

Effects of Probiotics on Allergic Rhinitis Induced by Japanese Cedar Pollen: Randomized Double-Blind, Placebo-Controlled Clinical Trial

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Key Words

Japanese cedar pollen • Allergic rhinitis • Probiotic •
Lactobacillus casei • Fermented milk

Abstract

Background: *Lactobacillus casei* strain Shirota (LcS) has been found to exert antiallergic effects in animal experiments, but there is little information about its clinical effects in human patients with allergy. **Methods:** We performed a randomized double-blind, placebo-controlled study to investigate the effects of LcS in patients with allergic rhinitis triggered by Japanese cedar pollen (JCP). Participants were asked to drink fermented milk containing LcS (LcS group) or placebo (control group) for 8 weeks. Clinical symptoms and immunological parameters were compared between the two groups. **Results:** Symptom-medication scores (SMS) worsened in accordance with the increase in the amount of scattered JCP. In terms of the nasal and ocular SMS, there was no significant difference between the LcS group and the placebo group during the ingestion period. In the subgroup of patients with moderate-to-severe nasal symptom scores before starting the ingestion of test samples, supplementation with LcS tended to reduce nasal SMS. **Conclusion:** These results indi-

cate that fermented milk containing LcS does not prevent allergic symptoms in patients sensitive to JCP, but may delay the occurrence of allergic symptoms in patients with moderate-to-severe nasal symptom scores.

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Introduction

Recently, patients with allergic rhinitis have been increasing in Japan, and the incidence of allergic rhinitis caused by Japanese cedar pollen (JCP) is estimated to be in the range of 10–15% among Japanese people [1]. This tendency could be a serious problem from the standpoint of socioeconomic aspects, because the allergic symptoms are unpleasant for patients and often cause various disturbances in their ordinary life during the particular season. Moreover, the medical bill for treating allergic symptoms is not negligible.

The general treatment of allergic rhinitis is administration of antihistamines, laser evaporation of the inferior turbinate, Vidian neurectomy and immunotherapy. These modalities are considered efficient but have some problems. Antihistamines sometimes cause side effects

Table 1. Characteristics of the participants in the LcS group and the placebo group

Characteristics	LcS group	Placebo group	Significance
Number of patients (male:female)	55 (22:33)	54 (21:33)	NS
Age, years	39.3 ± 8.0	39.5 ± 10.9	NS
Total IgE, IU/ml	198.9 ± 273.8	160.0 ± 247.0	NS
Anti-JCP IgE, IU/ml	15.6 ± 21.5	14.0 ± 15.7	NS
Nasal SMS	1.33 ± 0.72	1.42 ± 0.88	NS
Ocular SMS	0.94 ± 1.01	0.95 ± 0.97	NS
Swelling of nasal mucosa	1.24 ± 1.00	1.20 ± 0.86	NS
Color of nasal mucosa	1.45 ± 0.90	1.52 ± 0.91	NS
Amount of mucus	0.89 ± 0.76	0.89 ± 0.66	NS
Nature of mucus	1.45 ± 1.32	1.50 ± 1.31	NS

NS = Not significant; SMS = symptom-medication score.

such as sleepiness, thirst or gastrointestinal disturbance, and their dose and timing of administration should be strictly controlled. Laser evaporation of the inferior turbinate, Vidian neurectomy and immunotherapy cause a great burden to the patient, such as hospitalization, or sometimes a long ambulatory treatment period. Under these circumstances, food products to prevent or improve allergic symptoms that are easily available in ordinary life are required. Thus, yogurt, tea and herbs have been shown to potentially relieve the allergic symptoms [2–5].

Supplementation with milk fermented with *Lactobacillus paracasei* 33, *L. acidophilus* L-92 or *Bifidobacterium longum* BB536 has been shown to suppress the subjective symptoms and may modulate immunological parameters in allergic rhinitis patients [6–9]. These findings support the opinion that stabilization of the intestinal microflora by administration of probiotics may prevent the development of allergic rhinitis.

