Allergology International. 2009;58:179-185
DOI: 10.2332/allergolint.O-08-548

ORIGINAL ARTICLE

Clinical Efficacy of Halophilic Lactic Acid Bacterium *Tetragenococcus halophilus* Th221 from Soy Sauce Moromi for Perennial Allergic Rhinitis

Ikuko Nishimura[1], Toshinori Igarashi[1], Tadao Enomoto[2], Yoshihiro Dake[3], Yoshiaki Okuno[4] and Akio Obata[1]

ABSTRACT

Background: Recently, some common foods in daily life, especially lactic acid bacteria, have been found to have anti-allergic effects. We previously isolated a halophilic lactic acid bacterium, *Tetragenococcus halophilus* Th221, from soy sauce moromi, a mixture of koji and salt solution, and showed that it possesses an immunomodulatory activity that promotes T helper type 1 immunity.

Methods: To evaluate the anti-allergic effects of Th221, we performed a randomized, double-blind, placebo-controlled study in 45 subjects with perennial allergic rhinitis (PAR) treated by oral administration of Th221 (high dose, 60 mg/day, 15 subjects; low dose, 20.4 mg/day, 15 subjects) or a placebo (15 subjects) for 8 weeks.

Results: There were no significant differences among the groups that ingested Th221 and the placebo group regarding the disease severities, total nasal symptom scores and total nasal sign scores examined by physicians. However, the disease severity examined by physicians significantly improved in the high-dose group at the end of the trial compared with the beginning ($p < 0.05$). The total score for nasal symptoms of subjects who received a high dose of Th221 also showed a significant improvement at the end of the trial compared with the beginning ($p < 0.01$). According to the subjects' diaries, significant improvements in sneezing and rhinorrhea were observed during some periods in the high-dose group. The change in serum total immunoglobulin E improved significantly at the end of the trial compared with the beginning in this group ($p < 0.05$). The safety of Th221 treatment was confirmed by laboratory tests and inspection of the general condition of each subject.

Conclusions: Th221 can be expected to safely improve the symptoms of PAR.

KEY WORDS

clinical efficacy, immunoglobulin E, lactic acid bacteria, perennial allergic rhinitis, soy sauce

INTRODUCTION

Allergic rhinitis is a symptomatic disorder of the nose induced by immunoglobulin E (IgE)-mediated inflammation of the membranes lining the nose after allergen exposure,[1] first defined in 1929.[2] The three cardinal symptoms of nasal reactions occurring in allergy are sneezing, nasal obstruction and mucous discharge. Recently, the incidences of both perennial allergic rhinitis (PAR) and seasonal allergic rhinitis have been increasing worldwide,[1,3-6] leading to dramatic escalations in direct and indirect treatment costs.

PAR is mainly caused by house dust and mites, and its prevalence has reached 18.7% in the Japanese population, which is higher than that of cedar pollinosis (16.2%).[7] The therapeutic strategy for PAR usually involves removal and avoidance of antigens by environmental maintenance and pharmacotherapy. Inhibitors of chemical mediators, anti-histamines and topi-

[1]Research & Development Division, Kikkoman Corporation, Chiba, [2]Faculty of Medicine, Tottori University, Tottori, [3]Dake ENT Clinic, Wakayama and [4]Okuno ENT Clinic, Osaka, Japan.
Correspondence: Ikuko Nishimura, Research & Development Division, Kikkoman Corporation, 399 Noda, Noda City, Chiba Prefecture 278–0037, Japan.
Email: inishi@mail.kikkoman.co.jp
Received 27 February 2008. Accepted for publication 8 September 2008.

Table 1 Characteristics of the patients enrolled in the study

	High dose	Low dose	Placebo
n	15	15	15
Age (mean ± SE)	33.8 ± 2.0	36.7 ± 1.2	36.5 ± 2.8
Sex			
Male	7	9	4
Female	8	6	11
Disease severity			
Mild	2	2	2
Moderate	11	13	13
Severe	2	0	0

cal steroids are widely used, but their potential side effects after long-term application are causes for concern.

Recently, some common foods in daily life have been found to have anti-allergic effects. There is growing interest in this prospect, especially with regard to lactic acid bacteria (LAB).[8-11] We previously isolated a halophilic LAB, *Tetragenococcus halophilus* Th221, from soy sauce moromi, and showed that it possesses an immunomodulatory activity that promotes T helper type 1 (Th1) immunity.[12] Specifically, we found that Th1-dependent contact sensitivity was augmented, while Th2 immunity evaluated by allergen-specific IgE production was suppressed, following oral ingestion of Th221 in an in vivo mouse study. Based on these findings, we investigated the anti-allergic effects of Th221 in a randomized, double-blind, placebo-controlled clinical trial in subjects with PAR in the present study.

