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Research article

Pediococcus sp. KB1 and rosmarinic acid cooperatively improve the symptoms in a murine cedar pollinosis by inhibition of FcεRIα via splenic increase of IL-10 and IL-27 expression

Akina Omori[1], Takao Mori[2], Kazunobu Baba[1] and Tadaaki Miyazaki[1,*]

[1] Department of Synbiotics, Institute for Genetic Medicine, Hokkaido University, North, 15 West 7, Kitaku, Sapporo, Hokkaido, 060-0815, Japan
[2] LAB biotech Co., Ltd, Hokudai Business Spring Bidg, North 21 West 12, Kitaku, Sapporo, Hokkaido, 001-0021, Japan

* **Correspondence:** Email: miyazaki@pop.med.hokudai.ac.jp; Tel: +81117068095.

Abstract: Lactic acid bacteria and polyphenols are known to have various health-promoting effects. *Pediococcus* sp. KB1 (KB1) is a probiotic that was isolated from pickled suguki turnips and has been shown to have potential immunomodulatory activity and strong resistance to gastric acid. Rosmarinic acid (RA) is a phenolic compound commonly found in Lamiaceae plant species, known for its broad anti-inflammatory properties. However, the underlying mechanisms of the combined effects of KB1 and RA on pollinosis remain unclear. The aim of this study was to investigate the effects of oral administration of KB1 and RA on symptom amelioration and anti-inflammatory activity using a murine model of pollinosis. Our results showed that the KB1 + RA group had attenuated sneezing and nasal rubbing in our murine model of cedar pollinosis. The KB1 + RA group exhibited decreased serum histamine levels and reduced Cry j1-specific IgE levels. In both the KB1 and KB1 + RA groups, spleen tissue weight was significantly reduced. In addition, in the KB1 + RA group, increased mRNA expression levels of IL-10, IL-27, IFN-γ, and Foxp3 in the spleen, along with reduced FcεRI-α protein expression, were found. These observations suggest that the combined administration of KB1 + RA may alleviate symptoms by regulating the Th1/Th2 balance and anti-inflammatory cytokines. These results suggest that KB1 and RA may serve as potential natural anti-inflammatory agents for the management of pollinosis.

Keywords: Japanese cedar pollinosis; Cry j1; heat-killed *Pediococcus* sp. KB1; rosmarinic acid; anti-inflammation

Abbreviations: KB1: *Pediococcus* sp. KB1; RA: Rosmarinic acid; JCP: Japanese cedar pollinosis; IL-10: Interleukin -10; Th: T helper; IFN-γ: Interferon-γ; IgE: Immunoglobulin E; $Al(OH)_3$: Aluminum hydroxide; Tr1: T regulatory 1

1. Introduction

Allergic rhinitis is a major health challenge, affecting 10%–20% of the global population [1]. In Japan, Japanese cedar pollen is a primary trigger of seasonal allergic rhinitis (SAR), with 40% of the Japanese population reportedly suffering from cedar pollinosis [2]. This condition, first reported in 1960, significantly diminishes patients' quality of life, rendering it a serious societal issue [3]. During peak allergy season, individuals with cedar pollinosis endure more intense and prolonged symptoms than those with other forms of pollinosis [4]. The major allergens contributing to pollinosis in Japan are derived from Japanese cedar, with Cry j1 (a pectate lyase) [5] and Cry j2 (a polygalacturonase) [6] being the first identified major JCP allergens. Cry j1, a major cedar allergen, is routinely employed to establish murine pollinosis models, facilitating research on the pathogenesis, prevention, and treatment of pollinosis [5]. Despite substantial progress in the diagnosis and treatment of pollinosis, a fundamental cure remains elusive [4]. Current therapeutic interventions are limited to anti-pollinosis medications, such as antihistamines, which mitigate allergic symptoms by antagonizing H1 receptors. Although highly effective, antihistamines are associated with adverse effects such as drowsiness and impaired judgment, thereby compromising patients' quality of life [7]. This has led to a growing interest in functional nutrients, which are widely consumed and considered safe, albeit with generally milder efficacy compared to pharmaceutical agents [8]. Given that pollinosis is an inflammatory nasal disorder, therapies aimed at modulating inflammatory responses are pivotal.

Interleukin (IL)-10 is recognized as a potent anti-inflammatory cytokine that exerts a broad impact on both innate and acquired immunity. IL-10 was first described as a cytokine synthesis-inhibitory factor, originating from mouse Th2 cells, capable of inhibiting the activation of Th1 cells and their cytokine production [9]. Consequently, augmenting IL-10 production within the immune system represents a novel therapeutic approach for allergic diseases. In contrast, IL-27 directly mediates IL-10 transcription via the activation of two transcription factors, STAT1 and STAT3, which are recruited to the activated receptor [10]. IL-27, a cytokine with multifaceted influences on the immune response, modulates the differentiation of T helper (Th) cell subtypes (e.g., Th1, Th2, and Th17) via downstream transcription factor activity. This modulation results in Th1 promotion, Th2/Th17 suppression, and IL-10 induction. IL-27 is a heterodimeric cytokine composed of IL-27p28 and Epstein-Barr virus-induced gene 3, which exhibits sequence similarity to the p40 and p35 subunits of IL-12, respectively [11]. Furthermore, T-helper cells play a pivotal role in the initiation and progression of allergic diseases, as well as resistance against them [12]. Th1 cells produce cytokines, such as interferon-γ (IFN-γ), to promote cellular immunity, whereas Th2 cells generate cytokines, including IL-4 and IL-5, to drive humoral immunity through immunoglobulin E (IgE) production [13]. Consequently, rebalancing the Th1/Th2 axis from a Th2-dominant state toward a Th1-dominant state could potentially suppress both IgE production and inflammatory responses [14].

