Original Article

Lysed Enterococcus faecalis FK-23 oral administration reveals inverse association between tuberculin responses and clinical manifestations in perennial allergic rhinitis: a pilot study

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Summary.
Background: The interest in anti-allergy immunoregulation by lactic acid bacteria (LAB) has been growing over the last few decades. There is evidence to suggest that lysed *Enterococcus faecalis* FK-23 (LFK), a kind of LAB preparation, could relieve the clinical symptoms of Japanese cedar pollinosis. However, little is known about how LFK plays a role in combating allergy.
Objective: The aim of this study was to clarify whether improvement of clinical manifestations is associated with enhancement of tuberculin responses in patients with allergic rhinitis treated by LFK.
Methods: One gram of LFK per day was administered orally to fifty perennial allergic rhinitis patients in an open trial that lasted 28 days. Nasal symptoms and sign scores were rated before and after administration of LFK. Tuberculin responses and peripheral blood cells were also measured before and after LFK treatment.
Results: Purified protein derivative (PPD) skin test diameter was 2.14 ± 2.14 mm before LFK administration versus 7.26 ± 4.81mm at day 31 ($p<0.01$). A significant inverse correlation was recognized between PPD skin test diameters and total nasal scores in the nasal provocation test before and after treatment ($r= - 0.600$, $p<0.001$). Peripheral blood eosinophils were 248 ± 149 cells/ml before LFK administration and then they significantly decreased to 76 ± 98 cells/μl ($p<0.01$).
Conclusions: These finding may be interpreted as a result of improved clinical symptoms in allergic rhinitis after LFK oral treatment owing to the enhanced host's Th1-type immune responses and supression of the over-expression of Th2-dominated allergic responses.

Key words: allergic rhinitis; allergy; tuberculin responses; eosinophils; lactic acid bacteria; *Enterococcus faecalis*

Introduction

An inverse association between tuberculin responses and atopy has been observed in Japanese children, suggesting that BCG immunization, subclinical exposure to *Mycobacterium tuberculosis* in early childhood without clinical disease, or host characteristics may influence the Th1/Th2 balance with decreased atopy as a result [1]. Moreover, there is epidemiological evidence that shows the increasing tuberculosis notification rates associated with a stepwise decrease in symptoms of asthma and rhinoconjunctivitis in an international ecological study [2]. These data provide the first suggestion that a drug capable of enhancing tuberculin responses in allergic patients may lead to improvement of their clinical symptoms.
Lysed *Enterococcus faecalis* FK-23 (LFK) is a kind of lactic acid bacteria (LAB) preparation processed by bacteriolytic enzyme and heat treatment. It has been shown that LFK could inhibit active cutaneous anaphylaxis (ACA) [3] and allergen-induced peritoneal accumulation of eosinophils in mice [4]. In addition, there is clinical evidence that LFK was effective in the improvement of symptoms of Japanese cedar pollinosis [5] and rosacea [6]. However, little is known about how LFK plays a role in combating allergy. The aim of this study was to clarify if improvement of clinical manifestations is associated with enhanced tuberculin responses in patients with allergic rhinitis treated by LFK.

Material and Methods

Subject

A total of fifty adult patients (38 men and 12 women) with perennial allergic rhinitis were enrolled in this open trial performed at the International Research Centre for Nasal Allergy, Nanjing Medical University. They ranged in age from 18 to 53 years (32.6 ± 9.6 years). Patients were recruited on the basis of a clinical history of allergic rhinitis and the presence of positive skin test response to house dust mites. Patients were excluded if within the previous 4 weeks they had taken specific immunotherapy and the following drugs: long-acting antihistamine, intranasal or systemic corticosteroids. The exclusion criteria were the presence of concurrent positive tuberculin skin responses (mean diameter 5 mm and above) in the patients. Pregnant or lactating women were also excluded. No patients received any medication for their allergy during the 7 days' observation period and the whole length of LFK administration. The study was performed with the approval of the local ethics board and informed consent was obtained from all patients.

LFK administration

LFK (Nichinichi Pharmaceutical Co., Ltd., Mie, Japan) is a bacteriolytic and heat-treated *E. faecalis* FK-23 preparation with no viable cells. *E. faecalis* is isolated from one of the LAB strains in human commensal flora. Two packages of LFK per day were administered orally to all patients for 28 days. Each package contains 0.5 g of LFK and 1.5 g of dextrin. The daily dose in this study is equivalent to over 2.4 trillion cells.
As a functional supplementation, LFK has been in the Japanese market since 1992.

