Effect of Intralymphatic Allergen-specific Immunotherapy on House Dust Mite in a Murine Model of Allergic Rhinitis

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Research Article

Keywords: Allergens, Allergic rhinitis, Allergy immunotherapy, immunotherapy, rhinitis

Posted Date: May 31st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1659901/v1

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Abstract

Background

Intralymphatic immunotherapy (ILIT) is a promising alternative for the treatment of patients with allergic rhinitis, providing similar therapeutic efficacy to conventional allergen-specific immunotherapy (AIT). However, the allergic mechanism of ILIT is not completely known. The aim of this study was to determine the efficacy of ILIT in a house dust mite (HDM) mouse model of allergic rhinitis.

Methods

Six-week-old female BALB/c mice were divided into four groups. Four groups were established: Group 1 (G1, n=10), control group without allergy; Group 2 (G2, n=10), allergy group sensitized with HDM without ILIT; Group 3 (G3, n=10), allergy group with ILIT (L1: starting with HDM 1.25 mg/mL); and Group 4 (G4, n=10), allergy group with ILIT (L2: starting with HDM 2.5 mg/mL)

After the murine model of allergic rhinitis with HDM was established, mice were administered an intralymphatic injection through the inguinal lymph nodes with HDM once per week for 5 weeks. Thereafter, the levels of total serum IgE, interleukin IL-6, IL-13, IL-17A, IL-25, IL-33, and interferon (IFN)-γ in serum and spleen were measured using ELISA kits. Eosinophils in nasal mucosa were also evaluated via hematoxylin and eosin staining. Cytokine expression in nasal mucosa were determined using RT-PCR and the effects of ILIT on FoxP3(+) Treg cell in splenocytes were assessed using flow cytometry.

Results

ILIT significantly decreased serum total IgE and eosinophil counts in nasal mucosa; IL-6 IL-17A, IL-25, and IFN-γ in serum (P< 0.05); and IL-17A and IL-33 in splenocyte supernatant (P< 0.05). ILIT also reduced the mRNA expression of IL-4, IL-6, IL-17A, and IL-33 cytokines in nasal mucosa. FoxP3(+) Treg cell expression was significantly decreased in the allergic group and significantly increased in the ILIT group (P< 0.05).

Conclusion

Our results suggest that ILIT regulates the specific immunotherapy immunologic mechanism by downregulating Th1, Th2, and Th17 cytokines and upregulating FoxP3(+) T reg cells in the HDM allergic mouse model.

Introduction

Allergen-specific immunotherapy (AIT) is used as a desensitizing therapy for allergic diseases and serve as a potentially curative and specific treatment approach. AIT is the only disease-modifying treatment for allergic diseases.1 Subcutaneous immunotherapy (SCIT) has been the standard route for immunotherapy for many decades; however, several alternative routes of administration are currently being utilized,
including sublingual immunotherapy (SLIT), epicutaneous immunotherapy (EPIT), intralymphatic immunotherapy (ILIT), intranasal immunotherapy (INIT), and oral mucosal immunotherapy (OMIT).\textsuperscript{1}

Although SCIT and SLIT have been proven to be effective and safe interventions for allergic respiratory diseases, less than 5% of allergic patients receive SCIT or SLIT owing to their long duration, the risk of a hypersensitivity reaction, and the high cost of these treatments.\textsuperscript{2} ILIT has been proposed as a faster alternative, requiring as few as three injections administered over 4 weeks. Further, ILIT is a less time-consuming, safe, and effective alternative to conventional SCIT.\textsuperscript{3, 4}

Several recent studies have revealed that allergen-specific ILIT is a promising alternative for the treatment of patients with allergic rhinitis (AR), providing similar therapeutic efficacy to conventional AIT.\textsuperscript{5, 6} Notably, clinical data suggest that treatment with ILIT reduces the need for rescue medications, rapidly alleviates skin and nasal allergic symptoms, and prevents allergy aggravation for up to 3 years, without causing severe local or systemic adverse effects.\textsuperscript{3, 6} Previously, cervical ILIT was demonstrated to be safe and induced allergen tolerance after 3 injections with a significant improvement in AR.\textsuperscript{7}