KB-1 is a lactic acid bacterium (LAB) isolated from suguki, a traditional Japanese pickled turnip, that exhibits great potential for modulating immune responses, including allergic symptoms [15]. On the other hand, rosmarinic acid (RA), a polyphenolic phytochemical, is widely distributed in numerous herbal plants, including rosemary, oregano, blue perilla, lemon balm, basil, and mint. RA is well documented for its broad spectrum of pharmacological activities, such as anti-inflammatory, anti-allergic, and hepatoprotective effects [16]. For example, a study demonstrated that Lamiaceae plants, which contain RA, attenuate IgE secretion and inflammation via lymphocytes, indicating the potential of RA to modulate the immune system [17].

However, to our knowledge, no studies to date have investigated the immunomodulatory effects of KB1 in conjunction with RA during pollinosis. Our investigation focuses on lactic acid bacteria and polyphenols, particularly their antioxidant attributes, as potential anti-allergic food materials. This study specifically examines whether the oral administration of heat-killed KB1 in combination with RA, a compound found in Lamiaceae plants, suppresses inflammation and symptoms, both locally and systemically, induced by JCP allergens. We propose that KB1 + RA administration mitigates the severity of pollinosis via IL-27-mediated IL-10 upregulation in the spleen.

2. Materials and methods

2.1. KB1, RA, and Cry j1

Pediococcus sp. KB1 (1.5×10^{12} colony forming units/g) was obtained from Nissei Bio Co., Ltd (Hokkaido, Japan). The bacterial cells were suspended in distilled water and subjected to heat inactivation at 80 ℃ for 30 min. RA (96% purity by HPLC), supplied as a powdered dry extract, was acquired from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). It was dissolved in ethanol to create a stock solution, which was later diluted with distilled water before use. The ethanolic stock solution of RA was maintained at −80 ℃ prior to use. Cry j1 was obtained from Funakoshi Co., Ltd. (Tokyo, Japan).

2.2. Mice

Five-week-old specific-pathogen-free (SPF) male BALB/c mice (six per group) were acquired from CLEA Japan, Inc. (Tokyo, Japan). All animal procedures strictly adhered to the guidelines for the care and use of laboratory animals set forth by Hokkaido University. The protocols were approved by the Animal Experiment Committee of Hokkaido University. The mice were maintained in an SPF animal facility under controlled 12-h light/12-h dark conditions and provided *ad libitum* access to water and standard chow (CLEA Japan, Inc., Tokyo, Japan).

2.3. Experimental schedule

The mice were randomly allocated into five experimental groups. Cry j1-sensitized mice underwent initial sensitization on days 7, 14, and 21 via intraperitoneal injection of 0.5 μg of Cry j1 combined with 100 μg of aluminum hydroxide [$Al(OH)_3$] and dissolved in 0.2 mL of phosphate-buffered saline (PBS). The sensitization phase was followed by a 10-day challenge phase, during which mice received daily intranasal administrations of 0.5 μg of Cry j1 dissolved in 10 μL of PBS. The sham

group received 0.2 mL of PBS intraperitoneally, followed by 10 μL of PBS intranasally. On day 31, following intranasal Cry j1 administration, the frequency of sneezing and face scratching was quantified over a 10-min observation period. Throughout the 31-day study, heat-killed KB1 (10 mg/body/day) and/or RA (30 mg/kg/day) were orally administered in 0.2 mL of distilled water six days per week. Mice in both the sham and control groups (sensitized and challenged with Cry j1) received only 0.2 mL of distilled water. Since the blood concentration and effects of polyphenols often peak approximately 2 h after ingestion [10], the test substance was administered 1 h prior to Cry j1 sensitization to ensure maximum effect at the time of dissection. On the final day of the study, following euthanasia via isoflurane inhalation, the spleens and livers of the mice were harvested, rinsed with PBS, and examined (Figure 1).

(1) Sham group: Negative control; received PBS for both sensitizations.
(2) Control group: Cry j1-induced JCP group; sensitized and challenged with Cry j1.
(3) KB1 treatment group: Cry j1-challenged mice receiving KB1 (10 mg/body/day) by oral administration 1 h before challenge.
(4) RA treatment group: Cry j1-challenged mice receiving RA (30 mg/kg/day) by oral administration 1 h before challenge.
(5) KB1 + RA treatment group: Cry j1-challenged mice receiving KB1 (10 mg/body/day) and RA (30 mg/kg/day) by oral administration 1 h before challenge.

