Clinical Effects of *Lactobacillus acidophilus* Strain L-92 on Perennial Allergic Rhinitis: A Double-Blind, Placebo-Controlled Study

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ABSTRACT

Studies in animals have suggested that lactic acid bacteria alleviate allergic diseases, however, little information is available on their clinical effect on allergy in humans. Thus, we examined the efficacy of orally administered *Lactobacillus acidophilus* strain L-92 (L-92) on perennial allergic rhinitis. In a randomized, double-blind, placebo-controlled clinical trial, 49 patients with perennial allergic rhinitis were randomized to receive either 100 mL of heat-treated fermented milk containing L-92 (n = 25) or acidified milk without lactic acid bacteria (placebo; n = 24) for 8 wk. The severity of symptoms was evaluated based on the changes in the scores of clinical symptoms. Oral administration of milk fermented with L-92 resulted in a statistically significant improvement of nasal symptom-medication scores. Ocular symptom-medication scores of patients in the L-92 intervention group tended to improve compared with those in the placebo group. In addition, clear decreases of the scores of swelling and color of the nasal mucosa were observed in the L-92 intervention group at 6 and 8 wk after the start of ingestion of fermented milk. There were no significant differences in serum antihouse dust mite immunoglobulin E levels nor in T helper type 1/T helper type 2 ratio between the 2 groups. These results suggest that oral administration of L-92 can alleviate the symptoms of perennial allergic rhinitis, however, statistically significant changes were not shown in blood parameters.

(Key words: *Lactobacillus acidophilus*, perennial allergic rhinitis, double-blind placebo-controlled study)

Abbreviation key: L-92 = *Lactobacillus acidophilus* strain L-92, SMS = symptom-medication score, Th1 = T helper type 1, Th2 = T helper type 2.

INTRODUCTION

Allergic diseases such as pollen allergy, perennial allergic rhinitis, and atopic dermatitis are becoming serious social problems in Japan and other developed countries. One of the explanations for their increased prevalence is given by the well-known 'hygiene hypothesis', which postulates that the decrease of opportunities of exposure to immunostimulating pathogens in early childhood causes increased prevalence of allergic diseases (Strachan, 1989). Consequently, T helper type 1 (Th1) cells have developed insufficiently, and continuous marked skewing of the immune response toward cells of the T helper type 2 (Th2) lineage may promote humoral immunity, for example, IgE production and eosinophilia (Romagnani, 1994; Holt, 1995).

Allergic children in Estonia and Sweden were found to be less often colonized with lactobacilli compared with nonallergic children (Björkstén et al., 1999). Lactobacilli are thought to induce Th1 reaction and improve allergic diseases (Cross et al., 2001). Furthermore, orally administered heat-treated *Lactobacillus casei* (strain Shirota) was found to inhibit IgE production induced by ovalbumin in mice serum (Matsuzaki et al., 1998). Moreover, intraperitoneally injected heat-treated *Lactobacillus plantarum* L-137 was demonstrated to suppress IgE production in response to a casein allergy in mice (Murosaki et al., 1998). Oral administration of lysed *Enterococcus faecalis* FK-23 resulted in a decrease of peritoneal accumulation of eosinophils induced by ragweed pollen (Shimada et al., 2003).

In humans, *Lactobacillus rhamnosus* strain GG administered in the perinatal period reduced the incidence of atopic eczema in children at risk during the first 2 yr of life (Kalliomäki et al., 2001) and beyond infancy (Kalliomäki et al., 2003). *Lactobacillus rhamnosus* 19070-2 and *Lactobacillus reuteri* DSM 122460 improved moderately the clinical severity of eczema in children with atopic dermatitis (Rosenfeldt et al., 2003). However, *Lb. rhamnosus* strain GG did not alleviate the symptoms of patients with birch pollen allergy (Helin et al., 2002).

Received March 24, 2004.

Accepted November 8, 2004.

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Figure 1. Study protocol. The study was carried out between December 2, 2002 and February 24, 2003. Patients were asked to drink fermented milk daily for 8 wk between December 16 and February 10.

