

Sublingual Immunotherapy Attenuates Nasal Symptoms Upon Allergen Exposure in Murine Allergic Rhinitis Model via an Induction of IL-10 producing T cells in Submandibular Lymph Node

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Yinfei Qu, MD¹, Takaya Yamada, PhD², Noriaki Aoi, MD, PhD¹,
Ichiro Morikura, MD, PhD¹, Takafumi Fuchiwaki, MD, PhD¹, Yukie Hotta, MD¹,
Emmanuel Prokopakis, MD, PhD³, and Hideyuki Kawauchi, MD, DMSc¹ 

Abstract

Objective: Sublingual immunotherapy has been considered to be a painless and effective therapeutic treatment of patients with allergic rhinitis. Its mechanism of action has been elucidated, but there are still controversies among many reports between clinical efficacy and laboratory data. Therefore, its mechanism of action needs to be investigated further by using promising animal models such as rodents and monkeys.

Materials and Methods: Bearing this in mind, in our present study, we successfully constructed an effective murine model for sublingual immunotherapy (SLIT) in allergic rhinitis in which mice were sublingually administered ovalbumin (OVA), followed by intraperitoneal (ip) sensitization and intranasal (i.n.) challenge of OVA.

Results: To summarize our experimental data, nasal symptoms such as sneezing and nasal rubbing of sublingually treated mice were significantly attenuated in accordance with lower specific IgE antibodies in sera. Histological analysis of eosinophil recruitment in nasal mucosae reveals less allergic inflammation in sublingually treated mice. Interleukin-10 (IL-10) production and IL-10–specific mRNA gene expression of cultured submandibular lymph node (SMLN) cells with OVA, obtained from sublingually treated mice, were significantly higher than those of mice without sublingual treatment.

Conclusion: These results demonstrate that sublingually introduced antigens can actually attenuate nasal symptoms in a murine allergic rhinitis model upon allergen exposures. Furthermore, our immunological data might indicate an important role of IL-10 producing T cells in SMLN to control nasal allergic reaction.

Keywords

allergic rhinitis, sublingual immunotherapy, regulatory T cell, IL-10, Eosinophil

Introduction

Allergic rhinitis is one of the commonest types of allergic inflammation, affecting 10% to 25% of the world's population, and its prevalence has increased over the past decade. Allergic rhinitis is characterized by a predominant Th2 response, antigen-specific IgE synthesis, and infiltration of eosinophils into the nasal mucosa.¹ The type I allergic response is largely divided into 2 phases, namely, systemic response at the induction phase and allergic inflammation in the eliciting phase. The eliciting phase is further divided into 2 phases, namely, immediate phase and late phase. To consider the pathologies of type I allergic inflammation, we should now distinguish between the induction phase and the

eliciting phase even though there is always a mixture of these 2 phases in normal human nasal mucosa. The hygiene

¹Department of Otolaryngology, Faculty of Medicine, Shimane University, Izumo city, Japan

²Department of Experimental Animals, Center for Integrated Research in Science, Shimane University, Izumo city, Japan

³Department of Otorhinolaryngology, University of Crete, School of Medicine, Crete, Greece

Corresponding Author:

Hideyuki Kawauchi, MD, DMSc, Department of Otorhinolaryngology, Faculty of Medicine, 89-1 Enya-cho, Izumo city, Shimane 693-8501, Japan.

Email: Kawauchi@med.shimane-u.ac.jp

hypothesis in line with helper T cell dichotomy has been proposed by a large number of investigators, and many plausible hypotheses are available to understand allergic inflammation in comparison with infective inflammation.

More recently, regulatory T (Treg) cells, defined as CD4⁺CD25⁺ FoxP3⁺ T cell subpopulations, have been focused on as key players to downregulate excessive Th1 or Th2 pathologies such as autoimmune disease or allergy. These Treg cell subpopulations are considered to be efficiently developed in a circumstance of low hygiene and high pathogen/adjuvant burden. Treg cells producing IL-10 and/or TGF- β are induced not only in atopic patients by successful immunotherapy but also during natural allergen exposure in healthy people. Regulatory T cells can control and regulate all the effector mechanisms activated during allergy and the Th2 responses through the production of IL-10/TGF- β and/or cell-cell contact. IL-10 is a potent suppressor of total and allergen-specific IgEs, whereas it induces an antibody isotype switch toward IgG4. TGF- β also decreases IgE production and induces an immunoglobulin isotype switch towards IgA. IL-10 and TGF- β act directly or indirectly on the human airways to decrease both mucus production and airway hyperreactivity.

Allergen-specific immunotherapy also modifies cellular and humoral responses to the allergen. The ratio of Th1 cytokines to Th2 cytokines is increased following allergen-specific immunotherapy, and functional regulatory T cells are induced.² Production of blocking antibodies, IgG1, IgG4, and IgA, is induced, and these immunoglobulins compete with IgE for allergen binding, thereby decreasing the allergen capture and presentation that is facilitated by the IgE in complex with Fc ϵ RI or Fc ϵ R2.

SLIT has previously been shown in many clinical studies to modulate allergen-specific Th2 responses with a decrease in the IgE to IgG4 ratio, and its mechanism has been considered similar to that of subcutaneous immunotherapy.³⁻⁷ Here, we address some new findings of the immunological mechanisms of SLIT such as decrease in the IgE/IgG4 ratio, induction of IL-10-producing Tregs and allergen-specific T cell tolerance, and so on. Cosmi's et al⁸ have performed a clinical long-term study for SLIT in patients with perennial rhinitis with *Dermatophagoides* and came to the conclusion that the effects of SLIT is downregulation of Dp-specific IgE, enhancement of IFN- γ with Th cells, downregulation of Dp-specific proliferation, and enhancement of IL-10 production. On the other hand, in their clinical study of patients with birch pollenosis, Bohler et al⁹ found that transient induction of regulatory T cells immediately after SLIT is followed by long-term allergen-specific peripheral tolerance (anergy) and immune deviation (from Th2 to Th1 milieu). Then, why do we choose SLIT as a new therapeutic method rather than subcutaneous immunotherapy? SLIT requires much more amounts of allergen than subcutaneous immunotherapy (SCIT), but it has several benefits in comparison

with SCIT.¹⁰ SLIT is pain-free and safer than subcutaneous immunotherapy because the amount of mast cells is least in the sublingual mucosal tissue. Another benefit is that patients can carry out the immunotherapy procedure by themselves at home after reaching the allergen maintenance dose, thus necessitating fewer visits to the outpatient clinic. To solve the problem of SLIT requiring a high dose of allergen, improvement of the allergen delivery system is necessary. One possible way is to modify immunocompetent cells such as dendritic cells for the efficient induction of regulatory T cells and the production of allergen-specific IgGs.

