

Effect of lysed *Enterococcus faecalis* FK-23 (LFK) on allergen-induced peritoneal accumulation of eosinophils in mice

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Summary

Background The interest in anti-allergy immunoregulation by lactic acid bacteria has been growing for the last few decades. There is some evidence to suggest that lysed *Enterococcus faecalis* FK-23 (LFK) could relieve the clinical symptoms of pollinosis. However, the mechanism responsible for this phenomenon remains unknown.

Objective To identify the effect of LFK, a lysozyme treated and heat-killed preparation from the lactic acid bacteria *Enterococcus faecalis* FK-23 strain, on allergen-induced eosinophil accumulation.

Methods BALB/c mice were sensitized with ragweed pollen extract, and peritoneal accumulation of eosinophils was induced. A total of 60 mg (0.5 mL) LFK was orally administered to the experimental mouse every day during 21 days of the sensitization period. In addition, LFK 4 mg, 25 mg and 60 mg (each 0.5 mL) were also orally administered to a mouse of each group every day for 21 days. Saline was fed in a dose of 0.5 mL/mouse per day for the same duration as a control.

Results Compared with control mice, LFK-treated mice exhibited decreased ragweed pollen allergen-induced peritoneal accumulation of eosinophils ($P=0.013$), which showed a tendency to be in a dose-dependent fashion ($P=0.14$).

Conclusion The results provide laboratory evidence of the role for LFK, a lactic acid bacteria preparation, in combating eosinophil accumulation.

Keywords allergy, *Enterococcus faecalis*, eosinophils, lactic acid bacteria

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Introduction

Recently, increasing research attention has been paid to a role of lactic acid bacteria (LAB) in anti-allergy immunoregulation [1]. Studies on lysed *Enterococcus faecalis* FK-23 (LFK) by our group have shown that it could inhibit active cutaneous anaphylaxis (ACA) in mice [2], and relieve clinical symptoms in patients with Japanese cedar pollinosis [3]. In a recent clinical study, we have observed that the number of peripheral blood eosinophils reduced significantly after LFK treatment in patients with perennial allergic rhinitis (unpublished data). In addition, FK23, a heat-killed preparation made from the same strain of *E. faecalis*, has been demonstrated to be capable of some activities important for maintenance of human health, such as anti-tumour [4, 5], immunomodulated [6, 7] and anti-hypertensive effects [8].

Eosinophils are believed to be key effector cells in the pathogenesis of allergic disorders, and their recruitment into the

upper or lower airways appears to be essential for the clinical manifestations of allergen inhalation. Given the perceived importance of eosinophils in allergic inflammation, they may be logical therapeutic targets [9]. The purpose of this experimental study was to identify the effect of LFK on allergen-induced eosinophil accumulation.

Materials and methods

Preparation of LFK

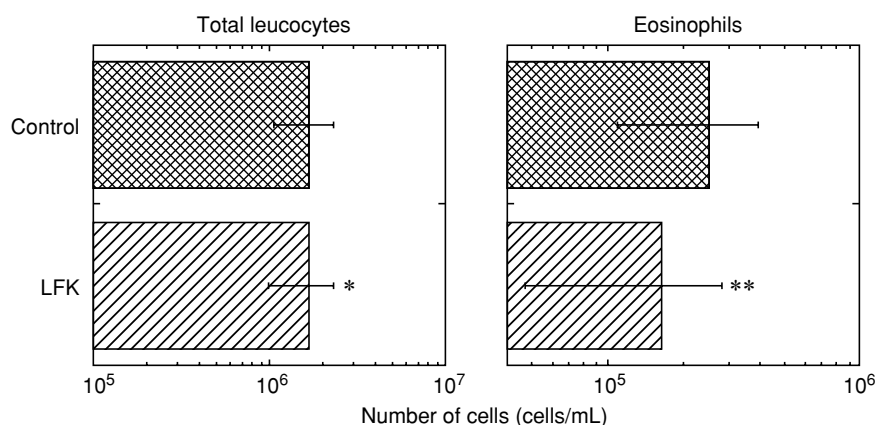
LFK was prepared as described previously [2]. Briefly, *E. faecalis* FK-23 was cultured for 24 h at 37 °C in a broth medium containing 2% glucose, 2% yeast extract, 2% meat extract and 4% K₂HPO₄. The cells were harvested by centrifugation, washed three times with distilled water and lysozyme treatment (added 1 mg/mL) at 37 °C for 2 h, and heated at 105 °C for 10 min, then lyophilized. The preparation is LFK.