As there are a few clinical trials, there is no sufficient information about the significance of lactic acid bacteria in allergic disorders. Previously, we reported that oral administration of *Lactobacillus acidophilus* strain L-92 (**L-92**) suppressed mice serum antigen-specific IgE levels (Ishida et al., 2003). This study was carried out to verify the antiallergic effect of L-92 in patients with perennial allergic rhinitis.

MATERIALS AND METHODS

Subjects

The subjects were screened by medical examination of the nasal cavity and blood test at Soiken Clinic (Osaka, Japan). After screening, 52 patients with perennial allergic rhinitis and high concentrations of anti-house dust IgE or antihouse dust mite IgE were selected. None of them had asthma or had received specific immunotherapy. The study protocol was in accordance with the Declaration of Helsinki, and it was approved by the Ethics Committee of Soiken Inc., and Soiken Clinic on Clinical Tests. All patients gave written informed consent to participate in the study.

Study Design

The study was double-blind and placebo-controlled. It took place between December 2, 2002 and February

24, 2003. Patients were randomized after a 1-wk run-in period into the intervention and placebo groups. Patients in the intervention group were asked to drink 100 mL of heat-treated milk fermented by *Lactobacillus acidophilus* L-92 (about 3×10^{10} counts/100 mL), and those in the placebo group to drink 100 mL of heat-treated milk controlled for the degree of acidity and sweetness daily for 8 wk (between December 16 and February 10). During the study period, a physician examined each patient's nasal cavity 7 times, and blood samples were taken at 5 determination points. Patients were instructed to keep a symptom and medication diary for 7 wk (wk -2 to -1, -1 to 0, 1 to 2, 3 to 4, 5 to 6, 7 to 8, and 9 to 10) (Figure 1).

Estimation of Subjective Symptoms

Nasal and ocular symptoms described in the patient's diary were classified as follows: sneezing, runny nose, stuffy nose, itchy eyes, and watery eyes, and were scored from 0 to 4 (Table 1). The highest score of sneezing, runny nose, and stuffy nose was used as the nasal symptom score, and the highest score of itchy eyes and watery eyes was used as the ocular symptom score. Patients were allowed to use medicines to relieve their symptoms. The medicines used were scored from 0 to 3 (Table 2). The sum of the symptoms score and medication score was used as the symptom-medication score (**SMS**). The SMS was defined by the Japanese Society of Allergology (Okuda et al., 2002).

Table 1. Scoring system for subjective symptoms.

Symptom	Symptom score				
	0	1	2	3	4
Sneezing ¹	0	1 to 5	6 to 10	11 to 20	≥21
Runny nose ²	0	1 to 5	6 to 10	11 to 20	≥21
Stuffy nose	None	Mild	Moderate	Severe	Violent
Itchy eyes	None	Mild	Moderate	Severe	Violent
Watery eyes	None	Mild	Moderate	Severe	Violent

¹Mean number of sneezing attacks in a day.

²Mean number of times patient blew nose in a day.

Table 2. Scoring system for medicines used.

Medicine	Score
Oral antihistamine	1
Oral histamine release inhibitor	1
Nose drops (excluding local steroids)	1
Eye drops (excluding local steroids)	1
Local administration of steroids	2
Oral antihistamines and local steroids	3

Table 3. Scoring of nasal cavity findings.

Findings	Score			
	0	1	2	3
Nasal mucosa				
Swelling	None	To middle concha	Between 1 and 3	Middle concha not observed
Color	Normal	Light red	Red	Bluish
Mucus in nasal cavity				
Amount	None	Adhesion level	Between 1 and 3	Filled
Attribute	None	Purulent	Viscous	Aqueous

Medical Examination

Nasal cavity findings, that is, swelling and color of the nasal mucosa, and amount and attribute of mucus were scored from 0 to 3 (Table 3).

Blood Examination

Blood samples collected were used to determine the concentration of total IgE, antihouse dust IgE, and antihouse dust mite IgE. The number of Th1 cells per Th2 cells (Th1/Th2 ratio), basophils, and eosinophils were determined and other common characteristics were evaluated. All blood tests were performed at Osaka Kessei Research Laboratories, Inc., Osaka, Japan.