However, to this end, upon the sublingual immunotherapy, dynamic immunological events in nasal mucosae in relation with draining nearby submandibular lymph nodes (SMLNs) or distant mucosal sites of lymphoid organs of patients with allergic rhinitis has never been elucidated except allergen-specific serum antibody titers or peripheral blood T cell cytokine profiles. Therefore, in our present study, we constructed murine models of SLIT, that is, preventive SLIT model in which mice were sublingually treated with allergen before systemic and intranasal (i.n.) allergen challenge in allergic rhinitis.

As a result, we successfully demonstrated a significant decrease of nasal symptoms with less eosinophil recruitments in nasal mucosae in mice treated with preventive SLIT. Furthermore, our immunological data might indicate an important role of IL-10 producing T cells in SMLN to control nasal allergic reaction.

Materials and Methods

Mice

Female BALB/c mice were purchased from CLEA Japan (Tokyo, Japan). All mice were maintained under specific pathogen-free conditions and received ovalbumin (OVA)-free diet in the laboratory animal research center of Shimane University. These mice were 5 to 6 weeks of age at the beginning of individual experiments. Animal care and experimental procedures were approved by the Animal Research Committee of Shimane University and conducted according to the Regulations for Animal Experimentation at Shimane University (approved number: IZ27-59).

Induction of Allergic Rhinitis

For the induction of allergic rhinitis, we employed a previously described protocol with some modifications in the quantity of antigens and the sensitization schedule.¹¹ In brief, BALB/c mice were presensitized by means of an intraperitoneal (ip) injection of 25 μ g of OVA (Grade V; Sigma Chemical Co., St. Louis, Missouri, USA) with 1 mg of aluminum hydroxide hydrate gel (Alum; LSL Co., Tokyo, Japan) in 200 μ L of PBS once a week for 3 weeks. Thereafter, mice were challenged by i.n. administration of

400 µg of OVA in 4 µL of PBS into bilateral nostril, twice daily, for 14 consecutive days.

Sublingual administration of antigens

Mice were sublingually administered with OVA (Grade V; Sigma Chemical Co.) 3 times weekly on days 0, 7, and 14 (Figure 1). Each group of mice was sublingually administered with 4 µL of PBS containing 500 µg of OVA or PBS only before an ip sensitization and i.n. challenge of OVA. For sublingual administrations of antigens, mice were anesthetized by ip injection of sodium pentobarbital (50 mg/kg) and then 4 µL of PBS alone, or PBS containing 500 µg of OVA was applied on sublingual mucosa under the tongue. Mice were held with their heads placed in a horizontal position for 20 minutes to prevent them from swallowing antigens.

Observation of nasal symptoms

Mice were intranasally challenged with OVA or PBS twice daily for 14 consecutive days. After the final i.n. challenge with OVA, mice were placed into an observation cage (1 animal/cage) for about 2 minutes. Clinical symptom was evaluated by counting the frequencies of sneezes and scratches in 2 minutes by employing the method of Sugimoto et al.¹²

Measurement of OVA-specific IgE in sera

Blood specimens were obtained from the inferior caval vein. The serum samples were stored at -20°C until use. OVA-specific IgE level in serum was analyzed using DS mouse IgE ELISA kit (OVA; DS Pharma Biomedical, Osaka, Japan).

Histologic examination

Mice were killed 12 hours after the last nasal challenge with OVA. The heads were removed and fixed in 10% formaldehyde solution for 24 hours at room temperature. After fixation, the heads were decalcified in 5% formic acid for 36 hours at room temperature and neutralized in 5% sodium sulfate solution for 12 hours at room temperature. Coronal nasal sections were then stained with hematoxylin and eosin, and the number of eosinophils in each side of the posterior edge of the nasal septum was counted microscopically in a high-power magnification field.

Eosinophil counts

Sections of nasal mucosa were stained with hematoxylin and eosin to assess inflammatory cell infiltration.

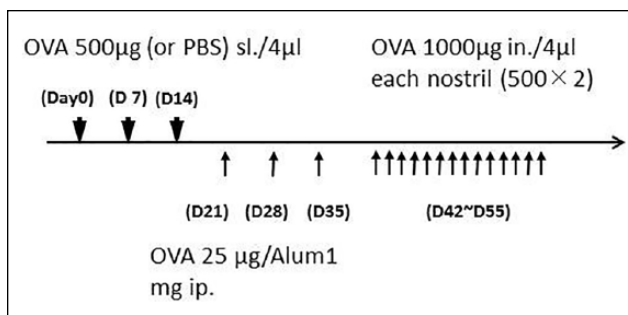


Figure 1. Experimental protocol, as described in the "Materials and Methods" section.

Eosinophils were defined morphologically by the presence of 2-lobed nucleus and eosinophilic granules in the cytoplasm. Eosinophils were counted under a microscope at $\times 400$ magnification. Four randomly chosen high power fields (HPFs) of each section were examined to yield the mean number of eosinophils per HPF of subepithelial connective tissue. Eosinophils in each section was counted, and the average number of eosinophils per HPF was also calculated. All slides were checked and confirmed by 2 investigators who were blinded to the experimental conditions.