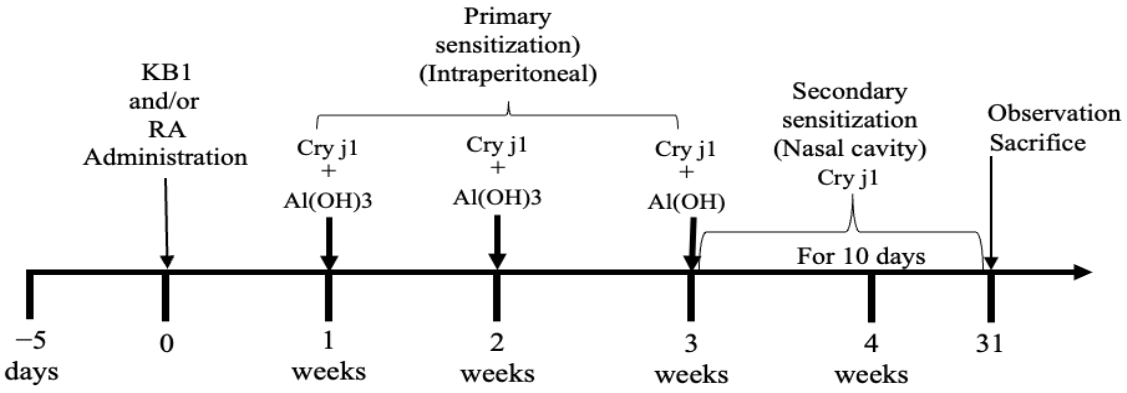

Figure 1. Schematic diagram of the experimental protocol for Cry j1- induced JCP mouse model. BALB/c mice immunized by intraperitoneal injection of Cry j1 + aluminum hydroxide were challenged by intranasal injection. Test group mice were orally administered with KB1 (10 mg/body/day) and/or RA (30 mg/kg/day) throughout the experimental period. Blood and fecal sampling and behavioral observation were performed on the indicated days. The detailed experimental procedure is described in Section 2.

2.4. Total leukocyte enumeration

For total leukocyte counts, 10 μL of blood was mixed with 90 μL of Turk's staining solution (FUJIFILM Wako Pure Chemical Corporation), and leukocytes were counted using a hemocytometer under a light microscope. The distinct staining properties of Turk's solution enabled clear differentiation of leukocytes, facilitating a straightforward and unambiguous cell count.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Heparinized blood, collected during experiments, was centrifuged to separate plasma from the blood cells. Serum samples were subsequently analyzed for cytokine concentration via ELISA. Specifically, serum levels of histamine and Cry j1-specific IgE were quantified using commercial ELISA kits, following the respective manufacturers' protocols. Mouse Histamine ELISA Set (Enzo Life Sciences, Inc., USA) was employed for histamine analysis, and Mouse IgE ELISA Set (FUJIFILM Wako Pure Chemical Corporation) was utilized for the analysis of Cry j1-specific IgE.

2.6. Total RNA extraction and quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA was isolated from approximately 50 mg of spleen tissue from each mouse group using the TRIzol reagent (FUJIFILM Wako Pure Chemical Corporation). RNA quality and concentration were determined spectrophotometrically using a NanoDrop 1000. Subsequently, 1 μg of total RNA was reverse transcribed into cDNA using the cDNA Synthesis Kit (TAKARA BIO INC, Japan). RT-qPCR was performed on a StepOnePlus system (Life Technologies, USA) using TB Green® Premix Ex Taq™ II (TAKARA BIO INC, Japan) according to the manufacturer's instructions. PCR conditions were as follows: 95 °C for 30 s, followed by 45 cycles of 95 °C for 5 s, and 60 °C for 30 s. All reactions were performed in quadruplicate. The data were automatically analyzed by the system, and amplification plots were obtained. We used the comparative Ct method to calculate relative mRNA expression. We verified the Ct values of both the control and target samples by normalizing them to the *GAPDH* gene. RT-qPCR results were calculated using the ΔΔCt method (amount of target = $2^{\wedge\Delta\Delta Ct}$). Primer sequences are listed in Table 1.

Table 1. Primer sequences (5'–3') used for quantitative real-time PCR.