L. casei strain Shirota (LcS) suppresses the IgE production of splenocytes by enhanced interleukin 12 secretion by macrophages in vitro [10], and its administration prevents the elevation of the IgE level and induction of anaphylactic symptoms after sensitization with ovalbumin in animal models [11, 12]. Therefore, it is worthwhile examining whether LcS could improve allergic symptoms in humans. We evaluated the effect of fermented milk containing LcS in patients with allergic rhinitis to JCP in a randomized double-blind, placebo-controlled study. This report shows that ingestion of fermented milk containing LcS did not prevent clinical symptoms or abnormal immunological parameters in patients allergic to JCP, but may delay the occurrence of subjective symptoms in patients with moderate-to-severe nasal symptom scores.

Materials and Methods

Subjects

To carry out this study, we recruited participants enrolled for human studies in the nontreated subject bank of Soiken Inc. (Osaka, Japan), which is an organization for evaluating the functions of foods or food-derived materials in humans. We explained the aim and protocol of this study, asked if they were willing to participate and screened for subjects having specific IgE for JCP by a scratch test using allergen extract (Torii Pharmaceutical Co. Ltd., Tokyo, Japan) and a radioallergosorbent test. Exclusion criteria were as follows: use of antihistamines or antiallergic medication at the time of the screening test; any recent history of acute rhinitis, sinusitis, nasal polyp, hypertrophic rhinitis, septal deformity or asthma; severe disorder of the liver, kidney, heart, respiratory organs, endocrine glands or metabolism; treatment with hypsensitization therapy; frequently drinking dairy products containing lactic acid bacteria, and cow's milk allergy. The study was performed in accordance with the Declaration of Helsinki and approved by the local ethics committee, which is independent of Soiken Inc. Written informed consent was obtained from all the participants.

Study Design

The study was performed in a randomized double-blind, placebo-controlled manner. Experiments were performed from January 22 to April 15, 2005. Randomization was performed by doctors, who were not involved in this study design. All of the enrolled subjects were randomly assigned to the LcS group or the placebo group according to computer-generated permuted-block randomization. There was no obvious difference in the two groups (table 1). The LcS group drank fermented milk containing LcS (4×10^{10} CFU/80 ml) and the placebo group drank unfermented milk. The composition of fermented milk containing LcS and placebo was the same except that the placebo did not contain LcS, and the lactic acid level of placebo was adjusted to the level of fermented milk containing LcS. Before delivery to the participants, we confirmed that the fermented milk contained more than 5×10^8 CFU/ml of LcS, and both fermented milk and placebo had no contamination with other bacteria.

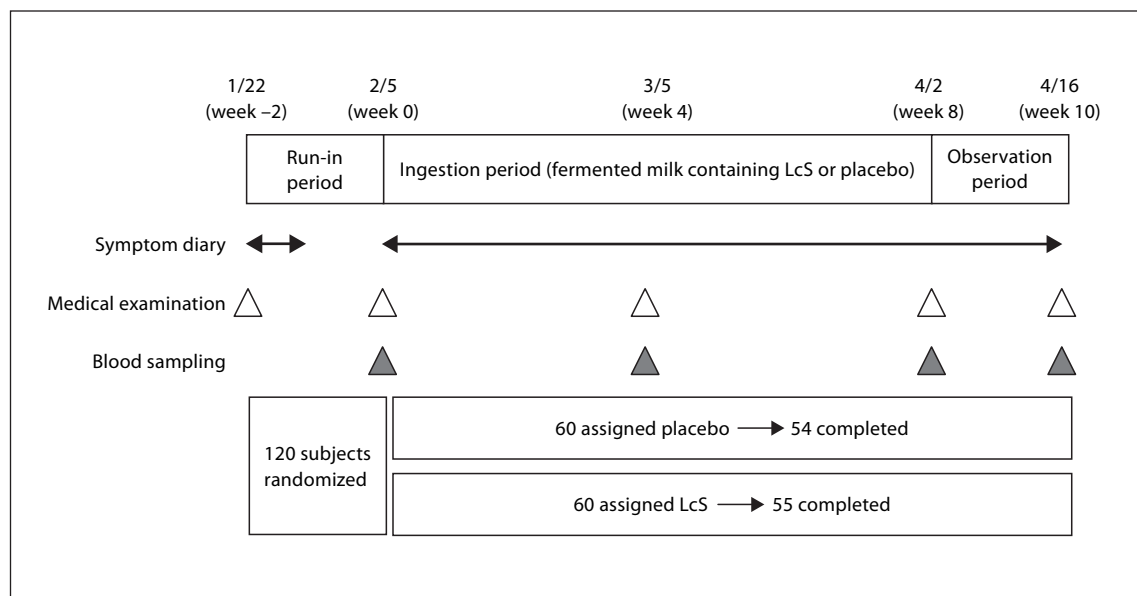


Fig. 1. Study protocol. The clinical trial was carried out from January 22 to April 15, 2005. Participants were asked to drink fermented milk containing LcS or placebo for 8 weeks, and to record their symptoms in a diary every day. Medical examination was conducted 5 times, and blood samples were taken 4 times during the study.