Lymphocyte isolation from spleens and SMLNs

Spleen and CLN were removed, and single-cell suspensions were prepared dissolved in PBS by mechanical dissociation.¹³ Splenic lymphocytes and SMLN lymphocytes from individual tissues were isolated by gentle teasing using needles through a 40 µm nylon mesh.

Analysis of Cytokine Production by splenic or SMLN cells

For in vitro culture of lymphocytes, cells were dissolved with complete RPMI medium containing 10% fetal bovine serum (FBS), 5µM 2-Mercaptoethanol (2-ME), 10 U/ml of penicillin, and 100 µg/ml of streptomycin. Cells were then cultured at a density of 1×10^5 cells/well in the presence of 1 mg/ml of OVA with splenic feeder cells (5×10^5 cells/well) that were pretreated with mitomycin-C (Sigma Chemical Co.) in round-bottom 96 wells microculture plates for 72 hours in an incubator with 37°C, 5% CO₂. Culture supernatants were collected and examined for the production of cytokines (IL-10, TGF-β) by cytokine ELISA kits (R&D Systems Inc., Minneapolis, Minnesota, USA).

Reverse Transcription PCR

Total RNA was extracted from cultured lymphocytes using TRIzol reagent (Invitrogen, Carlsbad, California, USA).

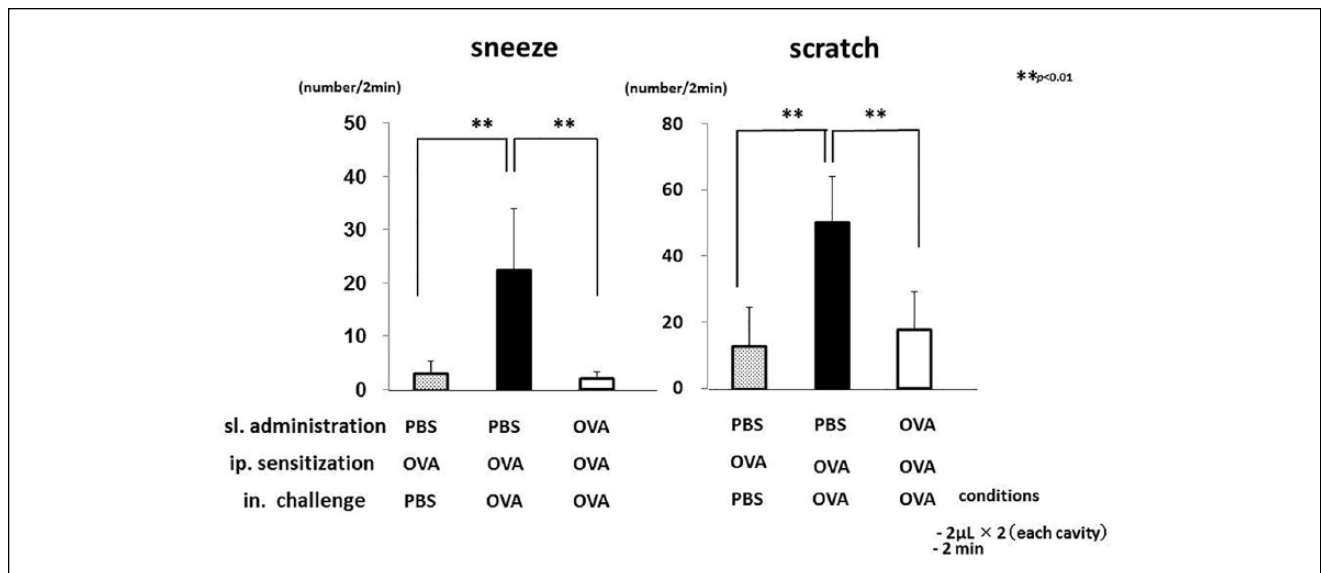


Figure 2. The numbers of sneezes and scratches. The number of (left) sneezes and (right) scratches were counted 2 minutes after the final ovalbumin intranasal challenge. Data are geometric M $\pm$ SD; 5 mice were used in each group.

* $p < .05$. ** $p < .01$, Dunnett's test.

To detect messenger RNA (mRNA) of cytokines, RNA was reverse transcribed using the primers of these genes, and such cDNA was further amplified by PCR methods. cDNA was obtained by reverse transcription using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster, California, USA). Real-time PCR was performed using commercial primer of mouse β -actin, Foxp3, IL-10, and TGF- β (Applied Biosystems). Real-Time PCR analysis Total RNA was isolated from cultured lymphocytes. Quantitative real-time PCR was performed using TAKARA code TP900 with commercial primers (β -actin, Foxp3, IL-10, TGF- β) for TaqMan Gene Expression Assays (Applied Biosystems). Messenger RNA expression levels for specific genes were normalized as a ratio relative to β -actin. The PCR was carried out with an initial denaturation for 30 seconds at 95°, followed by 40 cycles of 1 minute at 60°, and 1 minute at 75°. Their sequences are as follows: Foxp3 (sense, 5'-AG TGCCTGTGTCCTCAATGGTC-3'; antisense, 5'-AG GGCCAGCATAGGTGCAAG-3'), TGF- β (sense, 5'-GT GTGGACAACATGTGGAAGCTCTA-3'; antisense, 5'-CG CTGAATCGAAAGCCCTGTA-3'), and IL-10 (sense, 5'-GCCAGAGCCACATGCTCCTA-3'; antisense, 5'-GA TAAGGCTTGGCAACCCAAGTAA-3'). To quantify the amount of IL-10 RNA, a real-time PCR, based on SYBR green fluorescence, was performed using SYBR Premix Ex Taq polymerase and Takara real-time Thermal Cycler Dice (Takara, Shiga, Japan). The aforementioned primers were used to specifically amplify respective genes.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD) or standard error (SE) and evaluated by analysis of variance (ANOVA) with Dunnett's t test or Scheffe test. P values $< .05$ were assumed to be statistically significant.