	Forward primer	Reverse primer
IL-10	CGGGAAGACAATAACTGCACCC	CGGTTAGCAGTATGTTGTCCAGC
IL-27	TCTCGATTGCCAGGAGTGAACC	AGTGTGGTAGCGAGGAAGCAGA
Foxp3	CCCATCCCCAGGAGTCTTG	ACCATGACTAGGGGCACTGTA
IFN γ	ACACCTGATTACTACCTTCTTC	CTTCCTGAGGCTGGATTC
IL-12	CTTTGATGATGACCCTGTG	GAGTCTCGCCATTATGATTC
FcεRI α	ATTGTGAGTGCCACCGTTCA	GAAGGAGCAGCCAATCTTGC
GAPDH	TTCACCACCATGGAGAAGGC	GGCATGGACTGTGGTCATGA

2.7. Western blot analysis

Spleen tissues from each group were homogenized at 4 ℃ in RIPA buffer (FUJIFILM Wako Pure Chemical Corporation) supplemented with phosphatase inhibitors (Roche Diagnostics, Switzerland) to extract total proteins. Protein lysates were then resolved by 10% SDS-PAGE and subsequently transferred to nitrocellulose membranes. The membranes were then blocked with 5% skim milk for 30 min and were incubated with primary antibodies at room temperature for 1 h. After washing, the membranes were incubated with HRP-linked anti-mouse IgG (Cell Signaling Technology, USA) for 1 h at room temperature. The primary antibodies utilized were anti-FcεRI-α (Santa Cruz

Biotechnology, USA; 1:500) and anti-FcεRI-γ (Santa Cruz Biotechnology, USA; 1:500). β-actin (Cell Signaling Technology, USA; 1:4000) served as a loading control. Western blot signals were detected using enhanced luminol-based chemiluminescence (Bio-Rad Laboratories, USA) and visualized with a LAS-1000 gel imaging system (FUJIFILM Wako Pure Chemical Corporation). Relative band intensities were normalized to the mean intensity of the β-actin band.

2.8. Statistical analysis

All experimental data were statistically analyzed by one-way ANOVA followed by Tukey's multiple comparison test using IBM SPSS Statistics 29 (IBM Corp., Armonk, NY, USA) and expressed as mean ± SEM. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. sham group. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control group.

3. Results

3.1. Effects of KB1 and RA on pollinosis symptoms

To elucidate the effects of KB1 and RA, we initially developed a Cry j1-induced pollinosis mouse model through intraperitoneal sensitization with Cry j1 and aluminum hydroxide. The detailed administration schedule for KB1 and/or RA is presented in Figure 1. In the established Cry j1-sensitized mice, sneezing and nasal rubbing behaviors were quantified over a 10-min period. As expected, the control group exhibited a significant increase in pollinosis symptoms compared with the sham group. Although no significant reduction in the frequency of sneezing and nasal rubbing was observed with either KB1 or RA alone, the combined administration of KB1 + RA resulted in a significant decrease in these behaviors, suggesting a potential attenuation of allergic symptoms (Figure 2).

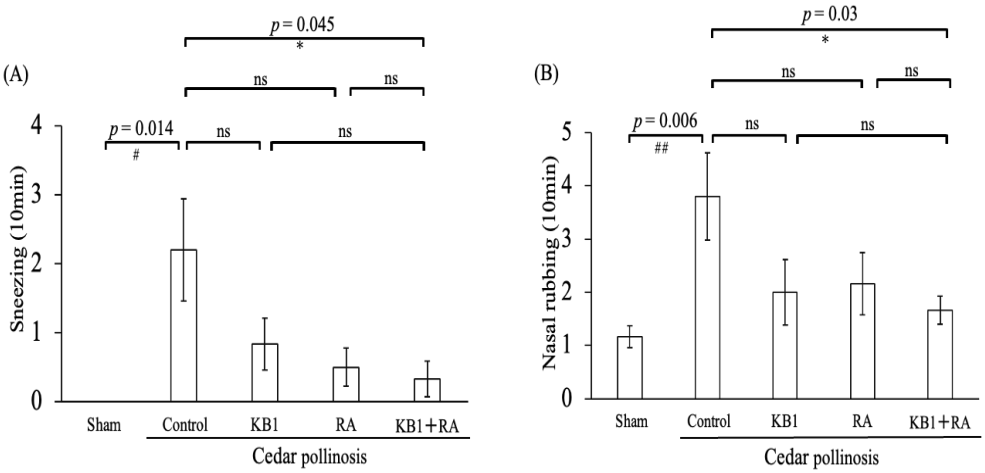

Figure 2. KB1 and/or RA attenuates allergic symptoms in Cry j1-induced JCP mice. (A) Number of sneezes. (B) Number of nasal rubbing events. Nasal symptoms were observed and counted for 10 min after antigen challenge. Data are presented as mean ± SEM from 6 mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}p < 0.01$, $^{\#\#}p < 0.01$ compared to sham group. $^{*}p < 0.05$ compared to control group.

3.2. Inflammatory markers and organ weights in pollinosis

To further characterize inflammatory symptoms in pollinosis, we quantified leukocyte numbers and measured the weight of spleen and liver tissues (Table 2). As demonstrated in Figure 3, a significant enlargement of the spleen was observed in Cry j1-induced control mice compared to the sham group. Conversely, a reduced spleen weight was observed in both the KB1 and KB1 + RA groups, compared to the control group (Table 2). Importantly, no significant differences were found in leukocyte numbers or liver weight between the sham and control groups (Table 2).

Table 2. Effect of oral administration of heat-killed KB1 and/or RA on leukocyte and tissue weight.