According to a recent study, ILIT failed to mitigate nasal symptoms and improve quality-of-life or intradermal test scores in patients with grass pollen allergy, despite modulating the IgE and IgG4 levels to some extent.\textsuperscript{8} ILIT does not exhibit profound therapeutic efficacy in AR induced by house dust mite (HDM), cat, or dog allergens.\textsuperscript{9} Hence, the efficacy of ILIT in AR remains controversial.

Some mechanisms, including the decrease in antigen-specific IgE and increase in antigen-specific IgG4 in sera and the activation of regulatory T cells (Tregs) and regulatory B cells (Bregs), have been suggested to be involved in the effectiveness of SCIT and SLIT.\textsuperscript{10} Tregs have been identified as key regulators of immunological processes in peripheral tolerance to allergens. Naturally occurring FoxP3(+) Treg cells and inducible type 1 Treg (Tr1) cells contribute to the control of allergen-specific immune responses via several major schemes.\textsuperscript{11}

Naive T cells can differentiate into Th1, Th2, Th9, Th17, or Th22 type memory and effector cells. During the development of allergic disease, IFN-\gamma is the major cytokine in Th1 cells. Th2 cells produce IL-4, IL-5, IL-13 and such as innate cytokine, IL-25, IL-31, and IL-33 contribute to Th2 responses. Th17 cells are characterized by IL-17A, IL-17F, IL-6, IL-8, TNF-\alpha, IL-22, and IL-26 expression. The strong relationship between the levels of innate cytokines and the development of allergic disease was recently reported.\textsuperscript{11, 12, 13}

The direct administration of allergen extracts into lymph nodes increases specific IgG production and T-cell responses using significantly lower allergen doses.\textsuperscript{14} AIT affects the long-term development of allergic rhinoconjunctivitis and induces clinical tolerance primarily by stimulating Tregs and attenuating T helper 2 responses and the synthesis of blocking antibodies.\textsuperscript{11} ILIT in an allergic mouse model was found to more significantly reduce eosinophil infiltration in the nasal mucosa, and total and OVA-specific IgE levels than intranasal and sublingual administration.\textsuperscript{15} However, the efficacy of ILIT in allergic
diseases lacks preclinical validation. Furthermore, no studies have determined the therapeutic efficacy of ILIT in HDM-induced AR. The allergic mechanism of ILIT is not completely known and the changes in cytokine levels need to be assessed.

We established an ILIT model in allergic mouse and sought to determine the changes in immunologic and inflammatory cytokines after ILIT using a house dust allergen mouse model.

Methods

Animals

Six-week-old female BALB/c mice were purchased from Orient Bio Inc. (Seongnam, Korea). All animal experiments were carried out in accordance with the Gachon University Animal Research Guidelines, and the study was approved by the Institutional Review Board of Gachon University (LCDI-2017-0105).

Establishment of a murine model of allergic rhinitis

Six-week-old female BALB/c mice were divided into four groups. After a 1-week acclimation period, mice in the control group were treated with phosphate-buffered saline (PBS) 20 µL nasal drops while mice in the experimental groups were sensitized with HDM nasal drops (HDM 50 µg in PBS 20 µL per mouse) five times per week for 5 weeks. Four groups were established: Group 1 (G1, n = 10), control group without allergy; Group 2 (G2, n = 10), allergy group sensitized with HDM without ILIT; Group 3 (G3, n = 10), allergy group with ILIT (L1: starting with HDM 1.25 µg/mL); and Group 4 (G4, n = 10), allergy group with ILIT (L2: starting with HDM 2.5 µg/mL) (Fig. 1).