Results

Sublingual Administration of OVA before systemic sensitization significantly attenuated nasal symptom

On days 0, 7, and 14, each group of mice was sublingually administrated with 4 μ L of PBS containing 500 μ g of OVA or PBS only, and then from day 21, mice were intraperitoneally sensitized with 25 μ g of OVA with 1 mg of aluminum hydroxide hydrate gel 3 times at 1-week interval (Figure 1). From day 42 to day 55 after systemic sensitization, each group of mice was intranasally challenged with 4 μ L of PBS containing 1 mg of OVA in the bilateral nostrils. Over 2 minutes after the final i.n. challenge of OVA or PBS, the numbers of sneeze and scratch were counted precisely (Figure 1).

Numbers of sneeze and scratch after final i.n. challenge significantly increased in mice intranasally challenged 14 days with systemic sensitization, in comparison with those only with systemic sensitization (Figure 2). Sublingual immunotherapy with OVA before systemic sensitization

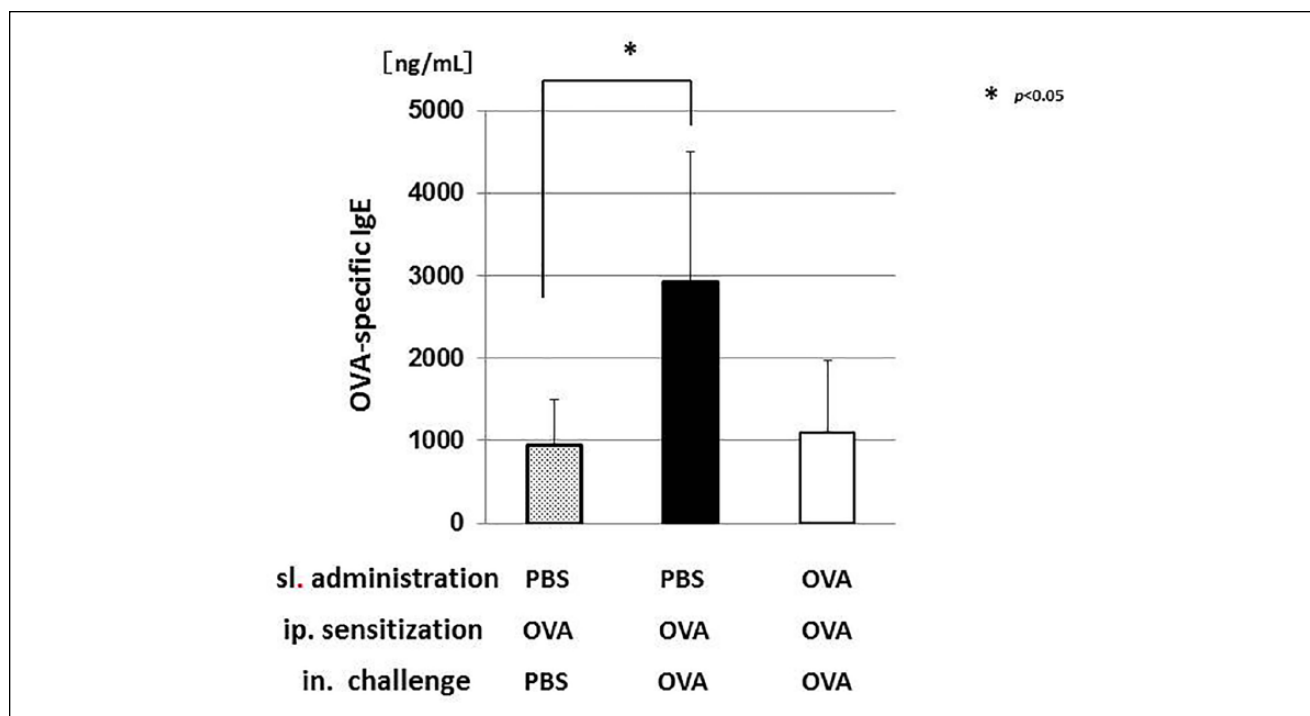


Figure 3. Antigen-specific allergic responses in sublingually treated mice with allergic rhinitis. Mice were sublingually administered with PBS alone or ovalbumin (OVA) followed by intraperitoneal sensitization and nasal challenge with OVA. OVA-specific IgE levels in serum were assayed by sandwich ELISA. Data are expressed with mean value $\pm$ SD as a representative of 3 independent experiments, containing 5 mice in each group. Significance was evaluated by Dunnett's *t* test.

**p* < .05.

significantly decreased in number of sneeze and scratch compared with that of sublingually PBS-treated mice (Figure 2). These data actually indicate that our allergic rhinitis model is efficient to see the effect of SLIT and SLIT is also effective to prevent nasal symptoms of allergic rhinitis.

Sublingual Administration of OVA Before Systemic Sensitization Suppressed OVA-Specific IgE Production in Serum

Antigen-specific IgE antibody titers in sera significantly increased in mice intranasally challenged with OVA post systemic sensitization compared with those only with systemic sensitization. Mice sublingually administered with OVA before systemic sensitization and i.n. challenge showed a decreased level of serum antigen-specific IgE compared to mice with allergic rhinitis without sublingual administration with OVA, even though this difference was not statistically significant (Figure 3).

Histological finding

Coronal sections of nasal mucosae derived from each group of mice were stained with hematoxylin and eosin and observed under the microscope to see the allergic

inflammation. As shown in Figure 3, Eosinophil infiltration and epithelial cell damage were prominent in nasal mucosae of mice intranasally challenged 14 days with systemic sensitization. In contrast, eosinophil infiltration and epithelial cell damage were interestingly reduced in mice sublingually administered with OVA (Figure 4).