	Sham	Control	KB1	RA	KB1 + RA
Leukocyte ($\times 10^4$/mL)	509.2 ± 56.8	578.3 ± 61.9	535.8 ± 55.7	622.5 ± 61.2	548.3 ± 52.3
Spleen weight (mg)	107.9 ± 5.9	137.8 ± 6.2$^{###}$	123.1 ± 4.4*	130.6 ± 4.8	122.9 ± 3.9*
Liver weight (g)	1.59 ± 0.03	1.59 ± 0.03	1.55 ± 0.02	1.54 ± 0.02	1.59 ± 0.04

Data are presented as mean ± SEM from 6 mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison test. $^{###}p < 0.005$ compared to sham groups. $^{*}p < 0.05$ compared to control group.

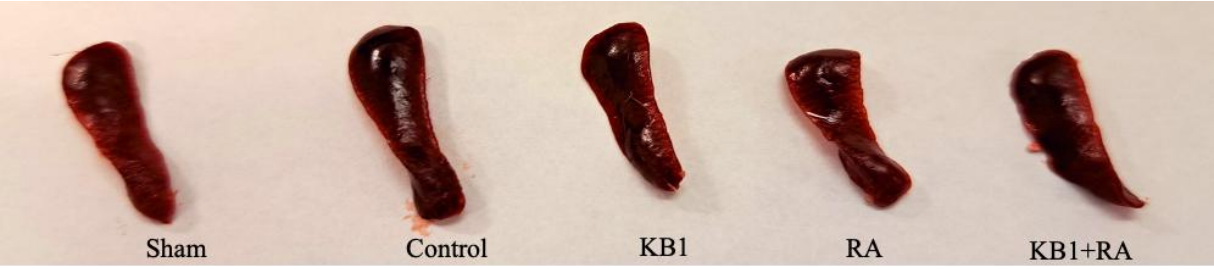

Figure 3. Pathological effect of oral administration of heat-killed KB1 and/or RA in the spleen tissue on Cry j1-induced JCP mice.

3.3. KB1 and RA reduce serum histamine and Cry j1-specific IgE levels.

Pollinosis is characterized by elevated serum levels of histamine and IgE; these biomarkers are commonly used to assess disease severity (Figure 4). We evaluated the effects of KB1 and RA, administered alone or in combination, on serum histamine and Cry j1-specific IgE in pollinosis mice. Control mice displayed significantly higher serum levels of histamine and Cry j1-specific IgE than the sham mice. KB1 administration reduced serum histamine levels, whereas RA administration decreased serum IgE levels. In contrast, co-administration of KB1 and RA significantly reduced both histamine and IgE levels.

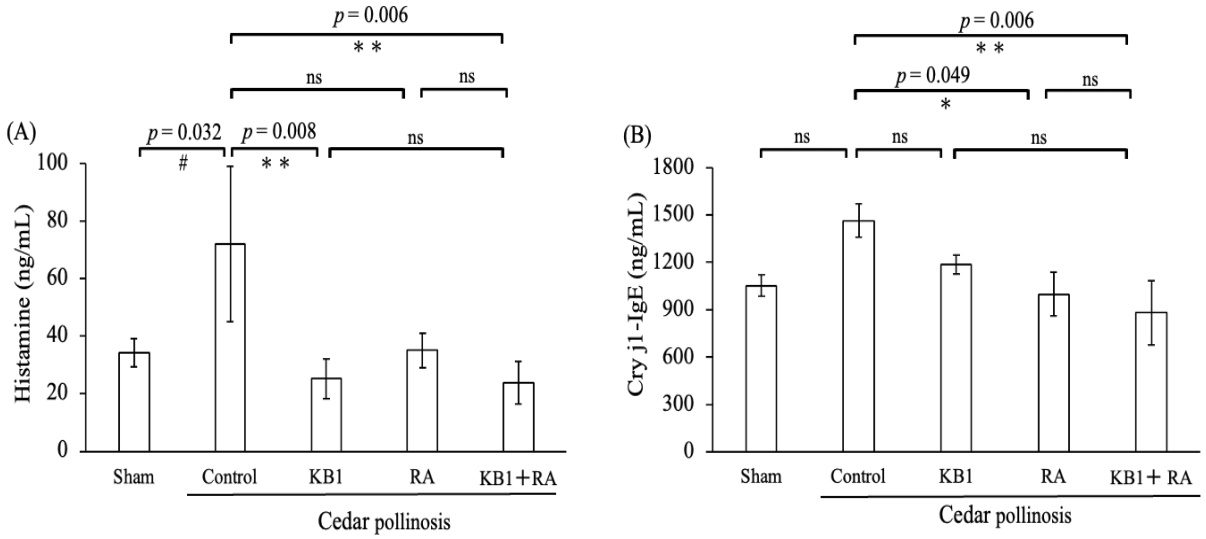

Figure 4. Effects of oral administration of heat-killed KB1 and/or RA on the production of histamine and Cry j1-IgE in the serum of Cry j1-induced JCP mice. (A) Histamine and (B) Cry j1-IgE in the serum were measured by ELISA. Data are presented as mean ± SEM from 4 mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}p < 0.05$ compared to sham group. $^{*}p < 0.05$, and $^{**}p < 0.01$ compared to control group.