Intralymphatic injection

One week after the end of HDM exposure, mice were administered intralymphatic injection (inguinal lymph node) once per week for 5 weeks. Briefly, after gas anesthesia with isoflurane, Evans blue dye was injected into the tail vein. Two days later, the hairs of mice were removed via shaving to locate the inguinal lymph node, and HDM was injected into the lymph node (Fig. 1).

Mice in group 1 and group 2 were administered 20 µL of PBS via the inguinal node for 5 weeks. Mice in Group 3 were administered the starting dose of HDM 1.25 µg/mL (L1) incrementally, as scheduled, once per week for 5 weeks and mice in group 4 were administered the starting dose of HDM 2.50 µg/mL dose (L2) incrementally, as scheduled, once per week for 5 weeks.

During intralymphatic injection, mice in group 1 only received a drop of PBS, while mice in the other groups received HDM nasal drops twice per week for 5 weeks to ensure sustained allergic stimulation. Mice were killed at 1 week after the last ILIT and their serum and other specimens were collected (Fig. 2).

Serum collection
Approximately 300–400 µL of blood was obtained from the ophthalmic artery of each mouse by orbital puncture. Blood samples were stored at 4°C for 2–4 h and centrifuged (2000 rpm; 4°C) to obtain serum.

**Collection of nasal specimens and spleens**

On the day of killing, the heads of mice were harvested and fixed in 10% paraformaldehyde for 24 h. The specimens were decalcified in a decalcifying reagent (Decalcified II; Surgipath Medical Industries Inc., Richmond, IL, USA) for 5 days and sectioned from behind the upper incisor to 2 mm in front of the incisive papilla of the hard palate. Tissues were embedded in paraffin, and cut into 4 µm sections.

Splenocytes were isolated from mouse spleen using a standard isolation technique and resuspended in RPMI 1640 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen, Grand Island, NY, USA), 50 µg/mL streptomycin, and 50 U/mL penicillin (Invitrogen). Dispersed cell suspensions from spleens were resuspended at 1 × 10^6 cells/mL and incubated with 50 ng/mL phorbol 12-myristate 13-acetate (PMA) and 500 ng/mL ionomycin in RPMI medium at 37°C for 6 h. The supernatants of these splenocytes were collected from each group for ELISA.

**Total IgE in serum**

The total IgE levels in mouse sera were determined using an ELISA kit (BioSource International Inc., Camarillo, CA, USA) in accordance with the manufacturer’s instructions.

**Eosinophil counts in nasal mucosa**

To determine the degree of eosinophil counts in the nasal mucosa, we stained nasal mucosal tissues with hematoxylin and eosin staining for 45 s. After washing, we stained tissues with eosin for 20 s. The numbers of eosinophil cells in five different optical fields were counted under a microscope (400× magnification).

**Cytokine levels in serum and the supernatant of splenocytes**

We measured the levels of the Th1 cytokine, IFN-γ; Th2 cytokine, IL-13; innate cytokines, IL-25 and IL-33; and Th17 cytokines, IL-17A and IL-6, in serum and the supernatant of splenocytes using the respective ELISA kits (BioSource International Inc., Camarillo, CA, USA) according to the manufacturer’s instructions.

**Cytokines in nasal mucosa**

Total RNA was isolated from nasal mucosal tissues using the RNeasy Plus Mini Kit (Qiagen). RNA 1 µg was reverse transcribed into cDNA using an iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer’s instructions. PCR was performed with specific primer sets for IL-6, IL-13, IL-17A, IL-25, IL-33, IFN-γ, and GAPDH. PCR products were examined on 2% agarose gels (Table 2).
Table 1
Dose administered to each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>HDM drop</th>
<th>1st ILIT</th>
<th>2nd ILIT</th>
<th>3rd ILIT</th>
<th>4th ILIT</th>
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<td>G1 control</td>
<td>PBS 50 µl/mouse</td>
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<td>PBS 20 µl</td>
<td>PBS 20 µl</td>
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<tr>
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<td>HDM 50 µg/mouse</td>
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<td>PBS 20 µl</td>
<td>PBS 20 µl</td>
<td>PBS 20 µl</td>
<td>PBS 20 µl</td>
</tr>
<tr>
<td>G3 Allergy + ILIT(L1)</td>
<td>HDM 50 µg/mouse</td>
<td>HDM</td>
<td>HDM</td>
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<tr>
<td>G4 Allergy + ILIT (L2)</td>
<td>HDM 50 µg/mouse</td>
<td>HDM</td>
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ILIT: Intralymphatic immunotherapy