Assessment of nasal scores

Scores of nasal symptoms (sneeze attack, nasal discharge and nasal obstruction) and signs (swelling of inferior turbinate, watery secretion and description of nasal discharge) were rated independently before the treatment and after 28 days' administration, based on a "four-point scale method" (0 = no symptom or sign, 1 = mildness, 2 = moderation, 3 = seriousness) according to Okuda's standard [7]. For nasal provocation test, a small paper disc soaked in the allergen extract of house dust mites was placed on the inferior turbinate, as described in literature [8].

Measurement of tuberculin responses

Tuberculin responses of all patients were measured by a standard method. Basically, single needle intradermal injection of purified protein derivative (PPD) was applied to patients before and after LFK treatment. Long and short diameters (mm) of the skin redness were measured 48 hours after the injection and the arithmetic mean of both diameters was calculated.

Peripheral blood cell count

The blood samples from all patients were collected before and after LFK treatment. Peripheral blood cell counting was carried out by autoanalyzer.

Statistical analysis

The Statview Computer Package (Abacus Concepts, USA) was used for all analyses. Data were expressed as mean ± standard deviation (S.D.). Paired Student's t-test was performed before and after treatment for nasal scores, tuberculin responses and blood cell count. The frequency of tuberculin responses enhancement was analyzed by Fisher's exact test. A p-value < 0.05 was considered statistically significant.

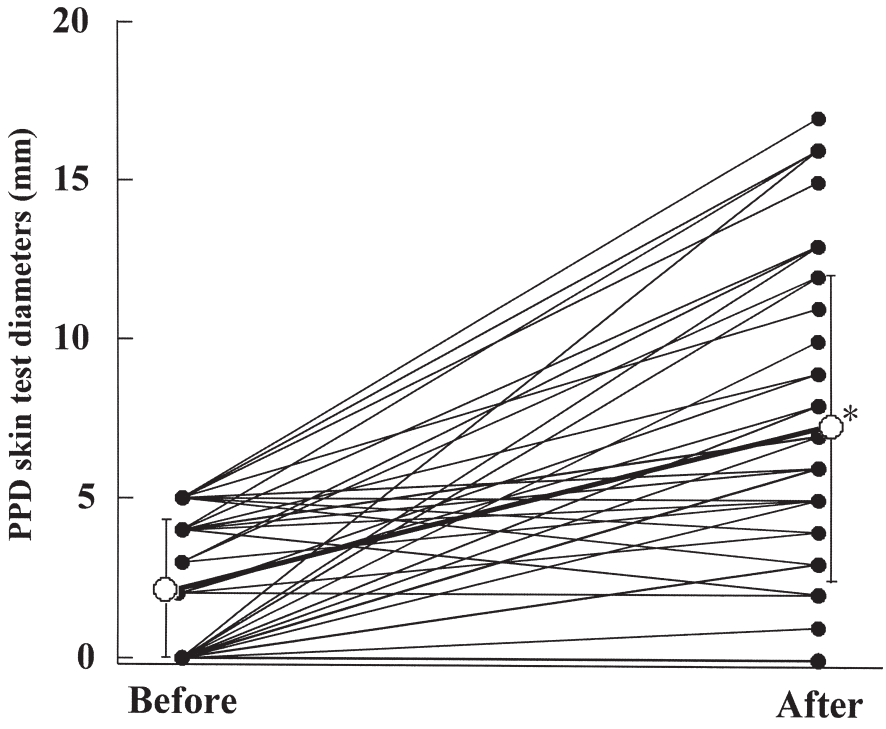

Figure 1. PPD skin test diameters in perennial allergic rhinitis patients before and after LFK treatment
n=50, mean ± S.D.
* p<0.01 compared with the previous LFK administration.

Results

Tuberculin responses and nasal scores

PPD skin test diameter was 7.26 ± 4.81mm at day 31, though it was 2.14 ± 2.14 mm before LFK administration (Figure 1). There was a significant difference before and after treatment (p<0.01). Conversely, the total nasal symptom scores decreased significantly from 15.7 ± 1.6 to 10.4 ± 2.4 (p<0.01). The number of improvements and the improvement rate of nasal symptom and sign scores after LFK administration are shown in Table 1. Clinical symptoms improved after treatment, especially sneezing. A significant inverse correlation was recognized between PPD skin test diameters and total nasal scores in the nasal provocation test before and after LFK treatment (r= -0.600, p<0.001), as shown in Figure 2.