To assure the viability of LcS, we prepared fermented milk every week during the study period. Furthermore, we checked the acidity and sugar level in LcS-containing fermented milk and confirmed that these values were within standardized levels. We did not check the intestinal microflora in the volunteers in this study. However, it has already been verified that LcS can be detected in feces after supplementation of LcS-containing fermented milk [13–15]. We asked all the participants not to change their ordinary lifestyle during the study. The participants drank 80 ml of placebo or fermented milk containing LcS daily for 8 weeks. The schedule of the study is shown in figure 1. Participants were asked to record their nasal and ocular symptoms and medication in a diary during the study period. Moreover, they underwent medical examination by an otolaryngologist 5 times during the study.

Evaluation of Symptoms and Medical Examination of Subjects

The scores of nasal and ocular symptoms have been defined by the Japanese Society of Allergology [1, 16]. Briefly, sneezing, runny nose, stuffy nose, itchy eyes and watery eyes were each scored from 0 to 4 according to the severity of symptoms by the participants, and the medication score was estimated based on the efficacy of medicines (table 2). The medication score, which was described in the guidelines of the Japanese Society of Allergology, is determined by medication usage (table 2). The clinical condition of the nasal cavity (swelling and color of nasal mucosa, amount and nature of mucus) was scored from 0 to 3 for each feature according to the severity by the otolaryngologist (table 3). The symptom-medication score (SMS) was calculated by summing the symptom score and the medication score.

Table 2. Symptom score and medication score for evaluating the subjective symptoms

a Symptom score

Score	Sneezing ^a	Runny nose ^b	Stuffy nose	Itchy eyes	Watery eyes
0	0	0	none	none	none
1	1–5	1–5	mild	mild	mild
2	6–10	6–10	moderate	moderate	moderate
3	11–20	11–20	severe	severe	severe
4	>21	>21	violent	violent	violent

b Medication score

Score	Medicine
1	oral antihistamine, oral histamine release inhibitor, nose or eye drops (without steroids)
2	local administration of steroids
3	oral antihistamine plus local administration of steroids

Sneezing = Average number of sneezing attacks in a day; runny nose = average number of times patient blew nose in a day.

Table 3. Standard for doctors of otolaryngology to assess the nasal cavity

Score	Swelling of mucosa	Color of mucosa	Amount of mucus	Nature of mucus
0	none	normal	none	none
1	middle concha observable	light red	adhesion level	purulent
2	between 1 and 3	red	between 1 and 3	viscous
3	middle concha unobservable	bluish	filled	aqueous

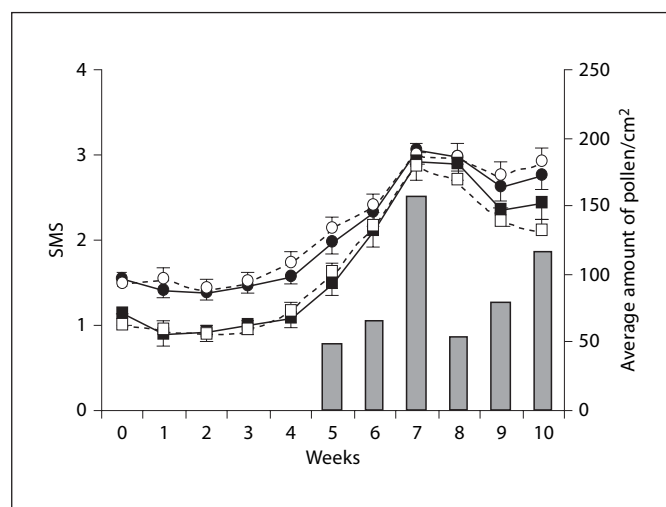


Fig. 2. Nasal and ocular SMS throughout the study period. Nasal SMS in the LcS group (●) and placebo group (○) and ocular SMS in the LcS group (■) and placebo group (□) are shown as means $\pm$ SE. Filled columns show the average amount of pollen (JCP and hinoki pollen) scattered in the area where the studies were carried out.