METHODS

SAMPLE PREPARATION

T. halophilus Th221 was heat-killed by incubation at 90℃ for 15 minutes. Three kinds of tablets weighing 200 mg were prepared as follows: placebo tablets containing no Th221; low-dose tablets containing 3.4 mg of Th221 per tablet; and high-dose tablets containing 10 mg of Th221 per tablet.

SUBJECTS

Forty-five PAR subjects (20 males, 25 female; age range: 16–60 years) were enrolled in this clinical study. The eligibility criteria for PAR were confirmation of positive results in at least 2 of the following 3 tests according to the Guidelines for the Management of Allergic Rhinitis in Japan (2002)[13]: (1) allergen skin test or detection of serum allergen-specific IgE against house dust or mites; (2) nasal provocation test; and (3) eosinophil count in nasal discharge.

The exclusion criteria were as follows: (a) combination with other nasal diseases; (b) sensitization to other allergens that may influence the study; (c) complication by systematic diseases; (d) simultaneous attendance of another clinical trial; (e) pregnancy or lactation; (f) poor health condition when consuming soy sauce or LAB; and (g) inappropriate cases for the trial as defined by physicians.

The present study followed the tenets of the Declaration of Helsinki. In addition, written informed consent was obtained from all subjects prior to participation in the study.

The detailed characteristics of the study subjects are shown in Table 1. Each subject had a history of PAR of more than 3 years. Among the 45 subjects with PAR, 6 cases (13.3%) were diagnosed as mild, 37 cases (82.2%) as moderate and 2 cases (4.4%) as severe according to the scores of the 3 main nasal symptoms (sneezing, rhinorrhea and nasal obstruction) on the basis of the guidelines.

STUDY DESIGN

A randomized, double-blind, placebo-controlled trial was performed at the following 3 institutions: Japanese Red Cross Society Wakayama Medical Centre (Wakayama); Dake ENT Clinic (Wakayama); and Okuno ENT Clinic (Izumisano, Osaka). The trial was performed between October 2005 and December 2005 in order to avoid any influence of the Japanese cedar pollen season. The study was conducted at TTC Co. Ltd. and approved by the Ethics Committee of the Shinjuku Oiwake Clinic (Shinjuku, Tokyo).

METHODS

The subjects were randomly divided into 3 groups. The first group received 6 placebo tablets per day (placebo group; $n = 15$), the second group received 6 low-dose tablets containing 3.4 mg of Th221 per day (low-dose group; total dose: 20.4 mg/day; $n = 15$) and the third group received 6 high-dose tablets containing 10 mg of Th221 per day (high-dose group; total dose: 60 mg/day; $n = 15$). Each subject received 2 tablets *t.i.d.* for a course of 8 weeks. After the administration was completed, there was a follow-up period of 1 week, totaling a duration of 9 weeks. The clinical characteristics of the subjects in the 3 groups are shown in Table 1. There were no significant differences among the characteristics of the subjects.

During the study period, each subject visited a doctor 3 times: at the beginning of the trial, during the trial (week 4), and at the end of the trial (week 8). Subjects were asked to record sneezing, rhinorrhea, nasal obstruction and quality of life (QOL) on a daily basis. A questionnaire was conducted regarding the QOL in their daily lives and responses were evaluated using a 5 grade system based on the guidelines (Table 2).

The primary endpoints set before the beginning of the study were disease severity, nasal symptoms (sneezing, rhinorrhea and nasal obstruction) severities, QOL, nasal signs (mucosal swelling, mucosal

Table 2 QOL questionnaire sheet

Morning	Afternoon	Evening	
−	4 +	+	4 + : Cannot work, study or complete housework 3 + : Interference with work, study or housework 2 + : Between 3 + and + + : Little interference with daily life − : No problems

color, discharge volume and discharge character) and allergic parameters (serum total IgE, serum allergen-specific IgE, eosinophil count in nasal discharge and neutrophil count in nasal discharge).

Disease severities, nasal symptoms (sneezing, rhinorrhea and nasal obstruction) severities and QOL were scored by doctors using the subjects' diaries. Each subject's nasal signs (mucosal swelling, mucosal color, discharge volume and discharge features) were also scored by doctors based on the guidelines.