Experimental animals

Female BALB/c mice were purchased from SLC Inc., Shizuoka, Japan. Five-week-old mice were used for all experiments. They were fed on pellet diet CE-2 (Clea Inc., Tokyo, Japan) and

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Fig. 1. Effect of LFK on peritoneal accumulation of total leucocytes and eosinophils in ragweed pollen allergen-sensitized mice. Each value represents mean $\pm$ SD. * $P=0.78$, ** $P=0.013$, as compared with control.



received tap water that had been filtrated through a PF filter. All animals were housed in cages with a 12-h light/dark cycle. Temperature and humidity were controlled at $25.0 \pm 1.0^\circ\text{C}$ and $55.0 \pm 5.0\%$.

Allergen-induced cell accumulation

The animal model was prepared according to the procedure of Kaneko et al. [10]. BALB/c mice were sensitized with the commercially prepared ragweed pollen extract 1/100 w/v (Torii Pharmaceutical Co. Ltd, Tokyo, Japan); 0.1 mL of this dilution was injected subcutaneously on days 0 and 1, and 0.2 mL was injected subcutaneously on days 6, 8 and 14. The mice were challenged on day 20 by the intraperitoneal injection of 0.2 mL of the dilution of ragweed pollen extract. Peritoneal cells were harvested 24 h after challenge with 4 mL of phosphate-buffered saline containing 1.0% fetal calf serum and 5 U/mL heparin. An appropriate phosphate-buffered saline dilution of the infusion was added to Turk's solution, and the total number of blood cells was counted with a haemocytometer under a microscope. For this purpose, 50 μL of the peritoneal cell suspension (5×10^5 cells/mL) was smeared on a microscope slide after centrifugation. A differential cell count was carried out under a microscope after fixation and staining with May–Grunwald Giemsa dye.

Administration of LFK

LFK 60 mg (0.5 mL) was orally administered to the experimental mouse ($n=29$) every day in LFK group, during 21 days of the sensitization period. Saline was fed in a dose of 0.5 mL/mouse per day for the same duration as a control ($n=26$).

In addition, to explore the dose-dependent effect of LFK on allergen-induced peritoneal accumulation of eosinophils, LFK 4 mg, 25 mg and 60 mg (each 0.5 mL) were also orally administered to mice of each group ($n=6$, respectively) every day for 21 days. The administration of saline for the control group ($n=6$) was the same as above.

Statistical analysis

Data were expressed as the mean $\pm$ standard deviation (SD). The SPSS 10.0J was used for analysis. Statistical differences between the groups were determined by unpaired Student's *t*-test and one-way analysis of variance (ANOVA). Differences were considered significant if the *P*-value for the effect was less than 0.05.

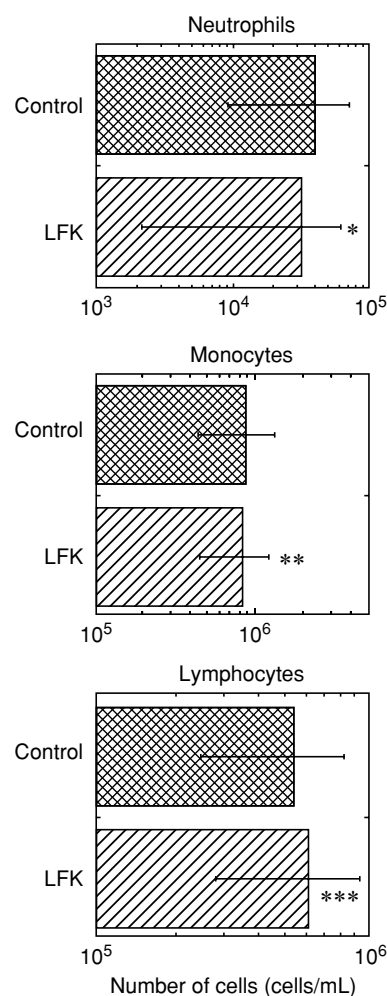


Fig. 2. Effect of LFK on peritoneal accumulation of neutrophils, monocytes and lymphocytes in ragweed pollen allergen-sensitized mice. Each value represents mean $\pm$ SD. * $P=0.31$, ** $P=0.80$, *** $P=0.37$, as compared with control.