Blood Examination

Blood samples were collected 4 times during the study, and anti-JCP IgE, eosinophil number, eosinophil cationic protein (ECP) and the balance of Th1 cells to Th2 cells (Th1/Th2 ratio) were determined. The anti-JCP IgE level was evaluated by the radioallergosorbent test. The ECP level was measured by radioimmunoassay (Unicap system; Roche Diagnostics KK, Sweden). Peripheral blood mononuclear cells were stimulated with phorbol myristate acetate plus ionomycin for 4 h in the presence of brefeldin A, and stained with anti-CD4 antibody. After cells had been fixed and permeabilized, the accumulated γ -interferon and interleukin 4 in CD4+ T cells were stained and measured by a flow cytometer. The ratio of γ -interferon+ CD4+ T cells to interleukin-4+ CD4+ T cells was expressed as Th1/Th2 balance.

Statistical Analysis

SMS were averaged each week. Differences in SMS and description of the nasal mucosa between the groups were evaluated by the Mann-Whitney U test. Differences in immunological pa-

rameters were assessed by the unpaired Student's t test. Data were analyzed using SPSS software (version 11.5, SPSS Inc., Chicago, Ill., USA).

Results

Scattering of JCP

The Osaka Prefectural Institute of Public Health and the Japan Weather Association reported that much JCP was scattered in the spring of 2005, and the level of JCP reached 10–30 pollen/cm² on the first 10 days of March (4–5 weeks after the start of ingestion), being maximal (approx. 600 pollen/cm²) in the last 10 days of March (7 weeks). Thereafter, the amount of scattered JCP gradually declined, although it was still detected at more than 10–30 pollen/cm² until the beginning of April (9 weeks). Furthermore, hinoki pollen started to scatter from the beginning of April, causing a biphasic change in the average amount of total pollen in the spring of 2005 (fig. 2).

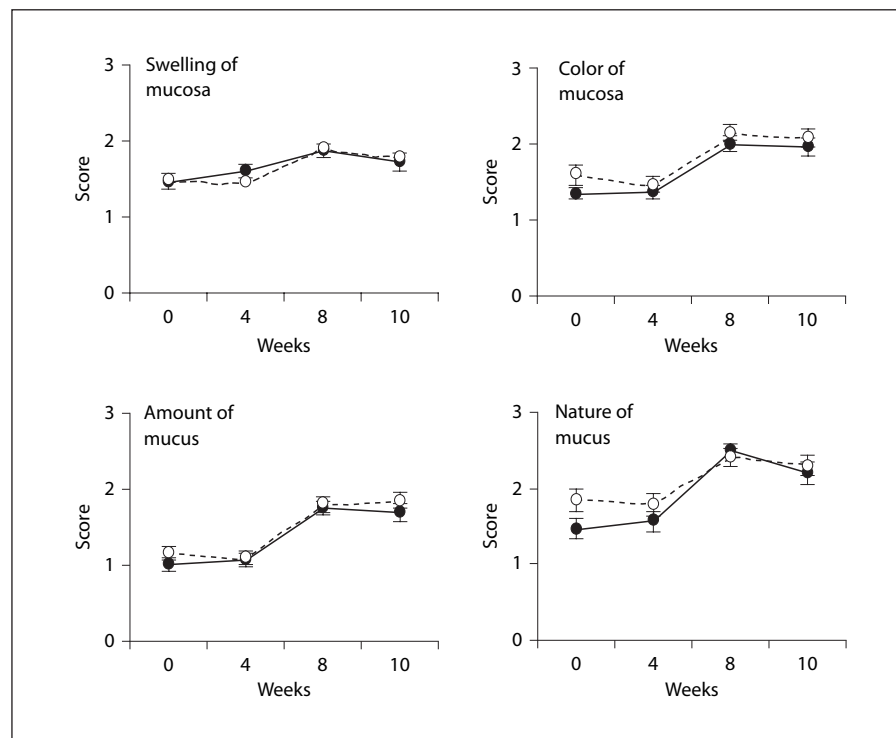
Study Population

One hundred and twenty subjects were enrolled, but 11 subjects declined to take part in the study for personal reasons. As a result, 109 subjects (54 subjects in the placebo group and 55 in the LcS group) completed the study and their data were analyzed (fig. 1). There was no difference between the groups in terms of age, total and anti-JCP IgE level, and severity of allergic symptoms before the study (table 1).