Total nasal symptom scores and total nasal sign scores were obtained by adding symptom scores (sneezing, rhinorrhea and nasal obstruction) and sign scores (mucosal swelling, mucosal color, discharge volume and discharge character), respectively.

Medication scores were recorded according to drug characteristics using the following guidelines: second-generation antihistamines, mast cell stabilizers, vasoconstrictors and mast cell-stabilizing eye drops, 1 point each; topical ocular and nasal steroids, 2 points each. Any concurrent use of drugs that could influence the evaluation of efficacy was prohibited.

When evaluating the subjects' diaries, the number of sneezes and rhinorrhea events recorded in the diaries were added up weekly. Nasal obstruction and QOL were also quantified and added up weekly. Evaluations were made for 8 weeks plus a follow-up period of 1 week, totaling a period of 9 weeks.

Peripheral blood and urine routine examinations were performed for each subject after the 3 visits to their physician. Serum total IgE, serum allergen-specific IgE (house dust, *Dermatophagoides pteronyssinus*), eosinophil count in nasal discharge, neutrophil count in nasal discharge and TARC (Th2 marker) were also measured.

STATISTICAL ANALYSIS

Data are expressed as means ± SE. Disease severities evaluated by physicians and eosinophil counts in nasal discharge were evaluated within groups and between groups using one-sample and two-sample Wilcoxon tests, respectively. Intragroup and intergroup differences in nasal symptom scores, nasal sign scores, total nasal symptom scores, total nasal sign scores, diagnostic scores and rates of score changes using the subjects' diaries were evaluated using one-sample and two-sample t-tests, respectively. Medication scores were recorded by drug characteristics and evaluated using a two-sample Wilcoxon test. Differences were considered to be significant at $p < 0.05$.

RESULTS

DISEASE SEVERITIES, TOTAL NASAL SYMPTOM SCORES AND TOTAL NASAL SIGN SCORES

Disease severities found by physicians based on the subjects' diaries (sneezing, rhinorrhea and nasal obstruction) during the study are shown in Table 3. Although no significant differences could be seen between any 2 groups, disease severity significantly improved in the high-dose group at the end of the trial compared with the beginning ($p < 0.05$). No significant changes were found during the trial in the placebo and low-dose groups. There were no significant differences between any 2 groups regarding nasal symptom (sneezing, rhinorrhea and nasal obstruction) severities, QOL and nasal sign scores (mucosal swelling, mucosal color, discharge volume and discharge character).

The total nasal symptom scores during the study are shown in Table 4. A comparison of the high-dose and placebo groups revealed a tendency toward improvement in the high-dose group ($p = 0.091$), and the score significantly improved in the high-dose group at the end of the trial compared with the beginning ($p < 0.01$). Regarding the total nasal sign score, there were no significant differences among the groups that ingested Th221 and the placebo group, and significant improvements were observed in all 3 groups at the end of the trial compared with the beginning ($p < 0.01$).

The medication scores recorded by drug characteristics were evaluated using a two-sample Wilcoxon test, and no significant differences were observed among the groups.

EVALUATION OF THE SUBJECTS' DIARIES

Because 1 subject in each of the low-dose and high-dose groups forgot to record their details at the beginning of the trial, they were excluded from the analysis. In addition, 2 subjects in the placebo group and 3 subjects in the high-dose group whose sneezing or rhinorrhea increased by more than 100 number of times, compared with the beginning of the trial, were excluded. Therefore, an analysis was carried out for 13 subjects in the placebo group, 14 sub-

Table 3 Disease severities examined by physicians (presented as numbers of subjects)

Group	Time (weeks)	Very severe	Severe	Moderate	Mild	No symptoms	Intragroup *p* value	Intergroup *p* value
	0	0	2	6	5	2		
Placebo	4	0	1	6	7	1	0.755	
	8	0	0	6	7	2	0.245	
	0	0	1	6	7	1		
Low dose	4	0	0	4	11	0	0.401	0.654
	8	0	2	2	10	1	0.595	0.684
	0	0	2	6	3	4		
High dose	4	0	1	3	9	2	0.401	0.654
	8	0	1	1	8	5	0.029*	0.453

The disease severities were examined by physicians using the subjects' diaries (sneezing, rhinorrhea and nasal obstruction) during the study. *$p < 0.05$ compared to the beginning of the trial.