**FoxP3(+) Treg cell in splenocytes**

Mouse splenocytes (2 × 10^5 cells/well in 1 mL in 24-well plates) were cultured in the presence and absence of 50 µg/mL HDM extract at 37°C for 5 days. Subsequently, cells were stimulated with 0.1 µg/mL PMA (Sigma-Aldrich) and 1 µg/mL ionomycin (Sigma-Aldrich) in the presence of 1 µM monensin (Sigma-Aldrich) at 37°C for 4 h. To block FcRs, the cells were incubated with anti-mouse CD16/CD32 monoclonal antibody (BD Biosciences, San Diego, CA, USA) in HBSS buffer containing 2% fetal calf serum for 15 min on ice. After washing, the cells were fixed with Fix/Perm buffer (BD Biosciences) and then incubated with anti-mouse Th17/Treg phenotyping mix (BD Biosciences) at room temperature for 30 min. The stained cells were analyzed on a BD FACSAria system (Becton-Dickinson, Oakville, ON, Canada), and flow cytometry data were analyzed using FlowJo (Tree Star Inc., Ashland, OR, USA).

**Statistical analysis**

Data were analyzed using Student’s t-test. *P*-value < 0.05 was considered statistically significant.

SPSS statistics 19 software was used for the statistical analysis.

**Results**

**Effect of ILIT on the total IgE levels**
Serum total IgE levels were significantly higher in the allergy group (G2) than the control group (G1) \((P<0.001)\). The ILIT group with L1 dose (G3) had significantly reduced total IgE level compared to the allergy group without ILIT (G2) \((P<0.05)\). However, the ILIT group with L2 dose (G4) did not have a significant reduction in the total IgE level compared to the allergy group (G2) (Fig. 3).

**Effect of ILIT on the infiltration of eosinophils in the nasal mucosa**

A significantly increased number of infiltrated eosinophils was found in the nasal mucosa of the allergy group without ILIT (G2) compared with the control group (G1) \((P<0.05)\). The groups administered ILIT (G3, G4) exhibited a reduced number of infiltrated eosinophils in the nasal mucosa compared with the allergy group without ILIT (G2) (Fig. 4).

**Effect of ILIT on the cytokine levels in serum and the supernatant of splenocytes**

In serum, all Th2 cytokines, namely IL-13, IL-25, and IL-33, were increased in the allergy group (G2). Although ILIT decreased the expression of IL-13, IL-25, and IL-33, only the expression of IL-25 was significantly decreased in G4 \((P<0.05)\). The levels of the Th17 cytokines, IL-6 and IL-17A, were significantly increased in the allergy group. After ILIT, the level of IL-17A significantly decreased in the G3 and G4 groups \((P<0.05)\). Further, IL-6 was decreased in G4 and significantly decreased in G3 \((P<0.05)\) after ILIT. The Th1 cytokine, IFN-\(\gamma\), was significantly increased in the allergy group. However, after ILIT, IFN-\(\gamma\) was decreased in G3 and significantly decreased in G4 \((P<0.05)\) (Fig. 5).

In the supernatant of splenocytes, IL-33 was significantly decreased in G4 after ILIT \((P<0.05)\). However, no difference in the level of IL-25 was found between the groups. The levels of IL6 and IL-17 were significantly increased in the allergy group while IL-17A was significantly decreased in G4 \((P<0.05)\) after ILIT. The levels of IFN-\(\gamma\) did not differ between the groups after ILIT (Fig. 6).