The number of eosinophils infiltrating into the nasal mucosa

The number of eosinophils infiltrating into the nasal mucosa was semi-quantitatively counted with histologic sections in each group of mice. The numbers of eosinophils were significantly decreased in the OVA sublingually treated mice in comparison with those of allergic rhinitis without sublingual treatment (Figure 5).

IL-10 production of cultured SMLN cells was significantly upregulated by sublingual administration of OVA

Given the suppressed antigen-specific IgE production in serum and less Th2 type of allergic inflammation of nasal mucosa observed in mice treated with preventive SLIT to intranasally administered antigen, we hypothesized that

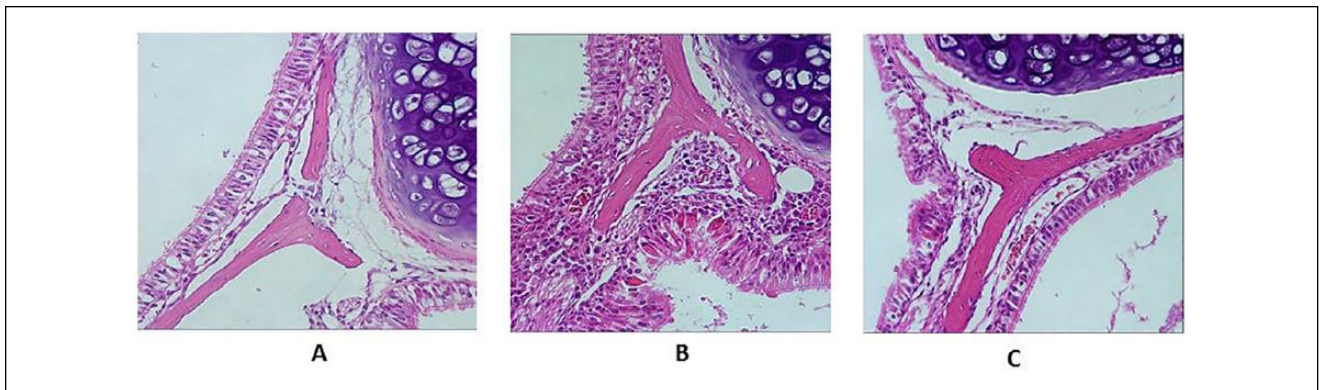


Figure 4. Effect of sublingual immunotherapy on allergic inflammation in murine nasal mucosa. Representative sections of nasal mucosae are seen at a magnification of $\times 400$ after hematoxylin and eosin staining. (Left) Normal control. (Middle) allergic rhinitis induced mice. (Right) Ovalbumin sublingually treated mice.

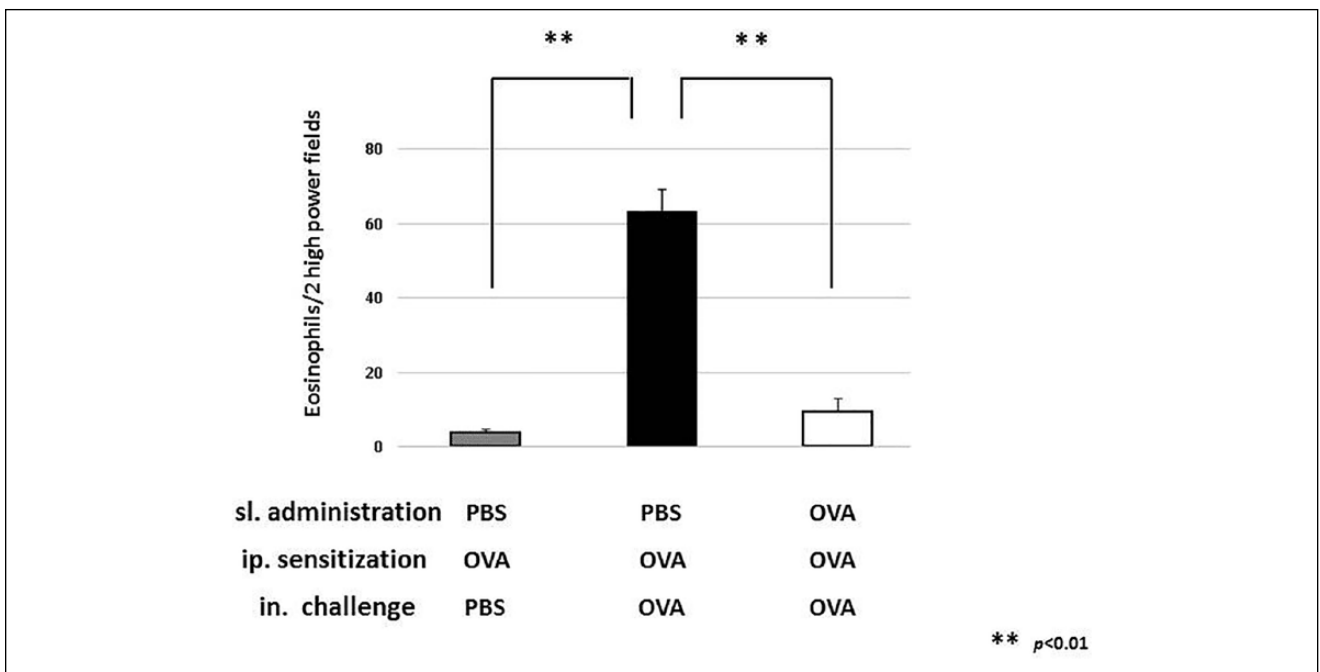


Figure 5. The number of eosinophils infiltrating into the nasal mucosa. Data are mean value $\pm$ SE. Five mice were used in each group. The number of eosinophils was significantly decreased in the ovalbumin sublingually treated group (group C) compared with allergic rhinitis group (group B).