Subjective Symptoms

Nasal and ocular SMS began to rise during the first 10 days of March in 2005 (4 weeks after the start of ingestion) and increased in association with the increase in scattered JCP. Deterioration of nasal SMS was delayed 1 week in the LcS group compared with the placebo group, but the difference in nasal SMS between the groups was not significant during the study (fig. 2). However, when the patients were divided into two categories ('mild' and

Fig. 3. Score of condition of nasal mucosa throughout the study. Data are presented as means $\pm$ SE. ● = LcS group; ○ = placebo group.



‘moderate-to-severe’) based on the nasal symptom score before the study, the nasal SMS in moderate-to-severe cases in the LcS group was lower at 4 and 5 weeks than in the placebo group (LcS group $n = 13$; placebo group $n = 11$; data not shown).

Medical Examination of Nasal Cavity

Scores of swelling and redness of the nasal mucosa, and the amount and nature of mucus deteriorated in association with the increase in scattered JCP. However, none of these scores differed between the groups (fig. 3).

Blood Examination

Immunological parameters associated with allergic symptoms increased in response to the amount of scattered JCP. Compared with the value before ingestion, the ECP level rose by 4 weeks and the anti-JCP IgE level and eosinophil number increased by 8 weeks. No difference was detected in immunological parameters between the two groups during the study (fig. 4). We divided the participants into mild cases and moderate-to-severe cases based on the nasal symptom score before the study and performed the statistical analysis, but there was no significant difference between the two groups (data not shown).

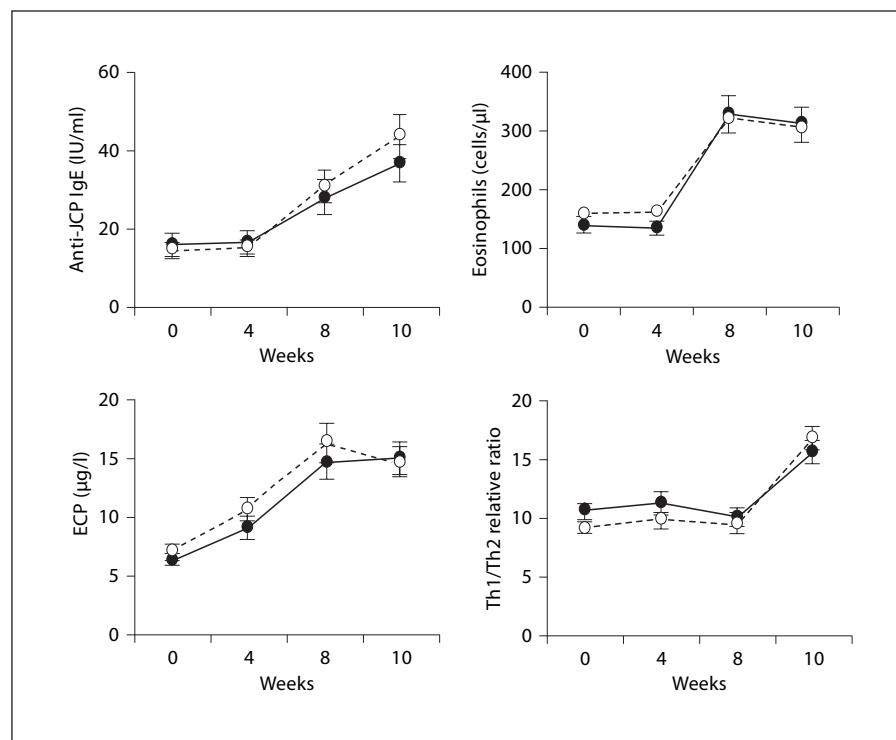
Side Effects

Ten subjects suffered from a cold, 3 subjects developed diarrhea, and 1 subject vomited during the study. All of these disorders were transient and there was no concern that they were related to the ingestion of fermented milk containing LcS (data not shown).