**Effect of ILIT on the cytokine expression levels in nasal mucosa**

AR mice exhibited higher mRNA expression of IL-4, IL33, IL-6, IL-17A, and IFN-\(\gamma\) than control mice. However, ILIT reduced the mRNA expression levels of these cytokines in G3 and G4 (Fig. 7).

**Effects of ILIT on FoxP3(+) Treg cell levels**

HDM exposure significantly reduced FoxP3(+) Treg cell levels in the allergy group (G2) \((P<0.005)\). ILIT exhibited a significantly higher FoxP3(+) Treg cell expression in G3 than G2 \((P<0.05)\) (Fig. 8).

**Discussion**

Previously, ILIT was revealed to be safe, conferred desensitization to seasonal and nonseasonal allergens, alleviated allergic rhinitis symptoms, and reduced medication use in 17 clinical trials for AR.\(^4\)
Preseasonal ILIT for grass pollen allergic adolescents may be clinically effective for decreasing symptoms and medication use during grass pollen season and mountain cedar pollen.\textsuperscript{10,16}

ILIT induces moderate-to-severe systemic reactions and is associated with pain at the injection site. A previous meta-analysis had limitations, such as a small number of participants and long-term outcomes.\textsuperscript{4} The relatively small number of subjects might have contributed to the insignificant efficacy of ILIT for the treatment of AR.\textsuperscript{9} ILIT induces severe systemic and/or local hypersensitivity reactions when aqueous allergen extracts are employed.\textsuperscript{17}

Intraperitoneal injection of ovalbumin (OVA) was employed in previous studies to establish an allergic mouse model.\textsuperscript{18} In this study, we evaluated the ability of ILIT to alleviate HDM-induced allergy in a murine model of AR. We used HDM nasal drops to establish a mouse model of AR, which accurately resembled the pathophysiology observed in AR patients. Previous efforts to assess the effect of ILIT in preclinical allergy models also used other substances instead of the actual allergen.\textsuperscript{19}

To our knowledge, our study is the first experimental ILIT study using the HDM allergy mouse model, thereby increasing the clinical relevance of our findings. However, our study had some limitations. Herein, we did not determine the specific change in HDM IgE level after ILIT, which might critical for evaluating the allergic mouse model.

Upon exposure to foreign antigens or allergens, antigen-presenting cells in secondary lymph nodes activate antigen-specific lymphocytes, initiating immune responses against foreign insults. However, in areas of the body, besides the lymphoid organs, antigens can be invisible to the immune system.\textsuperscript{20,21} Therefore, direct injection into the lymph nodes is the most effective method for immunization. In this study, we used Evans blue to accurately locate the lymph nodes, thereby bypassing the requirement for skin incision, which might cause undesired inflammatory reactions.\textsuperscript{22,23}

Many clinical studies have assessed the potential benefit of ILIT in patients with allergic inflammation via the inhibition of Th2 immunity and enhancement of Th1 Treg responses. Senti et al.\textsuperscript{3} was the first to report that ILIT reduced the serum levels of allergen-specific IgE and skin reactivity to allergens. ILIT in pollen-allergic young adults exhibited an immune-modulating effect by increasing allergen-specific IgG and IgG4 levels without inducing allergen specific IgE.\textsuperscript{6,24} In this study, we found that serum total IgE levels increased after HDM exposure and ILIT decreased serum IgE levels in AR mice. Further, the decrease in serum IgE levels after ILIT was found to be accompanied by a profound decrease in the number of infiltrating eosinophils, highlighting the potential value of ILIT in the treatment of allergies.