** $p < .01$, Dunnett's t test.

regulatory mechanism could be attributed to the suppression of Th2 type of allergic inflammation in these mice. To clarify this hypothesis, we focused on the regulatory network and its cytokine profile of sublingually treated mice after systemic sensitization and subsequent i.n. challenge of antigens. Thus, IL-10 and TGF- β production was measured in vitro by OVA stimulation of lymphocytes isolated from SMLN in each of group of mice. IL-10 and TGF- β production by cultured SMLN cells was evaluated by ELISA assay. IL-10 level in culture supernatants was significantly reduced with allergic

rhinitis group in comparison with control group of mice, which underwent only systemic sensitization but not i.n. challenge (Figure 6). However, very interestingly, IL-10 production by SMLN cells in sublingually treated group of mice recovered to the same level as control group of mice. But in contrast, TGF- β level in culture supernatants of cultured CLN cells was not different among each group of mice. These data indicate that antigen-specific IL-10-producing T cells induced in SMLNs play an important role in suppressing allergic inflammation in nasal mucosa of sublingually treated mice.

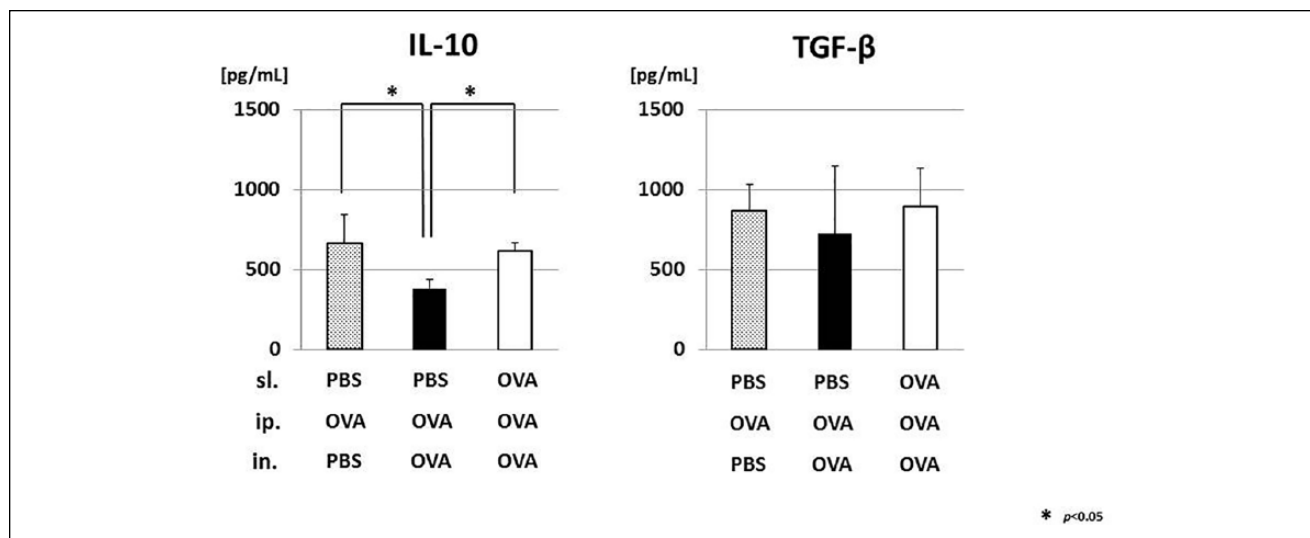


Figure 6. Cytokine production in submandibular lymph node (SMLN) cells. IL-10 and TGF- β production by cultured SMLN cells was evaluated by ELISA assay. Representative results from 3 independent experiments containing 5 mice in each group are shown. IL-10 level in culture supernatants was significantly reduced with allergic rhinitis group. But, IL-10 production by SMLN cells in sublingually treated group of mice recovered to the same level as control group of mice. Significance was evaluated by Dunnett's t test. * $p < .05$.