Table 4 Total nasal symptom scores (combination of sneezing, rhinorrhea and nasal obstruction) in perennial allergic rhinitis subjects during the trial

Group	Time (weeks)		
	0	4	8
Placebo	3.13	3.07	2.67
Low dose	3.20	2.87	3.07
High dose	2.73	2.93	1.67**

A placebo, low dose of Th221 (20 mg/day) or high dose of Th221 (60 mg/day) was administered for 8 weeks. **$p < 0.01$ compared to the beginning of the trial.

jects in the low-dose group and 11 subjects in the high-dose group.

The mean rates of changes from the baseline scores for sneezing during the study are shown in Table 5. There were no significant changes between any 2 groups. However, the rate of change in sneezing in the high-dose group showed a significant improvement during the first week ($p < 0.05$) and second week ($p < 0.05$), compared with the beginning of the trial. No significant changes were observed in the placebo group or low-dose group compared with the beginning of the trial.

The mean rates of change from the baseline scores for rhinorrhea during the study are shown in Table 6. In comparison with the placebo group, the rate of change in rhinorrhea in the high-dose group significantly improved during the first ($p < 0.05$) and second week ($p < 0.05$). A significant improvement could also be found in the low-dose group during the first week ($p < 0.05$) in comparison with the placebo group. The rate of change in rhinorrhea in the high-dose group began to improve during the second week ($p = 0.062$) compared with the beginning of the trial. No significant changes could be observed in the placebo group or low-dose group, compared with the beginning of the trial.

No significant changes were found between any 2 groups regarding the rate of change in nasal obstruction and QOL. The rate of change in nasal obstruction in the high-dose group showed significant improvement by the fifth week ($p < 0.05$) compared with the beginning of the trial (data not shown). Significant changes were also observed in the placebo group at the third week ($p < 0.05$) and fourth week ($p < 0.05$) compared with the beginning of the trial (data not shown). No significant changes were found in the low-dose group compared with the beginning of the trial.

The rate of change in QOL in the low-dose group improved significantly at the 7th week ($p < 0.05$) compared with the beginning of the trial (unpublished data). However, no significant changes were observed in the placebo group or high-dose group compared with the beginning of the trial.

ALLERGIC TESTS

Since there were no significant improvements in serum allergen-specific IgE (house dust, *D. pteronyssinus*) (Table 7), it can be said that the improvement in symptoms induced by Th221 were not marked. In addition, no significant improvements were found in the following allergic markers in PAR subjects: eosinophil count in nasal discharge (Table 7); neutrophil count in nasal discharge; and TARC (Th2 marker). In the high-dose group, however, a significant decrease in the change in serum total IgE was observed at the end of the trial (week 8; $p < 0.05$) compared with the beginning (Fig. 1, Table 7).

SAFETY

No adverse effects were observed during the study. Common colds and diarrhea were reported by some subjects, however the degrees of symptoms were slight and disappeared during the study, and may have had no relationship with the trial.

Table 5 Mean rates of change from the baseline scores for sneezing evaluated using the subjects' diaries

Group	Time (weeks)									
	0	1	2	3	4	5	6	7	8	9
Placebo	0.00	− 2.54	− 1.38	− 4.23	− 1.54	1.46	− 0.54	1.54	− 3.00	6.15
Low dose	0.00	6.93	3.64	0.57	1.79	0.71	5.14	0.21	− 0.86	− 2.64
High dose	0.00	− 6.91*	− 8.09*	− 3.55	− 7.91	− 8.09	2.64	− 6.45	− 2.64	5.82

The first day of administration (baseline) is indicated as 0 weeks. A placebo, low dose of Th221 (20 mg/day) or high dose of Th221 (60 mg/day) was administered for 8 weeks. After the administration was completed, a follow-up period of 1 week was set up, giving a final time point of 9 weeks. *$p < 0.05$ compared with the baseline score in each group.

Table 6 Mean rates of change from the baseline scores for rhinorrhea evaluated using the subjects' diaries

Group	Time (weeks)									
	0	1	2	3	4	5	6	7	8	9
Placebo	0.00	5.31	4.54	− 0.08	− 1.54	3.54	8.46	15.23	6.15	7.15
Low dose	0.00	− 2.29^{+}	4.36	− 0.93	1.07	4.50	5.36	2.93	3.71	3.14
High dose	0.00	− 4.18^{+}	− 5.36^{+}	− 0.64	− 3.64	− 4.00	1.55	− 2.09	3.00	10.09

The first day of administration (baseline) is indicated as 0 weeks. A placebo, low dose of Th221 (20 mg/day) or high dose of Th221 (60 mg/day) was administered for 8 weeks. After the administration was completed, a follow-up period of 1 week was set up, giving a final time point of 9 weeks. $^{+}p < 0.05$ compared to the placebo group.