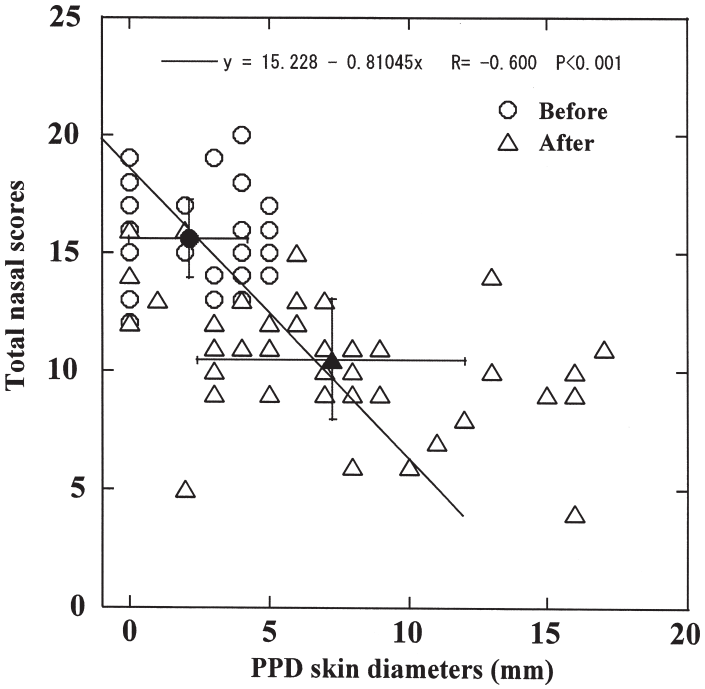

Figure 2. Relationship between total nasal scores and PPD skin test diameters in the patients with perennial allergic rhinitis before and after LFK treatment.
Plot of total nasal scores vs PPD skin test diameters in the same patients; n=50, mean ± S.D., (O) and (△) mean before and after LFK administration (r= -0.600, p<0.001).

Peripheral blood cells

Peripheral blood cells in all patients were also counted before and after treatment. Significant quantitative changes were observed in eosinophils and monocytes. Eosinophils decreased significantly from 248 ± 149 cells/μl to 76 ± 98 cells/μl (p<0.01), while monocytes