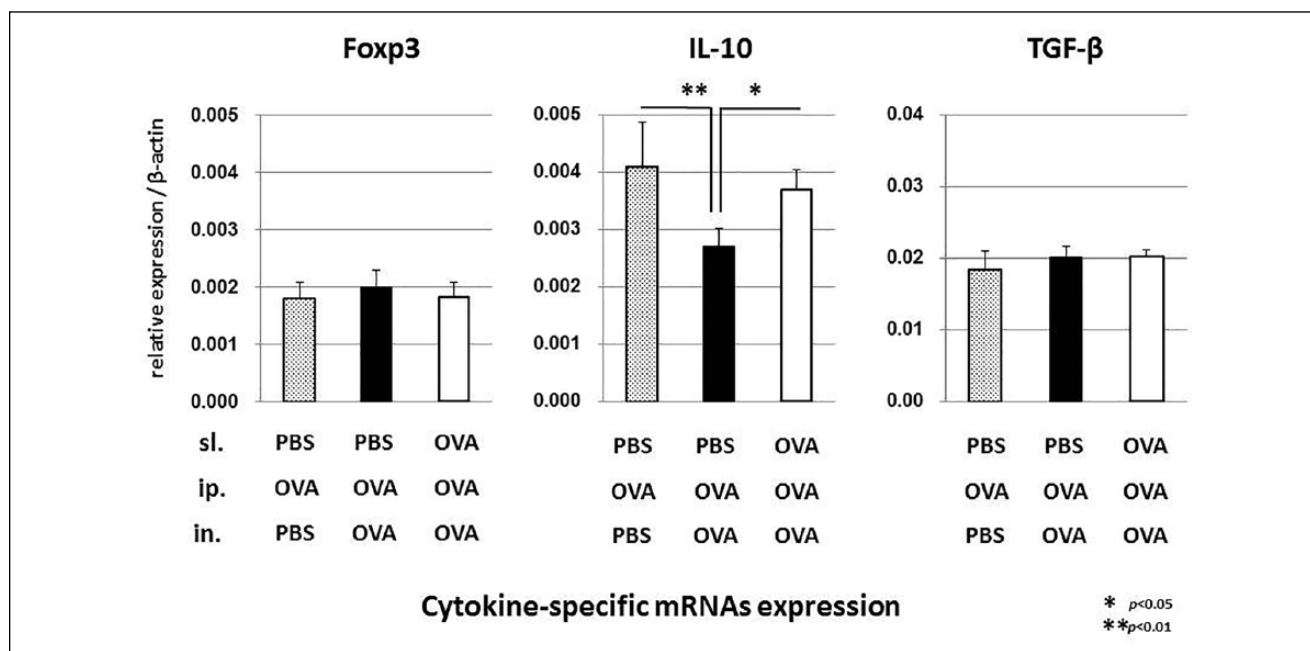


Figure 7. Foxp3, IL-10, and TGF- β specific messenger RNA gene expression of cultured submandibular lymph node (SMLN) cells of sublingually treated mice with allergic rhinitis. Messenger RNA expression of Foxp3, IL-10, and TGF- β in cultured lymphocytes of SMLN was determined by quantitative real-time PCR analysis. The expression of each molecule was normalized to the expression of β -actin. Representative results from 2 independent experiments containing 5 mice in each group are shown. Significance was evaluated by Dunnett's t test. * $p < .05$. ** $p < .01$.

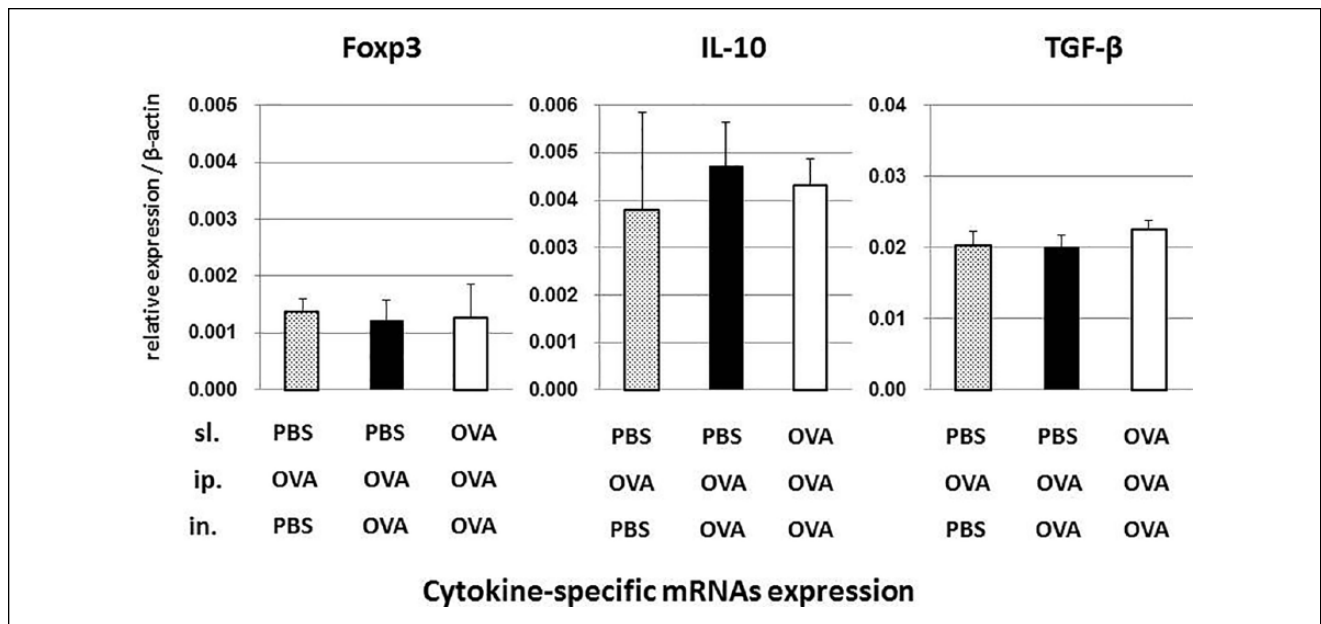


Figure 8. Foxp3, IL-10, and TGF- β specific messenger RNA gene expression of cultured spleen cells of sublingually treated mice with allergic rhinitis. Messenger RNA expression of Foxp3, IL-10, and TGF- β in cultured splenic lymphocytes was determined by quantitative real-time PCR analysis. The expression of each molecule was normalized to the expression of β -actin. Representative results from 2 independent experiments containing 5 mice in each group are shown. No significant difference was found among each group by Dunnett's *t* test.

Sublingual Administration of OVA Led to Upregulation of IL-10 Specific mRNAs in submandibular lymph node cells

To examine Treg-associated molecules-specific mRNA expression of cultured CLN cells or splenic cells, we performed quantitative real-time PCR analysis of Foxp3, IL-10, and TGF- β in cultured lymphocytes isolated from SMLNs and spleens of each group of mice. As a result, sublingual administration of OVA led to upregulation of IL-10-specific mRNAs in SMLN cells obtained from allergic rhinitis group of mice but neither Foxp3 nor TGF- β (Figure 7). On the other hand, sublingual administration of OVA did not alter the mRNA expression pattern of Foxp3, IL-10, and TGF- β in cultured spleen cells obtained from allergic rhinitis group of mice (Figure 8).

These data also indicate that sublingual administration of allergen may enhance suppressive function of IL-10-producing T cells in the draining SMLNs, although it has no influence on the number of those cells in spleen.