3.4. Effects of KB1 and RA on mRNA expression of anti-inflammatory cytokines and allergy-related factors in the spleen tissue

Given the importance of anti-inflammatory cytokines and allergy-related factors in mitigating pollinosis pathogenesis and symptoms such as nasal inflammation, we investigated the mechanisms underlying the alleviation of inflammation and pollinosis symptoms by the administration of the KB1 and RA mixture. We quantified splenic mRNA expression of cytokines pivotal for anti-inflammatory responses and Th1/Th2 balance, such as IL-10, IL-27, Foxp3, IFN-γ, IL-12, and FcεRI-α (Figure 5). Our results showed that both KB1 and RA administration groups exhibited elevated splenic levels of *IFN-γ* and *IL-12* mRNAs. Interestingly, the KB1 + RA group demonstrated a very high level of IL-10, IL-27, and IL-12. Thus, the alleviation of pollinosis symptoms appeared to correlate with increased mRNA levels of these genes.

3.5. Effects of KB1 and RA on splenic FcεRI expression at mRNA and protein levels

IgE-bound FcεRI is aggregated in the presence of antigens, triggering mast cell activation and subsequent histamine release. We investigated the effects of KB1 and RA administration on both the mRNA and protein expression of this high-affinity Fc receptor. Although a reduction was observed in the RA administration group, the decrease was more pronounced in the KB1 administration group and in the KB1 + RA combined group. Western blot analysis showed that splenic levels of FcεRI-α and FcεRI-γ were significantly decreased in both the KB1 and KB1 + RA administration groups compared with the control group (Figure 6).