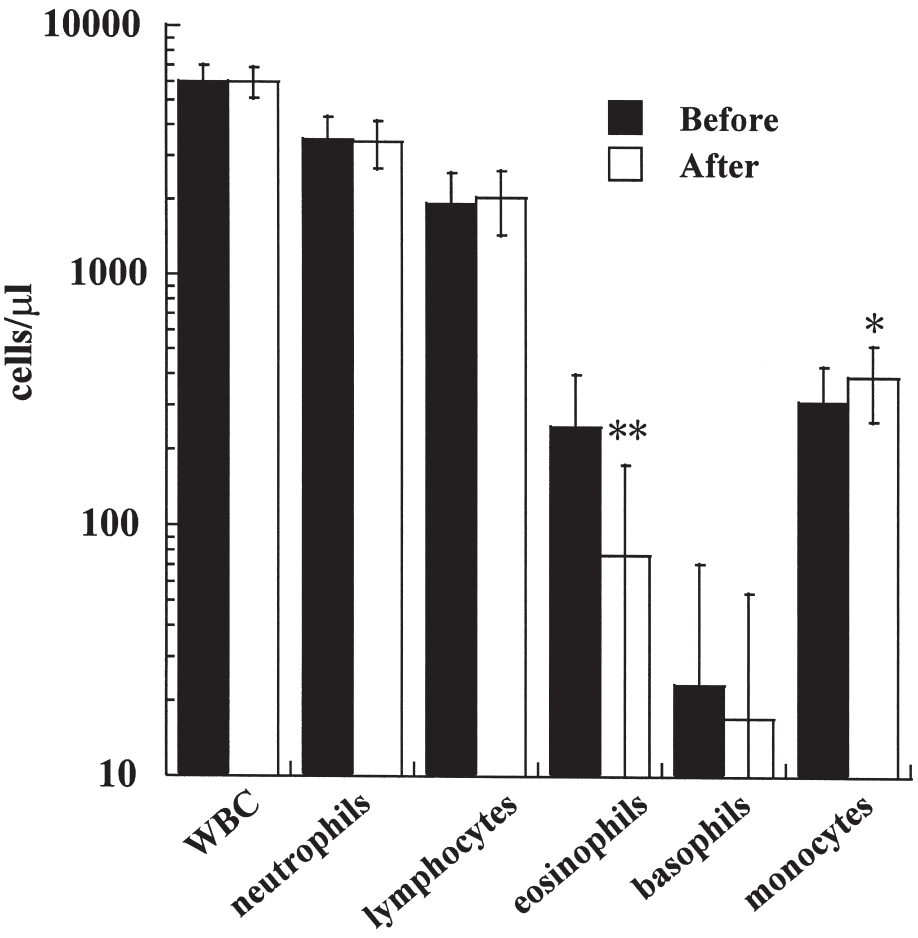

Figure 3. Peripheral blood cells in perennial allergic rhinitis patients before and after LFK treatment n=50, mean ± S.D.,
* $p < 0.05$;
** $p < 0.01$ compared with the previous LFK administration.

Table 1. Effect of LFK treatment on perennial allergic rhinitis

Symptoms & Signs	n	Improvement					Improvement rate (%)
		Marked	Moderate	Slight	Unchanged	Worse	Moderate and better
Sneeze attack	49	16	2	22	9	0	36.7
Nasal discharge	49	12	2	18	17	0	28.6
Nasal obstruction	44	2	1	20	21	0	6.8
Swelling of inferior turbinate	49	2	1	32	14	0	6.1
Quantity of watery secretion	50	4	2	21	23	0	12.0
Character of Nasal discharge	50	4	0	7	35	4	8.0
Nasal provocation reaction	49	0	2	20	27	0	4.1

increased from 310 ± 123 cells/μl to 389 ± 130 cells/μl ($p<0.05$), as shown in Figure 3.

Overall safety

No safety problem was identified during the LFK administration.

Discussion

The increase in the prevalence of allergic disease during the last few decades is likely to be explained by changes in the environment, including reduced microbial exposure and altered food consumption [9]. The introduction of scientifically composed probiotic functional foods for prophylactic or therapeutic purposes could be a solution. Recently, the role of intestinal microflora, such as several strains of LAB in priming the immune system during ontogeny to limit allergy has been brought to attention [10, 11]. Epidemiological studies have shown a higher incidence of allergy expression in early childhood among children who have low enteric populations of LAB, supporting the notion that appropriate microbial colonization of the gut can lower the risk of developing allergy [12, 13]. There is also clinical evidence that appropriate gut-colonizing microbes can control the development of atopy [14, 15]. In further studies, some LAB strains were shown to increase the IL-10 and IFN-γ levels[16], whereas they inhibit the secretion of IL-4 and IL-5 [17] by mononuclear cells from allergic patients, suggesting a possible use of LAB for combating allergy. A recent randomized placebo-controlled trial by Kalliomaki et al. [18] proved that one such strain, *Lactobacillus rhamnosus* GG, was effective for prevention of early atopic disease in Finnish children at high risk.

In the present study, we tried oral administration with LFK in patients with perennial allergic rhinitis. We observed a significant inverse correlation between PPD skin test diameters and total nasal scores ($r= -0.600$, $p<0.001$) in the nasal provocation test before and after 28 days treatment. Simultaneously, peripheral blood eosinophils were significantly decreased ($p<0.01$) after LFK treatment.

It has been found that the incidence of allergic disorder is significantly higher in Japanese schoolchildren with negative tuberculin responses than in those with positive tuberculin responses [1]. Thus if we could enhance the tuberculin responses, which works as an indicator of effects mediated by Th1-type immune responses, with certain reagents in allergic patients, it may be able to suppress the Th2-type immune responses and lead to an improvement of the clinical symptoms of allergy.

Recently, Sudo et al. [19] showed that IgE/IgG2a ratio in kanamycin-treated mice significantly decreased by supplementation with *E. faecalis*. In our previous experimental study, we have shown that LFK could inhibit the allergen-induced eosinophil accumulation in mice [4]. Taken together, it is likely that LFK, a preparation of lysed *E. faecalis* FK-23, can enhance host's Th1-type immunity and suppress the over-expression of Th2-dominated allergic responses. Also, alteration of gut mucosal immunity after LFK oral treatment might explain our finding.

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