Table 7 Effects of Th221 treatment on serum IgE and eosinophil counts of perennial allergic rhinitis subjects

	Group	Week 0	Week 4	Week 8
Total IgE (IU/ml)	Placebo	381 ± 130	372 ± 117	343 ± 101
	Low dose	377 ± 73	391 ± 86	358 ± 71
	High dose	1122 ± 745	1021 ± 643	1021 ± 643
Change in total IgE (%)	Placebo	100.0	105.4 ± 6.5	101.1 ± 3.8
	Low dose	100.0	100.7 ± 3.5	95.0 ± 3.0
	High dose	100.0	100.9 ± 2.6	94.1 ± 2.1*
House dust-specific IgE (Class)	Placebo	2.5 ± 0.3	2.5 ± 0.3	2.5 ± 0.4
	Low dose	2.7 ± 0.3	2.7 ± 0.3	2.7 ± 0.3
	High dose	3.1 ± 0.3	3.1 ± 0.3	3.1 ± 0.3
D. pteronyssinus-specific IgE (Class)	Placebo	2.6 ± 0.3	2.5 ± 0.4	2.7 ± 0.4
	Low dose	2.7 ± 0.3	2.7 ± 0.3	2.7 ± 0.3
	High dose	3.0 ± 0.4	3.0 ± 0.4	3.1 ± 0.4
Eosinophil count (%)	Placebo	5.5 ± 0.9	4.7 ± 0.8	5.2 ± 1.0
	Low dose	4.7 ± 0.8	4.2 ± 0.5	4.9 ± 0.8
	High dose	5.1 ± 0.8	4.7 ± 0.7	5.3 ± 0.8

Each value represents the mean ± SE. *$p < 0.05$ compared to the beginning of the trial.

DISCUSSION

PAR attacks in all seasons and predominates in younger children, rendering it harder to cure. Although a variety of medicine for PAR have been developed and applied clinically, PAR subjects typically need to use these drugs for the entire year with consideration for side effects. In addition, there are only a few kinds of medicine for PAR which are appropriate for younger children.

With this in mind, it may be interesting to identify foods with anti-allergic effects, as these could be expected to reduce dependence on drugs to some extent. One such promising food is LAB.[8] Several clinical trial reports have indicated that intake of LAB improves the symptoms of some kinds of allergies.[9-11]

In the present study, we evaluated the anti-allergic effects of the LAB *T. halophilus* Th221, which we previously isolated from soy sauce moromi.[12] The clinical findings by physicians improved significantly in the high-dose group during the latter half of the trial. Furthermore, during this period, the subjects' diaries revealed a decrease in the rates of change from the baseline scores for sneezing at weeks 4, 5, 7 and 8 (Table 5) and rhinorrhea at weeks 4, 5 and 7 (Table 6) in the high-dose group, although the changes were

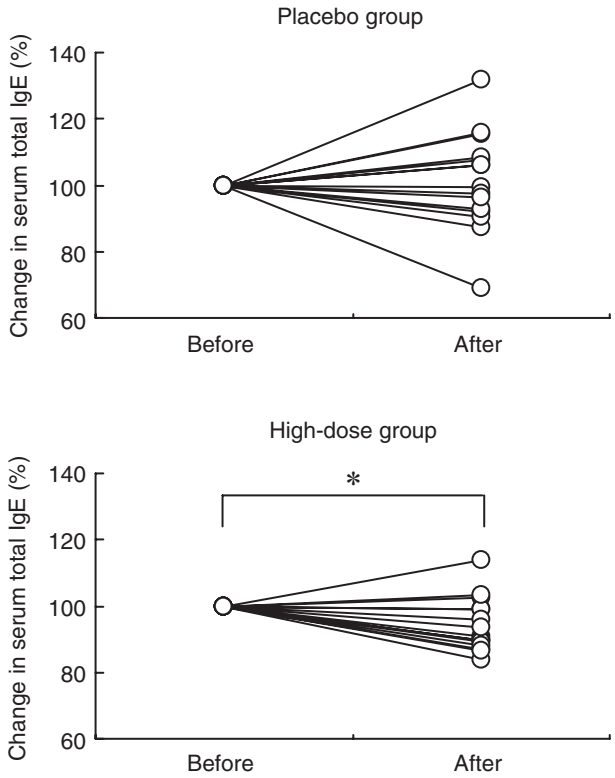

Fig. 1 Rate of change in serum total IgE in the placebo group and high-dose group (60 mg of Th221 per day) before and after the trial. $*p < 0.05$ compared with the beginning of the trial.