Discussion

In this study, we successfully constructed a murine model of allergic rhinitis in which nasal symptom such as sneeze and scratch can be effectively induced for a quantitative analysis to evaluate sublingual immunotherapy (SLIT) in murine allergic rhinitis. In our experimental protocol, 4 μ L

of PBS containing OVA as a model antigen was employed for preventive SLIT model. Previous study demonstrated that FITC-labeled OVA remained exclusively located on the sublingual mucosa for 2 hours and was undetectable in the esophagus and the intestine when the total volume of saline containing OVA sublingually applied was less than 10 μ L.¹⁴ In our study, sublingual administration of allergens to the mice, before systemic and subsequent intranasal sensitization, so called preventive SLIT, showed significantly attenuated nasal symptom together with suppressed allergic responses such as specific IgE production in serum and Th2 type of allergic inflammation of nasal mucosa, and these results correspond with previous findings reported by other investigators.¹⁵ And very interestingly, it is clearly demonstrated that histological quantitation of eosinophil infiltration into nasal mucosa actually corresponds to the nasal symptom such as sneezing and scratching in this murine allergic rhinitis model. Our semiquantitative analysis of eosinophil infiltration confirmed actual correspondence in the degree of nasal symptoms with severity of allergic inflammation of nasal mucosa in this murine model of allergic rhinitis, similar to extrapolating human conditions. Our experimental allergic rhinitis model can also be available to investigate the efficacy of various treatment strategies to extrapolate into human conditions. Our previous data from the SLIT model with daily once i.n. challenge have already demonstrated that the amount of Th2 cytokines such as IL-4, IL-5, and IL-13 in culture supernatant of spleen and

SMLN cells with OVA were significantly lower in mice sublingually treated with OVA than in control mice.¹⁶ Furthermore, our previous study suggested the involvement of IL-10 expressing CD4⁺CD25⁺Foxp3⁺ inducible Tregs of the draining submandibular lymph nodes in the suppression of Th2-mediated allergic responses in mice receiving SLIT. However, in that protocol, we were neither able to provoke enough number of nasal symptoms nor compare the difference in number of nasal symptoms among each group of mice. Therefore, in this study, with a small modification of experimental protocol, especially in i.n. antigen challenge, we successfully focused on quantitative analysis of nasal symptom and histological analysis of nasal mucosa together with an induction profile of Tregs in draining SMLNs and spleen, a distant lymphoid tissue in periphery.

Thus, in the present study, IL-10 and TGF- β production was measured in vitro by OVA stimulation of lymphocytes isolated from SMLN in each of group of mice. As a result, IL-10 level in culture supernatants were significantly reduced with allergic rhinitis group in comparison with control group of mice, which underwent only systemic sensitization but not i.n. challenge. However, very interestingly, IL-10 production by SMLN cells in sublingually treated group of mice recovered to the same level as control group of mice. In contrast, TGF- β level in culture supernatants was not different among each group of mice. Moreover, sublingual administration of OVA altered the mRNA expression pattern of IL-10, but neither Foxp3 nor TGF- β in cultured SMLN cells obtained from allergic rhinitis group of mice. In addition, sublingual administration of OVA did not alter the mRNA expression pattern of Foxp3, IL-10, and TGF- β in cultured spleen cells obtained from allergic rhinitis group of mice. Nevertheless, our data actually indicate that antigen-specific IL-10-producing T cells induced in SMLNs play an important role in suppressing allergic inflammation in nasal mucosa of sublingually treated mice. IL-10-producing inducible Tregs are reported to downregulate function and activity of eosinophils and suppresses IL-5 production by resting T_H0 and T_H2 cells.¹⁷ IL-10 is an anti-inflammatory cytokine that downregulates cellular immunity and allergic inflammation. IL-10 has been shown to modulate allergen-induced airway inflammation, inhibit eosinophil survival and IgE synthesis, and downregulate the expressions of IL-4 and IL-5 through T-helper type 2 lymphocytes.¹⁸ However, the definite role of IL-10 has not yet been clearly elucidated in allergic rhinitis. Recently reported data regarding the nasal release of IL-10 and other cytokines or chemokines in allergic rhinitis patient demonstrated a significant increase in IL-10 at 5 hours after nasal allergen challenge. However, it was significantly lower in allergic rhinitis patient than nonallergic control during natural pollen exposure, concluding that IL-10 may be related to the alleviation of nasal mucosal allergy.¹⁹

To explain the difference in IL-10 upregulation by preventive SLIT between draining SMLNs and spleen in

allergic rhinitis model, the concept of compartmentalization could be utilized to distinguish an induction site and effector site to deal with Th1/Th2 immune responses to the antigens.²⁰⁻²² Common mucosal immune system exerts a high degree of anatomical compartmentalization in relation with the induction and migration patterns of lymphocytes activated at different mucosal sites. The selective recruitments of mucosally activated lymphocytes to specific tissues are committed by cellular homing and chemokine receptors in combination with tissue-specific adhesion molecules and epithelial cell-derived chemokines that are, respectively, expressed in mucosal tissues. Main routes of mucosal immunization include perentoral, intranasal, and sublingual routes to activate antimicrobial defense mechanism and preferentially induce more effective mucosal immune responses at induction site rather than distant mucosal sites, as already reported by many investigators. On the other hand, the mechanism of sublingual administration to attenuate allergic responses at nasal mucosa remained yet to be clearly determined, so we have precisely performed our ongoing immunological experiments of SLIT to clarify the exact mechanism of it. Based on the concept of compartmentalization, our present data, in line with previous reports, strongly suggest that inducible IL-10-producing T cells can be efficiently induced in an antigen-specific fashion at the nasal mucosa with antigen stimulation and also neighboring draining SMLNs but not enough in distant lymphoid organs such as spleen. Now, we are going on to directly examine the cytokine profiles of nasal mucosal lymphocytes such as nasal proprial (NP) cells and nasal-associated lymphoreticular tissue (NALT) cells, despite the difficulty of harvesting enough numbers of lymphocytes from each mouse. Otherwise, flow-cytometric analysis and antigen-specific cytokine profile with cultured of nasal lymphocytes cannot be obtained.

In summary, nevertheless, the present study suggests the involvement of IL-10-producing T cells of the draining LN in the suppression of Th2-mediated allergic responses in mice receiving SLIT. For further improvement of SLIT, regulatory mechanism in nasal mucosa and relating lymphoid tissues should be further investigated to provide information for a novel and more safe strategy of SLIT against allergic rhinitis.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ORCID iD

Hideyuki Kawauchi  <https://orcid.org/0000-0003-4161-2591>

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