2.3 (1.6–4.7)	1.4 (1.0–2.6)
	BB536	2.8 (2.2–4.2)	3.1 (2.5–4.8)	2.0 (1.5–3.5)**	2.0 (1.3–4.2)**
Eosinophil rate (%)	Placebo	2.6 (2.3–5.3)	3.2 (3.0–4.9)	4.9 (3.9–7.6)*	5.4 (4.1–8.1)*
	BB536	2.4 (1.9–4.2)	2.5 (2.1–4.0)	3.1 (3.0–5.5)*	3.6 (3.1–7.1)

Data are presented as geometric means and 95% confidence intervals.

* $P < 0.05$.

** $P < 0.01$ (within-group difference from baseline, Wilcoxon's test after logarithmic transformation).

TARC, thymus- and activation-regulated chemokine; JCP, Japanese cedar (*Cryptomeria japonica*) pollen.

Table 4. Differences from baselines of blood parameters in the pollen season

	Group	Ratios to baseline levels (week 0)		
		Week 4	Week 8	Week 13
Number of subjects	Placebo	20	18	12
	BB536	22	22	20
Total IgE (IU/mL)	Placebo	1.09 (1.00–1.23)	1.03 (0.98–1.09)	1.36 (1.13–1.74)
	BB536	1.09 (1.03–1.18)	1.07 (1.01–1.14)	1.41 (1.17–2.00)
JCP-specific IgE (UA/mL)	Placebo	1.12 (1.00–1.30)	1.06 (0.98–1.15)	4.90 (3.94–6.43)
	BB536	1.13 (1.07–1.21)	1.11 (1.01–1.26)	3.66 (2.42–7.21) ⁺
IFN- γ (pg/mL)	Placebo	0.56 (0.48–0.90)	0.38 (0.18–1.33)	0.32 (0.26–0.52)
	BB536	0.77 (0.65–1.50)	0.43 (–0.48–3.12)	0.44 (0.40–0.70)
TARC (pg/mL)	Placebo	1.31 (1.13–1.83)	1.50 (1.27–2.05)	1.13 (0.84–1.67)
	BB536	0.88 (0.80–1.15) [*]	1.07 (0.95–1.35) [*]	0.85 (0.71–1.194)
IL-10 (pg/mL)	Placebo	1.32 (1.17–1.67)	0.92 (0.40–2.54)	0.65 (0.42–1.27)
	BB536	1.14 (0.99–1.47)	0.72 (0.63–0.97)	0.72 (0.64–0.90)
Eosinophil rate (%)	Placebo	1.25 (0.31–4.11)	1.99 (0.82–5.17)	2.06 (–2.34–12.81)
	BB536	1.03 (0.90–1.29)	1.28 (1.15–2.42)	1.41 (0.89–3.09)

Data are presented as geometric means and 95% confidence intervals.

⁺ $P < 0.1$.

^{*} $P < 0.05$ (vs. placebo, Mann–Whitney U -test).

TARC, thymus- and activation-regulated chemokine; JCP, Japanese cedar (*Cryptomeria japonica*) pollen.

group particularly at the early stage of pollen season [19]. A dramatic decline in plasma IFN- γ levels along with pollen dispersion was also observed in the present study (placebo group, significant decreases at weeks 4, 8 and 13; BB536 group, significant decreases at weeks 8 and 13), although no significant difference was found between these groups. As blood Th2 cytokines IL-4 and IL-5 were not measurable due to levels being below detection limits, TARC was measured as a Th2 marker in the present study. TARC is a member of the CC chemokines and is expressed transiently in phytohaemagglutinin-stimulated peripheral blood mononuclear cells (PBMCs) and constitutively and selectively in the thymus [23]. *In vitro* studies have shown that TARC induces selective migration of lymphocytes of the Th2 phenotype [24–26]. TARC level is well known as an objective parameter for disease severity in atopic dermatitis and represents a promising tool for treatment monitoring [27–30]. Plasma TARC might also offer a useful diagnostic marker for bronchial asthma and allergic rhinitis [31]. In the present study, plasma levels of TARC were found to be significantly elevated in the placebo group, but no marked changes were observed in the BB536 group. Significant differences in changed values from baseline compared with weeks 4 and 8 were noted between the two groups. These results indicate that a skewed immuno-response towards Th2 in T cells was induced by JCP sensitization in JCPsis subjects, and BB536 intake would modulate Th balance. A significant positive correlation was found between elevated TARC levels and subjective symptom scores during the pollen season among JCPsis subjects (data not shown). Furthermore, the Th2-skewed immuno-response (i.e. elevated

levels of IL-4 and IL-13) would then seem likely to induce JCP-specific IgE generation in B cells [32]. A marked elevation in JCP IgE and total IgE was found at week 13, the late stage of pollen dispersion, in JCPsis subjects. No marked change in JCP-specific IgE levels was noted in the 2004 trial [19]. As 2005 happened to be an unusually heavy year for pollen dispersion, this might be the reason for the different outcomes. We found, somewhat unexpectedly, that elevated IgE levels occurred at the late stage, but not at the peak stage of pollen dispersion. The reason is not clearly understood, but local IgE level is considered important for symptom development and only major changes might spill over into the circulation and be apparent in plasma. Furthermore, a time lag is known to exist from local to circulatory levels. Nevertheless, as a result of the immunomodulatory effects of BB536, JCP IgE elevation tended to be suppressed in the BB536 group compared with the placebo group ($P = 0.067$). The influence of skewed Th2 balance and the effects of BB536 intake were also found on leucocytic eosinophil levels. Eosinophils are known as a marker of inflammation, and are induced by IL-5 and may contribute to tissue remodeling processes in allergic diseases [33, 34]. Eosinophil rates were found to be significantly increased in the placebo group, but progressed at lower levels in the BB536 group. These results suggest that BB536 plays a role in modulating the skewed Th balance caused by pollen sensitization, which would result in inhibition of specific IgE increases and eosinophil generation in addition to symptom relief in the pollen season.

In the present study, flocculation of IL-10 levels in JCPsis subjects was controversial: IL-10 levels increased

at week 4, particularly in the placebo group, but decreased at weeks 8 and 13, particularly in the BB536 group. In the 2004 trial, IL-10 levels were found to change along with pollen dispersion [19]. These results probably indicate that degree of sensitization might influence flocculation of plasma IL-10 levels: mild sensitization (2004 season and the early stage of 2005) promotes IL-10 generation, whereas over-sensitization (middle and late stages of 2005) causes down-regulation of IL-10 generation. *In vitro* stimulation of PBMCs with LGG induces IL-10 [35], but 4-week administration of LGG in children with AEDS results in no net effect on blood IL-10 levels [22]. Furthermore, induced increases of blood IL-10 level were speculated as the reason for the negligible clinical effect of oral administration of probiotic mixes in children with AEDS [22]. In a murine model of allergic dermatitis, IL-10 promotes Th2 responses, suppresses Th1 responses and enhances eosinophil accumulation in the skin [36]. Conversely, increased IL-10 levels are associated with induction of oral tolerance in animal models [37, 38]. IL-10 as an immunomodulatory cytokine seems controversial and functions should be attributed with considerable caution.

In conclusion, the present study suggests the efficacy of BB536 in treatment of JCPsis allergy, probably through the modulation of Th2-skewed immune response, in confirmation of our previous findings [19].

Acknowledgement

We wish to thank Mr K. Yoshida of the Nutritional Science Laboratory, Morinaga Milk Industry Co. Ltd, for his helpful advice and technical assistance.

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