not significant. These results are consistent with the findings by physicians. According to the subjects' diaries, however, significant improvements were found in earlier periods for both the rate of change from the baseline scores for sneezing in the high-dose group, and the rate of change for rhinorrhea in the high-dose group compared with the placebo group, which were inconsistent with the physicians' findings. Moreover, significant changes were found in the rate of change for nasal obstruction in the placebo group at the third and fourth weeks, compared with the beginning of the trial. Therefore, the data from the subjects' diaries appear to be less consistent, and we consider it highly likely that significant early improvements included a placebo effect. In the present study, the change in serum total IgE significantly improved in the high-dose group at the end of the trial compared with the beginning, which supports the improvement found in clinical findings.

It has often been proposed that one mechanism for LAB-induced suppression of allergic responses is a reduction in IgE due to improvement of the Th1/Th2 balance.[8,14] For example, a clinical trial report that *Enterococcus faecalis* FK-23 improves the symptoms of PAR mentions that one of its mechanisms may involve improvement of the Th1/Th2 balance.[10] In that trial, tuberculin responses were also measured, and a significant inverse correlation was recognized between skin test diameters and symptoms. The authors interpreted these findings as being due to enhancement of host Th1-type immune responses and suppression of the overexpression of Th2-dominated allergic responses.

Some LAB strains have been proven to improve the Th1/Th2 balance in vitro and in vivo. For example, *Lactobacillus paracasei* KW3110, which was selected to show both high IL-12-inducing activity and high IL-4-suppressing activity, reduced antigen-specific IgE in an ovalbumin (OVA)-sensitized mouse model by improving the Th1/Th2 balance.[14] It was also reported that increased Th1 cytokine levels and decreased Th2 cytokine levels were found in splenocytes from OVA-sensitized mice that had ingested *Lactobacillus casei* Shirota.[15]

T. halophilus strain Th221 used in the present study was selected because it exhibited strong IL-12-inducing activity on mouse peritoneal macrophages.[12] A Th1-promoting activity was also manifested in an *in vivo* mouse study, because Th1-dependent contact sensitivity was augmented and Th2 immunity evaluated by allergen-specific IgE production was suppressed following oral ingestion of Th221. In the present clinical study, the change in total IgE significantly decreased, and this may be explained by improvement of the Th1/Th2 balance due to the Th1-promoting activity of Th221. However, the activity does not seem to be sufficiently strong to induce a significant decrease in serum allergen-specific IgE. In many clinical trials testing the beneficial effects of LAB or other foods, allergic symptoms have often improved, but a significant reduction in serum IgE is not commonly observed.[9,11,16,17] Therefore, it is worth noting that serum total IgE decreased due to Th221 in the present trial.

It has recently been reported that *Lactobacillus acidophilus* L-92 can induce apoptosis of Th2 cells, in addition to enhancing Th1 responses.[18] It will be interesting to investigate whether Th221 also possesses this activity. Furthermore, it has often been suggested that regulatory T cells[19] or Th17 cells[20] are related to allergic responses, but only the Th1/Th2 balance is usually taken into account. Therefore, we need to search for further mechanisms with this in mind.

Another possibility is that Th221 may have improved allergic symptoms by improving the intestinal microflora. The role for intestinal microflora, such as several strains of LAB, in priming the immune system during ontogeny to limit allergy has been pointed out.[21] Epidemiological studies have shown a higher incidence of allergy expression in early childhood among children with low enteric LAB populations, supporting the notion that appropriate microbial colonization of the gut can lower the risk of de-

veloping an allergy.[22,23] There is also clinical evidence that appropriate gut-colonizing microbes can control the development of atopy.[24] In addition, there is a report that administration of heat-killed LAB can improve intestinal microflora in adults.[25] Therefore, Th221 may contribute to the improvement of allergic symptoms via this mechanism.

The human gut is known to contain more than 1 kg of intestinal microorganisms.[26] It is extremely interesting and surprising that such a small amount of LAB, even if heat-killed, can lead to the improvement of allergic symptoms. It is also important to clarify which components of LAB provide beneficial effects. Further studies and development are required to provide beneficial and evidence-based foods to improve allergic symptoms.

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