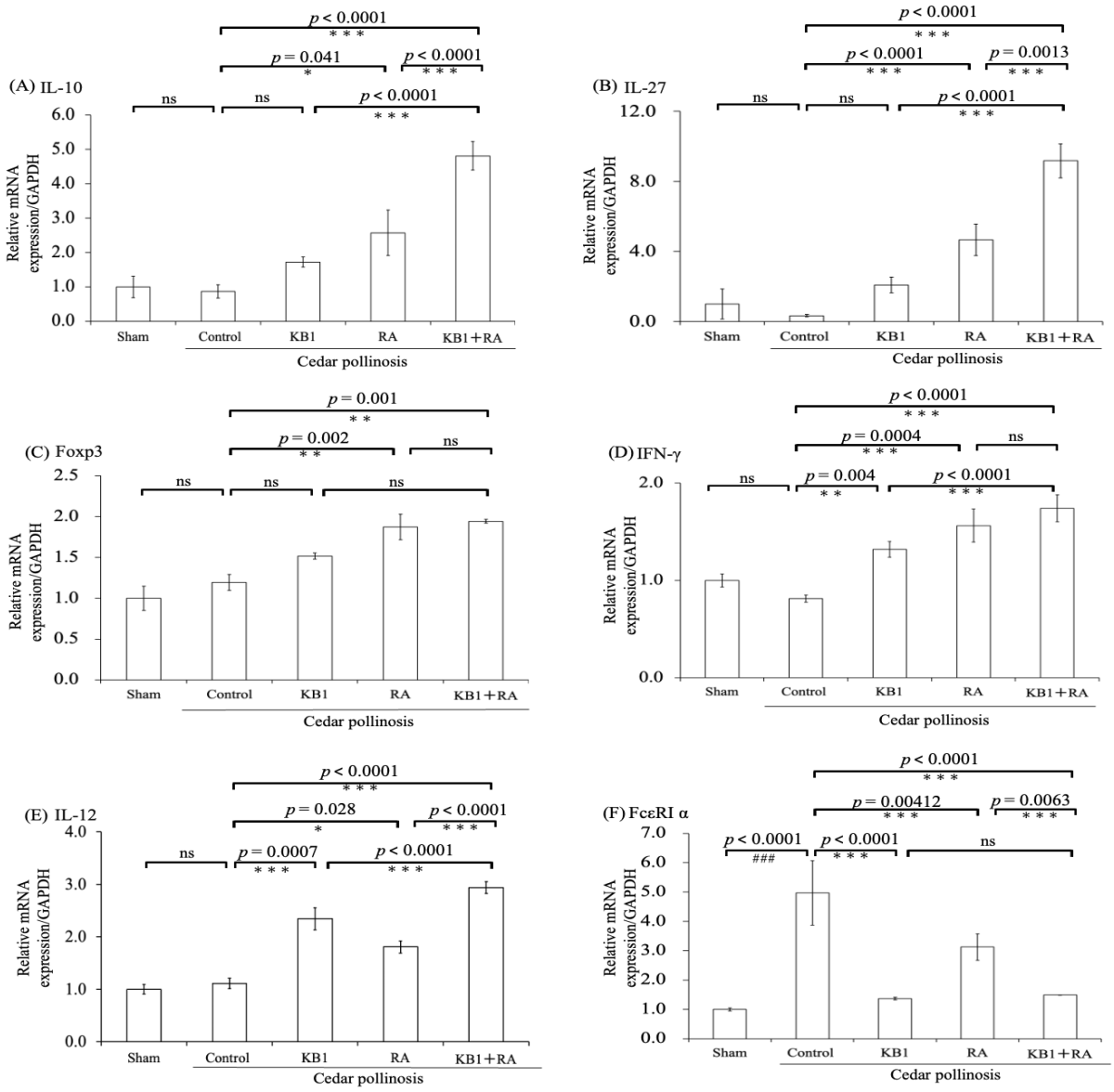

Figure 5. Effects of oral administration of heat-killed *Pediococcus* sp. KB1 and/or RA on mRNA expression of inflammatory cytokines and allergy factors in the spleen tissue of Cry j1-induced JCP mice. (A) IL-10, (B) IL-27, (C) Foxp3, (D) IFN-γ, (E) IL-12, and (F) FcεRI α mRNA expression in the spleen tissue were measured by real-time PCR. Data are presented as mean ± SEM from 6 mice in each group. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison test. $^{\#\#\#}p < 0.01$ compared to sham group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to control group.

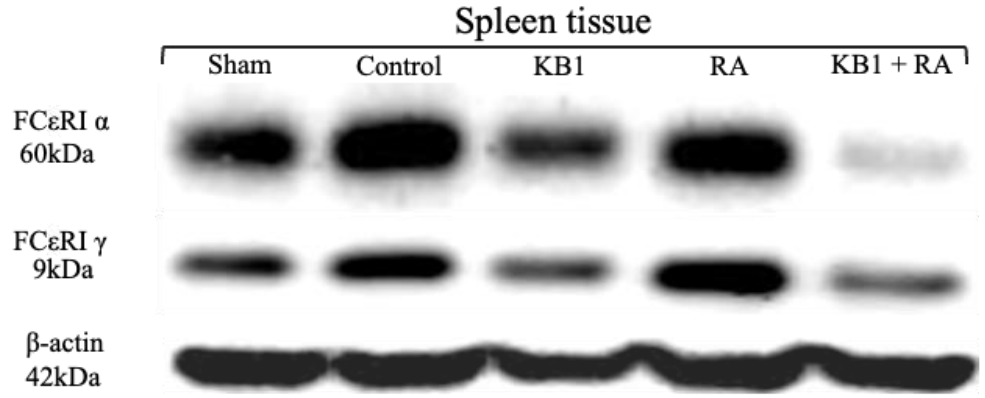

Figure 6. Effect of oral administration of heat-killed *Pediococcus* sp. KB1 and/or RA on high-affinity Fc receptor protein expression of spleen tissue of Cry j1-induced JCP. Western blot analysis of the expression of FcεRI α and FcεRI γ proteins in the spleen tissue of pollinosis mice.

4. Discussion

To the best of our knowledge, our study was the first to report that the KB1, a strain isolated from a Japanese traditional pickle vegetable, and RA, a phenolic compound found in Lamiaceae plants, show cooperative effects, robustly attenuating JCP symptoms in a five-week-old murine model. In recent years, heat-killed lactic acid bacteria, such as those used in our study, have been recognized as postbiotics, defined as non-viable microorganisms that confer health benefits independently of live bacterial colonization. Their anti-inflammatory activity is considered to be mediated by cell wall–derived components, including peptidoglycan fragments and lipoteichoic acids [18]. In contrast, polyphenols have recently been proposed to function as prebiotic-like compounds, as they confer beneficial effects on the gut microbiota through selective fermentation by specific microbial populations [19]. Moreover, polyphenols might interact with the gut microbiota to promote the growth of beneficial bacteria, such as *Bifidobacterium*, and suppress pathogenic bacteria [20]. In pollinosis, activated Th2 cells primarily produce inflammatory cytokines, such as IL-4, IL-5, and IL-13, leading to the symptoms of pollinosis [21]. The symptoms of pollinosis and associated systemic inflammation are closely linked to a fundamental shift in the Th1/Th2 balance [22]. Th1 cells secrete anti-inflammatory cytokines, including IFN-γ, IL-10, and IL-12, which regulate Th2-cell activation and proliferation [14]. In contrast, IL-4, a typical Th2 cytokine, primarily induces B-cell differentiation and enhances IgE production [21]. Consequently, the attenuation of allergic immune responses is directly associated with a rebalancing from Th2-dominant to Th1-dominant immunity [22]. In addition, activation of anti-inflammatory cells, including regulatory T cells (Tregs), plays an important role in suppressing pollinosis [23]. Our findings indicated that the combination of KB1 and RA more likely inhibited the production of antigen-specific IgE in the serum compared to groups treated with either KB1 or RA alone. In addition, we demonstrated that KB1 and RA suppressed not only serum histamine levels but also spleen weight and splenic swelling in pollinosis mice. The spleen is a crucial tissue as it serves as the site of antigen-specific T-cell responses and IgE antibody production by B cells, both of which drive systemic immune responses [24]. Furthermore, oral administration of the combination