Discussion

We investigated the effect of fermented milk containing LcS on allergic symptoms triggered by JCP in a randomized double-blind, placebo-controlled study. The results showed that nasal and ocular SMS worsened in accordance with the increase in scattered JCP, confirming that the subjective symptoms of allergic rhinitis appear swiftly in response to scattered JCP. We have also performed a similar study in the spring of 2004 when a very small amount of JCP was scattered. Most patients did not experience any problem related to allergic symptoms, and nasal and ocular SMS in the participants did not rise in the spring of 2004 (data not shown). Taken together, these observations demonstrate that subjective symptoms are good parameters to assess allergic rhinitis during the JCP season.

Fig. 4. Immunological parameters throughout the study period. Data are presented as means $\pm$ SE. ● = LcS group; ○ = placebo group.



Nasal and ocular SMS were not different between the LcS group and the placebo group. These results show that supplementation with fermented milk containing LcS during the JCP season does not prevent the exacerbation of seasonal allergic rhinitis. When the subjects were divided into two subgroups based on the severity of nasal symptom score before the start of ingestion, nasal SMS of the participants with moderate-to-severe nasal symptom scores was lower in the LcS group than in the placebo group at 4 weeks and 5 weeks, indicating that LcS may delay the onset of allergic symptoms in patients with a moderate-to-severe condition. In previous studies, we found that a daily drink of 80 ml of fermented milk containing LcS caused recovery of natural killer cell activity in humans [17–19]. Therefore, we considered that 80 ml of fermented milk containing LcS could be sufficient for modulating human immune function. However, it remains to be elucidated whether more than 80 ml may improve the results in placebo-controlled trials using participants with moderate-to-severe nasal symptoms in future investigations.

The clinical effects of probiotics on allergic rhinitis have been examined in several studies. Fermented milk containing *L. paracasei* LP-33 reduced the subjective symptoms in patients with perennial allergic rhinitis

more efficiently than fermented milk containing *Streptococcus thermophilus* and *L. bulgaricus* [6]. Fermented milk containing *L. acidophilus* L-92 improved the subjective symptoms in not only perennial allergic rhinitis, but also Japanese cedar pollinosis, compared with heat-treated milk, although the immunological parameters were not different between the L-92 group and the placebo group [7, 8]. Furthermore, the clinical symptoms in Japanese cedar pollinosis were relieved in patients ingesting yogurt fermented with *B. longum* BB536, *S. thermophilus*, and *L. delbrueckii* compared with those ingesting yogurt fermented with *S. thermophilus* and *L. delbrueckii*, and the immunological parameters were modulated by *B. longum*-BB536-fermented yogurt [9]. These results suggest that some probiotic strains and/or their fermentation products are responsible for improvement of allergic rhinitis. In contrast, supplementation with capsules containing *L. rhamnosus* GG did not improve the clinical symptoms of birch-pollen-allergic patients [20]. Therefore, further investigation is required to elucidate the antiallergic effects of probiotics on allergic rhinitis. Viljanen et al. [21] have reported that administration of *L. rhamnosus* GG did not improve the clinical scores in infants with food allergy, but this probiotic was considered effective for the improvement of symptoms in the IgE-sensi-

tized subgroup. Our results also suggest that supplementation with LcS might delay the onset of allergic symptoms in subjects with moderate-to-severe symptom scores. Altogether, the effect of probiotics on allergic rhinitis may vary dependent on the disease condition.

The effects of probiotics to modulate immunological parameters associated with allergic symptoms should be elucidated. It was reported that LcS suppressed IgE production in vitro and reduced the antiovalbumin IgE level in wild-type mice and transgenic mice expressing ovalbumin-specific T-cell receptor [10–12]. However, no difference was detected in immunological parameters between the LcS group and the placebo group in this clinical trial. Probiotics may possibly improve subjective symptoms even if immunological parameters such as the allergen-specific IgE level or Th1/Th2 imbalance are not normalized. In that sense, it is of great interest that sup-

plementation with *L. rhamnosus* 19070–2 and *L. reuteri* DSM stabilized the intestinal barrier function and decreased gastrointestinal symptoms in children with atopic dermatitis [22]. Moreover, involvement of natural killer T cells and regulatory T cells in the induction and control of allergic responses has been proposed [23, 24]. Therefore, other mechanisms besides suppression of IgE production or normalization of Th1/Th2 imbalance could be involved in the antiallergic activity exerted by probiotics in humans.

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