The proportion of activated and resting Treg cells increased between screening and 4 weeks after the second injection. However, no significant differences were observed over time for allergen-induced IL-4, IL-5, IL-10, IL-13, IFN-\(\gamma\), CXC motif chemokine ligand 10, and CC motif chemokine ligand 17 secretion.\textsuperscript{25} Our results revealed that ILIT decreased the Th2 cytokines, IL-13, IL-25, IL-33 in serum. Further, the Th1 cytokine, IFN-\(\gamma\), was found to be decreased by ILIT. These results are similar to those of a previous study.
where systemic cytokine (IL-4, IL-5, IL-6, IL-17, and IFN-γ) production and local cytokine (IL-4 and IL-5) production were found to be significantly reduced after intralymphatic injection in an OVA-allergic mouse model using RT-PCR. In our study, ILIT significantly reduced the expression of the Th17 cytokines, IL-6 and IL-17, in serum and nasal mucosa. These results are also consistent with those of a previous study, which revealed that ILIT downregulated Th17 expression in splenocytes in an OVA-mouse model.

The Treg cells, FoxP3(+) CD41CD25, contribute to the control of allergen-specific immune responses; suppression of dendritic cells that support the generation of effector T cells; suppression of effector Th1, Th2, and Th17 cells; and suppression of allergen-specific IgE and induction of IgG4.

In our study, AR mice exhibited significantly decreased levels of FoxP3(+) cells. Importantly, ILIT significantly increased FoxP3 cell levels in the G3 and G4 groups, suggesting that ILIT can promote immune tolerance by activating Treg cells. ILIT of the MAT-Fel d1 vaccine increased IL-10 and FoxP3 expression. However, there was no significant difference in the number of Foxp3 + Treg cells among PBMCs between the ILIT and control groups.

In summary, we established a new ILIT-treated HDM AR mouse model and revealed that ILIT decreased serum total IgE level and eosinophil infiltration in the nasal mucosa. ILIT also decreased the expression levels of IL-13, IL-25, IL-33, IFN-γ, IL-6, and IL-17, and increased the expression of FoxP3(+) T reg cells. Our results suggest that ILIT regulates the specific immunotherapy immunologic mechanism by downregulating the Th1, Th2, and Th17 cytokines in an HDM allergic mouse model.

**Conclusion**

In this study, ILIT was found to inhibit the secretion of Th2 cytokines and reduce the total IgE level in the serum of AR mice. These findings suggest that ILIT is a promising immunotherapeutic approach for the treatment of AR and other allergic diseases. However, future clinical trials are required to confirm the clinical usefulness of ILIT.

**Declarations**

**Ethics approval and consent to participate**

Gachon University Animal Research Guidelines, and the study was approved by the Institutional Review Board of Gachon University

**Consent for publication**

The authors have consent for publication

**Availability of data and materials**

The data and materials are available.
Competing interests

The authors declare that they have no conflicts of interest.

Funding

This Research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI16C2319).

This work was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP; Ministry of Science, ICT & Future Planning) (No. 2017R1C1B5017255).

Authors' contributions

Joo Hyun Jung: Conceived and designed the analysis, Wrote the paper

Sang Min Lee: Wrote the paper

Min Young Cho: Collected the data

Heung Eog Cha: Performed the analysis

Seon Tae Kim: Conceived and designed the analysis, Contributed data or analysis tools

References


**Tables**

Table 2 is available in the Supplemental Files section.

**Figures**

**Figure 1**

Intralymphatic injection. (A) After gas anesthesia with isoflurane, Evans blue dye was injected into mice via the tail vein. (B) After two days, the hair of mice was removed via shaving to identify the location of the inguinal lymph node.
Six-week-old female BALB/c mice were divided into four groups. After a 1-week acclimation period, mice in the control group were treated with PBS 20 mL nasal drops while mice in the experimental groups were sensitized with HDM nasal drops (HDM 50 mg in PBS 20 mL per mouse) five times per week for 5 weeks. After one week, mice in group 1 and group 2 were injected with 20 μL of phosphate-buffered saline (PBS), mice in group 3 were injected with L1 dose (HDM 1.25 μg/mL), and mice in group 4 were injected with L2 dose (HDM 2.5 μg/mL) once per week for 5 weeks. During ILIT, mice in the group 1 only received PBS drops, while mice in the other groups were administered HDM nasal drop twice per week for 5 weeks for sustained allergic stimulation. At one week after the last ILIT, mice were killed and serum and other specimens were collected.