of KB1 and RA most prominently increased the splenic levels of *IL-10* and *IL-27* mRNAs compared to the KB1 and RA groups. IL-10, a potent anti-inflammatory cytokine, inhibits eosinophil survival and the release of proinflammatory cytokines, such as tumor necrosis factor α, during infection [9]. Therefore, IL-10 is crucial for immunoregulation in pollinosis, and its induction has emerged as a promising therapeutic strategy for pollinosis. While initially attributed to Th2 cells, IL-10 is now recognized to be produced by numerous cell types, with T regulatory 1 (Tr1) cells being a major source that regulates immune homeostasis [25]. Kyeong Eun Hyung et al. reported that heat-killed *Lactiplantibacillus plantarum* suppressed IL-4 production while promoting IL-10 production from antigen-stimulated splenocytes and other immune cells [26]. Nan Yang et al. demonstrated that epigallocatechin gallate (EGCG), a polyphenol found in green tea, likely ameliorated ovalbumin (OVA)-induced airway inflammation by enhancing IL-10 production [27]. IL-27, a member of the IL-12 cytokine family, is a dominant factor involved in the induction of IL-10-producing T cells and cooperates with transforming growth factor-β (TGF-β) to further enhance Tr1 differentiation [28]. These results suggest that the combination of RA and KB1 may alter the gut microbiome, leading to increased IL-27 production in the small intestinal lamina propria. Lactic acid bacteria have been reported to activate immune cells and induce cytokine responses, including members of the IL-12 family such as IL-27 [29]. Although the present study is based on analyses at the mRNA level, the changes in IL-10 and IL-27 expression are consistent with these previous reports. In addition, we showed that KB1 and RA significantly increased the splenic levels of Foxp3 and IFN-γ mRNAs, thereby hindering the Th2 immune response. An imbalance in regulatory T (Treg) cells has been shown to inhibit the suppression of T-cell activation and the maintenance of immune homeostasis, thereby contributing to the development of allergic diseases [30]. The transcription factor Foxp3 is a specific marker for Treg cells, and its expression level reflects the number and functional activity of these cells. Foxp3-deficient mice spontaneously exhibit several features associated with allergic disease, including allergic airway inflammation, atopic dermatitis–like skin disease, and markedly increased IgE levels [31,32]. Recent studies suggest that gut microbiota, including *Lactobacillus*, induce a specific subset of $CD4^+$Foxp3 Treg cells in the spleen [33]. Moreover, EGCG has been shown to increase *Foxp3* mRNA levels in the lung tissue in a mouse model of asthma [27]. In addition, many LAB strains activate Th1 responses via IFN-γ production [34]. Polyphenols have also been reported to activate natural killer cells and increase IFN-γ secretion [35]. IFN-γ plays a central role in promoting $CD4^+$ T-cell Th1 differentiation and blocking IL-4-dependent isotype switching in B cells [13]. These findings corroborate our results and indicate that LAB and polyphenols promote a favorable Th1/Th2 balance, supporting the coordinated regulation of Th1 and Treg responses. Future human intervention studies are needed to clinically validate the therapeutic value of RA and KB1 in various inflammatory diseases. In human clinical trials, approximately 8.0×10^{10} heat-killed lactic acid bacterium cells were found to exhibit therapeutic effects against JCP-associated symptoms [36]. Animal studies on RA (at doses of 30–270 mg/kg) significantly inhibited allergy symptoms and the levels of histamine and specific IgE in the OVA-induced allergy model of mice [17]. Furthermore, human clinical studies on RA have reported that daily doses of 50–200 mg alleviate SAR symptoms, such as abnormal serum IgE levels, and itchy nose, watery eyes, and itchy eyes, as well as total symptom scores [37]. Our results demonstrated a more pronounced immunomodulatory effect of the combination of KB1 and RA, suggesting that this mixture represents a promising nutritional supplement candidate for the treatment of pollen allergy. Given the established anti-inflammatory properties of RA and lactic acid bacteria, their combined effects might find application across a significantly broader spectrum of inflammatory diseases.

5. Conclusions

KB1 and RA alleviated pollinosis symptoms, and their combination produced a more pronounced improvement. Cooperative effects were observed in IgE levels as well as in the mRNA expression of anti-inflammatory cytokines and Treg markers. These findings suggest that the combination of KB1 and RA may represent a promising anti-inflammatory strategy for the management of pollinosis.

Use of AI tools declaration

The authors state that they have not utilized Artificial Intelligence (AI) tools when creating this article.

Author contributions

Akina Omori conceived and designed the experiments. Akina Omori performed the experiments and analyzed the data and wrote the manuscript. Takao mori contributed to the study design. Tadaaki Miyazaki and Kazunobu Baba reviewed and revised the manuscript. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work was appropriately investigated and resolved.

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Conflict of interest

Takao Mori is an employee of LAB Biotech Co., Ltd., which funded this study. The remaining authors declare no conflicts of interest.

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