Statistical Analysis

The symptom-medication score was expressed as a weekly average. ANOVA of a split-plot design was applied to detect time-dependent changes of the weekly average score. The Steel-Dwass test was used to verify the difference between the values in the 2 groups at each time point. As for the evaluation of nasal cavity findings, Steel-Dwass test was applied as a multiple comparison procedure. The Mann-Whitney *U*-test was used to compare the mean values as reference analysis because of repetition of the analysis. Blood data were subjected to ANOVA with a split-plot design to detect time-dependent changes, and the Scheffé test was used for a multiple comparison procedure. Data were analyzed using SPSS (ver.12.0J, SPSS, Inc., Chicago, IL)

Table 4. Clinical characteristics of intervention and placebo groups.

Characteristic	Intervention group	Placebo group
Number of patients	25	24
Sex (M:F)	13:12	13:11
Mean age (yr) $\pm$ SE	34.0 $\pm$ 3.4	36.9 $\pm$ 3.0
Severity of symptoms before study		
Severe	4	6
Moderate	12	9
Mild	9	9

and Juse-Stat Works (ver. 4.0; The Institute of Japanese Union of Scientists and Engineers, Tokyo, Japan).

RESULTS

Study Population

Initially, 52 patients were included in the study. Three patients withdrew from the study due to personal reasons. Thus, 49 patients completed the study. There was no significant difference between the 2 groups in terms of age, severity of symptoms, or serum IgE level (Table 4 and wk -2 data of Table 5).

Table 5. Blood test data (means $\pm$ SE).

Item/Week	Intervention group	Placebo group
Total IgE (IU/mL)		
-2	598.9 $\pm$ 160.4	576.6 $\pm$ 155.9
0	543.5 $\pm$ 144.2	503.6 $\pm$ 133.0
4	571.0 $\pm$ 152.4	483.1 $\pm$ 116.5
8	565.2 $\pm$ 152.5	498.3 $\pm$ 135.8
10	562.2 $\pm$ 151.4	504.0 $\pm$ 132.0
Anti house dust mite IgE (IU/mL)		
-2	33.1 $\pm$ 6.2*	28.7 $\pm$ 5.2
0	29.1 $\pm$ 5.4	31.8 $\pm$ 5.9
4	32.4 $\pm$ 6.2	26.6 $\pm$ 4.7
8	31.3 $\pm$ 6.0	26.5 $\pm$ 5.0
10	24.3 $\pm$ 4.6**	21.9 $\pm$ 4.0*
Anti house dust IgE (IU/mL)		
-2	28.7 $\pm$ 5.3	27.7 $\pm$ 5.3
0	27.4 $\pm$ 5.1	27.0 $\pm$ 4.9
4	31.7 $\pm$ 6.4*	28.7 $\pm$ 5.5
8	27.0 $\pm$ 5.1	23.9 $\pm$ 4.5
10	27.1 $\pm$ 5.5	19.8 $\pm$ 3.4
Th1/Th2 ratio		
-2	15.9 $\pm$ 2.7**	12.1 $\pm$ 1.9**
0	9.1 $\pm$ 1.6	7.6 $\pm$ 0.6
4	12.7 $\pm$ 1.6	10.6 $\pm$ 1.4
8	11.0 $\pm$ 1.2	10.0 $\pm$ 1.2
10	11.7 $\pm$ 1.6	9.9 $\pm$ 1.1
Eosinophils (cells/L)		
-2	342.9 $\pm$ 33.5	304.3 $\pm$ 29.3
0	358.4 $\pm$ 33.0	366.5 $\pm$ 56.7
4	367.0 $\pm$ 43.5	370.3 $\pm$ 31.4
8	302.4 $\pm$ 33.6	345.3 $\pm$ 28.0
10	320.4 $\pm$ 37.6	340.1 $\pm$ 34.2

* $P < 0.05$ and ** $P < 0.01$ vs. data at start of study (Scheffé test).