Results

Effect of LFK on peritoneal accumulation of eosinophils in ragweed pollen allergen-sensitized mice

As shown in Fig. 1, the total number of accumulated cells in control and LFK groups were 1.69×10^6 and 1.64×10^6

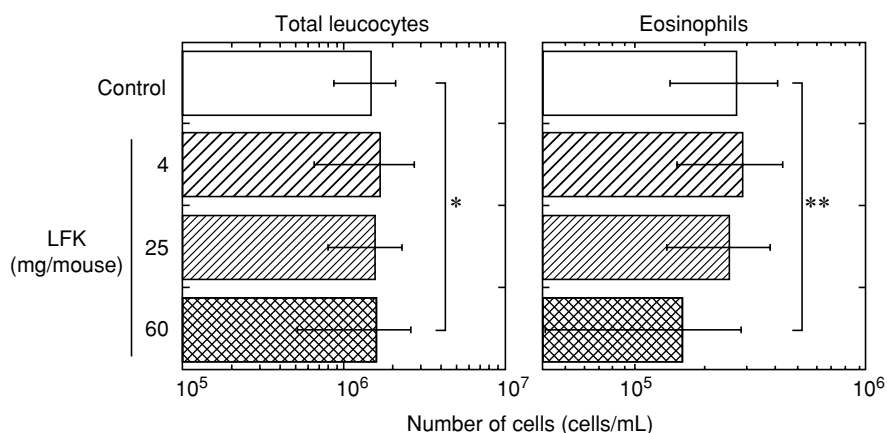


Fig. 3. Dose-dependent effect of LFK on peritoneal accumulation of total leucocytes and eosinophils in ragweed pollen allergen-sensitized mice. Each value represents mean $\pm$ SD of six mice. * $P=0.96$, ** $P=0.14$, as compared with control.

cells/mL, respectively ($P=0.78$). Interestingly, accumulation of eosinophils in control and LFK groups were 2.53×10^5 and 1.63×10^5 cells/mL, respectively ($P=0.013$). Compared with a decrease in the number of eosinophils, the number of lymphocytes increased in LFK-treated mice (see Fig. 2).

Dose-dependent effect of LFK on peritoneal accumulation of eosinophils in ragweed pollen allergen-sensitized mice

As shown in Fig. 3, the total number of accumulated cells in control mice and 4 mg, 25 mg and 60 mg LFK-treated mice were 1.47×10^6 , 1.66×10^6 , 1.52×10^6 and 1.53×10^6 cells/mL, respectively. No significant difference was observed among all groups ($P=0.96$). Accumulation of eosinophils in control mice and 4 mg, 25 mg and 60 mg LFK-treated mice was 2.76×10^5 , 2.92×10^5 , 2.55×10^5 and 1.60×10^5 cells/mL, respectively. It was inclined to be a dose-dependent fashion in combating eosinophil accumulation ($P=0.14$), but LFK did not have any effect on the number of neutrophils, monocytes and lymphocytes among each group.

Discussion

Over the past few decades, 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 [1]. Epidemiological studies have demonstrated 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 [11, 12]. There is also clinical evidence that appropriate gut-colonizing microbes can control the development of atopy [13, 14]. Further studies, utilizing splenocytes derived from allergen-primed mice, have demonstrated that the inclusion of LAB cells in allergen-stimulated cell cultures can increase production of IFN- γ , but reduces levels of allergen-driven IL-4, IL-5 and IgE [15, 16].

Extensive experimental studies and clinical investigations have implicated eosinophils in the pathogenesis of allergic diseases. Eosinophil accumulation into inflammatory sites should be viewed as a characteristic phenomenon of allergic inflammation [9]. In the present study, it has been demonstrated that LFK, a preparation of lysozyme treated and heat-killed *E. faecalis* FK-23 strain, inhibited the ragweed pollen

allergen-induced peritoneal accumulation of eosinophils and was inclined to be in a dose-dependent fashion in mice. However, FK23, a preparation of heat-killed *E. faecalis* FK-23 strain, did not show the same inhibition (data not shown). Interestingly, LFK and FK23 were differently treated preparations from the same strain of *E. faecalis* FK-23.

Although some attempts have been made to study the mechanism of LFK on inhibition of eosinophil accumulation, it remains uncertain. We have found that lysozyme treatment of the cell-wall compound of *E. faecalis* FK-23 could inhibit the peritoneal accumulation of eosinophils in mice [17]. In addition, LFK has an obvious effect on decreasing serum ovalbumin (OVA)-specific IgE levels, but not OVA-induced total IgE levels in experimental mice (data not shown). Furthermore, in a recent clinical study, we have observed that LFK could decrease the peripheral blood eosinophils and improve the tuberculin responses in patients with perennial allergic rhinitis (unpublished data).

Taken together, it is likely that LFK can prevent the over-expression of Th2-dominated immune responses. Also, alteration of gut mucosal immunity after LFK oral treatment might explain our finding. Further research on the molecular mechanism of LFK in combating eosinophilia and allergy is needed to clarify the association of orally delivered LAB and anti-allergy immunoregulation.

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