G1 (Group 1): control group without allergy, G2 (Group 2): allergy group without ILIT, G3 (Group 3): allergy group with ILIT (L1, HDM 1.25 μg/mL), G4 (Group 4): allergy group with ILIT (L2, HDM 2.5 μg/mL)
Total IgE level. The serum total IgE levels were significantly higher in the allergy group (G2) than the control group (G1). The total IgE of the ILIT group with L1 dose (G3) was significantly reduced compared to that of the allergy group (G2). However, the ILIT group with L2 dose (G4) did not display significantly reduced total IgE level compared to the allergy group (G2).

G1: control group without allergy, G2: allergy group without ILIT, G3: allergy group with ILIT (L1), G4: allergy group with ILIT (L2). *** $P<0.001$, ** $P<0.005$, * $P<0.05$, Error bar: Mean ±SD
Figure 4

Eosinophil counts in nasal mucosa. (A) Eosinophil infiltration increased in the nasal mucosa of mice in the allergic group. (B) The number of eosinophils significantly increased in the allergy group (G2) ($P<0.05$). Mice in the ILIT-treated groups (G3, G4) had a lower number of infiltrated eosinophils in the nasal mucosa than mice in the allergy group (G2)
G1: control group without allergy, G2: allergy group without ILIT, G3: allergy group with ILIT (L1), G4: allergy group with ILIT (L2). H&E stain, x400 magnification, black arrows: eosinophil. *** $P<0.001$, ** $P<0.005$, * $P<0.05$, Error bar: Mean ±SD.

Figure 5
Changes in serum cytokine levels. (A) IL-6 was significantly increased in the allergy group (G2); however, ILIT decreased IL-6 in the G3 group. (B) IL-13 was decreased after ILIT. (C) IL-17A was increased in the allergy group; however, and IL-17A was significantly decreased in G3 and G4. (D) ILIT significantly decreased IL-25 in the G4 group compared to the allergy group. (E) IL-33 levels did not differ between the groups. (F) ILIT significantly decreased the IFN-γ level in G4 compared to G2.

G1: control group without allergy, G2: allergy group without ILIT, G3: allergy group with ILIT (L1), G4: allergy group with ILIT (L2). *** $P<0.001$, ** $P<0.005$, * $P<0.05$, Error bar: Mean ±SD
Figure 6

Changes in the cytokine levels in the supernatant of splenocytes. (A) IL-6 was statistically significantly increased in the allergy group (G2); however, its level decreased in the ILIT group (G3). (B) IL-17A was significantly decreased in the ILIT group (G4). (C) IL-25 level did not significantly differ between the groups. (D) IL-33 was statistically significantly decreased in the ILIT group (G3, G4). (E) IFN-γ level did not significantly differ between the groups. (F)
Cytokine expression in the nasal mucosa. The allergy group had a higher mRNA level of IL-4, IL-6, IL-17a, and IL-33 than the control group based on RT PCR. After ILIT, the mRNA levels of these cytokines were decreased in G3 and G4 compared to G2.

G1: control group without allergy, G2: allergy group without ILIT, G3: allergy group with ILIT (L1), G4: allergy group with ILIT (L2). *** \( P<0.001 \), ** \( P<0.005 \), * \( P<0.05 \), Error bar: Mean ±SD
Figure 8

FoxP3(+) Treg cells. FoxP3(+) Treg cells were decreased in the allergy group (G2) compared to the control group (G1), but significantly increased in the ILIT group (G3).

G1: control group without allergy, G2: allergy group without ILIT, G3: allergy group with ILIT (L1), G4: allergy group with ILIT (L2). *** $P<0.001$, ** $P<0.005$, * $P<0.05$, Error bar: Mean ±SD

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table02.png