Figure 2. Daily averages of the symptom-medication score (SMS) throughout the study period. A) Nasal symptom-medication score. B) Ocular symptom-medication score. Each point and bar represent the mean daily average in each week and the standard error. Compared with the placebo group, in the intervention group the nasal SMS decreased significantly ($P < 0.05$), and the ocular SMS tended to decrease ($P < 0.1$) by ANOVA of a split-plot design. ** $P < 0.01$ vs. the start date (Steel-Dwass test).

Subjective Symptoms

Nasal SMS and ocular SMS of the L-92 group decreased during the ingestion period and increased again during the observation period (Figure 2). In contrast, nasal SMS and ocular SMS of the placebo group did not change much during the ingestion period. Compared with the score at the start date, nasal SMS of the L-92 group decreased significantly at wk 6 and 8 of ingestion ($P < 0.01$, Steel-Dwass). According to the split-plot design analysis of SMS changes, nasal SMS of the L-92 group was significantly different from that of the placebo group ($P < 0.05$), and ocular SMS of the L-92 group tended to decrease compared with the placebo group ($P < 0.1$).

Medical Examination of the Nasal Cavity

Scores of swelling and color of the nasal mucosa of the L-92 group decreased at wk 6 and 8 of ingestion, but not significantly compared with the start date (Figure 3a and b). Compared with the placebo group, swelling score of the nasal mucosa of the L-92 group was significantly lower at wk 8 of ingestion ($P < 0.05$), and color score of the nasal mucosa of the L-92 group was significantly lower at wk 6 of ingestion ($P < 0.01$). The score for the amount of mucus in the placebo group was significantly higher at wk 4 and 8 of ingestion ($P < 0.01, 0.05$, respectively), and that of the L-92 group was decreased (not significantly) at wk 6 and 8 of ingestion, compared with the start date (Figure 3c). The score for

the attribute of mucus in the placebo group was high, whereas that in the L-92 group decreased during the ingestion period, but not significantly (Figure 3d). However, scores of both amount and attribute of nasal mucus were different between groups before ingestion, therefore, it was considered that evaluation of the effect of L-92 was invalid in these terms.

Blood Examination

Compared with the values at the start of ingestion, antihouse dust mite IgE, antihouse dust IgE, and Th1/Th2 ratio showed significant changes at several times in both groups (Table 5). However, comparison between the 2 groups did not show significant differences by analysis with ANOVA with split-plot design. Many other parameters, such as basophil count, eosinophil count, etc., did not differ significantly between the 2 groups (data not shown).

DISCUSSION

We demonstrated that L-92 improved subjective symptoms in adult patients with perennial allergic rhinitis. The number of clinical studies about the efficacy of orally administered lactobacilli strains on allergic diseases is limited. In addition, most previous studies involved atopic infants or children. The findings of the present study indicate that lactobacilli can ameliorate the symptoms of type-I allergy not only in infants with an immature immune system but also in adults with

Figure 3. Score of nasal cavity findings. Each point and bar represent the average score and standard error. As compared with the start date, swelling and color scores of the mucosa of the inferior nasal concha and the scores of the amount and attribute of mucus decreased at wk 6 and 8 of ingestion, but not significantly, in the L-92 group. * $P < 0.05$ and ** $P < 0.01$ vs. the placebo group (Mann-Whitney U -test). † $P < 0.05$ and ‡ $P < 0.01$ vs. the start date (Steel-Dwass test).

fully developed immune systems. The prophylactic effects of probiotics on symptoms of allergy remain controversial. For example, *Lb. rhamnosus* strain GG did not alleviate the symptoms of patients with birch pollen allergy (Helin et al., 2002), whereas the clinical effect of the same strain on atopic dermatitis was confirmed (Kalliomäki et al., 2001). One of the reasons for the

difference in biological efficacy may be that the fluctuation in the severity of symptoms among patients with atopic dermatitis is comparatively smaller than that among patients with pollen allergy. During the pollination season, the symptoms of patients with pollen allergy are aggravated with the increase of pollen dispersal. As the level of severity of symptoms differs from

individual to individual, it is difficult to discriminate the effect of the test sample from the whole deviation of data. Another important reason is the difference regarding the validity of the clinical effects of lactic acid bacteria among species and strains. In fact, in vitro studies using human mononuclear cells have indicated that there are strain-dependent differences in the ability of lactic acid bacteria to induce immunoregulatory monokines such as interleukin 12 (Miettinen et al., 1998). Contribution of the species- and strain-specific nature of lactic acid bacteria on the efficacy of improving allergic symptoms should be considered.

In this study, scores of subjective symptoms and medical examination were improved during the relatively later period of ingestion. This indicated that, at least, the mechanism(s) behind improvement of allergic symptoms by L-92 ingestion might not be due to inhibition of the action of chemical mediator(s). It is well known that helper T cells play a critical role in the adaptive immune response. Murine and human helper T cells can be divided into 2 major subtypes, Th1 and Th2. T helper 1 cells produce interferon- γ and are essential for cell-mediated immune responses, whereas Th2 cells promote humoral immunity mediated by antibodies. Marked skewing of the immune response to the Th2 lineage can result in allergic disorders. Strain L-92 effectively suppressed serum antigen-specific IgE levels in mice (Ishida et al., 2003). However, in humans, no differences in serum IgE levels or Th1/Th2 ratio have been shown between placebo and intervention groups. These results are similar to those of the clinical trial conducted by Kalliomäki et al. (2001) using *Lb. rhamnosus* strain GG. We assume one of the reasons why differences in serum IgE levels and Th1/Th2 ratio between the 2 groups were not observed in our study was that they were too small to detect under the present experimental conditions. Another explanation could be an insufficient number of patients. On the other hand, interferon- γ production by cultured peripheral blood mononuclear cells from an asthmatic patient was found to increase when the patient was administered fermented milk (Wheeler et al., 1997). Thus, it might be possible to evaluate the effect of L-92 by investigating the production of cytokines by peripheral mononuclear cells.

In the case of human study, there is a possibility that the effect of improving allergy symptoms by L-92 might be not only due to the improvement of Th1/Th2 ratio but to other modes. As inferred from improvement of nasal symptoms, production of chemical mediators such as leukotrienes or inflammation factors have no relation to the improvement of Th1/Th2 ratio. We may need to pay attention to the function and number of peripheral eosinophils (Table 5). The antiallergic effect of L-92 was observed during the later period of ingestion in

this study, suggesting that L-92 influences host immune status at a slow and steady pace. For example, it may affect production of chemical mediators. This finding should be confirmed with future studies. There are several reports suggesting that the incidence of allergic asthma cannot be explained only by Th1/Th2 ratio (Kero et al., 2001). It is considered that immaturity of Th1 cells is not enough to explain the increase of the number of patients with allergic diseases and of those with autoimmune disorders (Herrick and Bottomly, 2003). Regulatory T cells have been a focus of attention as a factor regulating the host immune system in a negative way (Levings et al., 2002; Curotto de Lafaille et al., 2002). Two types of regulatory T cells have been reported so far. One is T regulatory 1 cells, which produce large amounts of interleukin 10 and inhibit antigen-specific T-cell responses (Groux et al., 1997; McGurk et al., 2002). The other is T helper type 3 cells, which contribute to oral tolerance and produce transforming growth factor β (Chen et al., 1994; Weiner, 2001). These anti-inflammatory T cells may have an important role in improving the symptoms of chronic allergic rhinitis. In fact, serum interleukin 10 has been shown to increase in children with atopic dermatitis after ingestion of *Lb. rhamnosus* GG (Pessi et al., 2000). Orally administered L-92 may not contribute to adjust the imbalance of Th1/Th2, but it may rather act on regulatory T cells. Further studies are needed to elucidate the mechanisms of the clinical effect of L-92 in patients with allergic diseases.

In conclusion, this study demonstrates the clinical effect of ingestion of L-92 on symptoms of perennial allergic rhinitis.

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