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ORIGINAL ARTICLE

# Sublingual Immunotherapy with House Dust Extract for House Dust-Mite Allergic Rhinitis in Children

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## ABSTRACT

**Background:** House dust extract is used in conventional immunotherapy for house dust-mite (HDM) allergic rhinitis in Japan. However, an alternative administration route is desired. The aims of the present double blind, placebo-controlled trial were to evaluate the therapeutic efficacy and safety of sublingual immunotherapy (SLIT) with house dust extract in pediatric patients with HDM allergic rhinitis.

**Methods:** The study population comprised 31 subjects (21 males and 10 females) aged from 7 to 15 years old. Twenty patients (the active group) received house dust extract and 11 received placebo via sublingual administration. Extract or placebo (1 ml) was administered at 10-fold dilution once weekly for 40 weeks. During the study period, the subjects recorded their daily nasal symptoms and use (dose and frequency) of other medications in a nasal allergy diary.

**Results:** The symptom scores in the active group began to decrease about 24 weeks after initiation of treatment and significant differences between the active and placebo groups were observed after 30 weeks. The average scores for the last four weeks of the study were significantly lower than those for the first four weeks in the active group but not in the placebo group. The only local adverse effect was a bitter taste reported by one patient. There were no other local or systemic adverse effects associated with SLIT.

**Conclusions:** Our results suggest that SLIT with house dust extract for more than 30 weeks is safe and effective treatment for HDM allergic rhinitis in children.

## KEY WORDS

children, double blind, house dust extract, house dust-mite allergic rhinitis, placebo-controlled trial, sublingual immunotherapy

## *INTRODUCTION*

An increased prevalence of allergic rhinitis has been found worldwide[1,2] and the onset age has decreased in pediatric patients.[2] Once allergic rhinitis develops in childhood, the disease does not remit easily[3] and may impair quality of life and school performance for many years.[4,5] Current therapy for allergic rhinitis includes allergen avoidance, symptomatic medication, and immunotherapy. Conventional allergen-specific immunotherapy via the subcutaneous route (SCIT) is effective for changing the natural course of allergic rhinitis and has long-term effects.[6-11] However, an alternative administration route is still required since the SCIT approach requires multiple injections and frequent visits to a physician's office, and can have severe systemic adverse effects.[12] A safer and simpler approach is particularly important for pediatric pa-

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**Table 1** Dose and dosing frequency

| | Week 1 | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|
| | 1000 fold | 100 fold | 10 fold | 10 fold |
| Day 1 | 0.2 ml | 0.2 ml | 0.2 ml | 1.0 ml |
| Day 2 | 0.4 ml | 0.4 ml | 0.4 ml | |
| Day 3 | 0.6 ml | 0.6 ml | 0.6 ml | |
| Day 4 | 0.8 ml | 0.8 ml | 0.8 ml | |
| Day 5 | 1.0 ml | 1.0 ml | 1.0 ml | |
| Day 6 | | | | |
| Day 7 | | | | |

tients.

Sublingual allergen-specific immunotherapy (SLIT) may offer a safe and effective alternative administration route and has attracted particular attention in Europe. The house dust mite (HDM) is the major allergen in pediatric patients and several randomized controlled trials of HDM in children have been reported.[13-16] Bahçeciler *et al.*[13] treated 15 pediatric patients with HDM extract for 6 months and showed that the mean daily doses of intranasal steroids decreased in these patients. Ippoliti *et al.*[14] similarly treated 86 pediatric patients for 6 months and found a significant reduction in rhinitis scores in the treatment group compared with patients who received a placebo.

In Japan, SCIT for HDM allergic rhinitis has been conducted with house dust extract but not with mite extract for more than 40 years and has been shown to be effective.[17] Although house dust extract contains not only mite allergens but also other antigens, such as cockroach, moth and mold, the Japanese Ministry of Health, Labour, and Welfare has only approved the use of house dust extract for immunotherapy. The mite extract widely used in western countries is not available in Japan. One milliliter of the house dust extract contains about 7 μg of Der f 1, one of the major allergens of *Dermatophagoides farinae*. The concentrations of other allergens have not been determined. To evaluate the efficacy of SLIT, a double-blind placebo controlled study was conducted in pediatric patients with HDM allergic rhinitis using the house dust extract used in Japan.

## METHODS

### SUBJECTS

The study population comprised 31 subjects (21 males and 10 females) ranging in age from 7 to 15 years old and living in Chiba, Hokkaido, or Akita. The subjects had a clinical history of HDM allergic rhinitis, but were otherwise healthy. Diagnosis of HDM allergic rhinitis was based on clinical history, positive allergen-specific skin tests (wheal diameter $\geq$10 mm) to house dust extract (Torii Pharmaceutical, Tokyo, Japan), and a serum house dust mite-specific IgE score $\geq$2 on the CAP-radioallergosorbent test (CAP-RAST, SRL Inc., Tokyo, Japan). Patients who had been treated with any allergen-specific immunotherapy (including with house dust) and those with severe or poorly controlled asthma were excluded. The study was conducted at Chiba University Hospital, Chiba Children's Hospital, Hokkaido University Hospital, and Akita University Hospital, in compliance with the Ethical Guidelines for Clinical Studies and Good Clinical Practice and the Declaration of Helsinki (2000 revision). The protocol was approved by the Ethics Committee of each hospital, and written informed consent was obtained from each subject prior to their participation in the study.

### HOUSE DUST EXTRACT

Extracts of house dust (Torii Pharmaceutical: lot number; ASCY) were used in the study. This extract contained 5 μg/ml of Der f 1.

### STUDY PROTOCOL

The study was performed as a placebo-controlled, double-blinded trial. The subjects were randomly allocated into two groups based on a table of random numbers produced by the Department of Pharmacy at Chiba University Hospital. An administrator who was not directly involved in the study was responsible for group allocation. The patients were randomly placed into active (treatment) and placebo groups at a ratio of 2 : 1. A group allocation number was given to each patient. This information was retained by the administrator and a member of the ethical committee who was also not directly involved in the study. The numbers were accessed with a key after completion of the study.

The active group (20 patients) received house dust extract and the placebo group (11 patients) received placebo by sublingual administration using the spit method (Table 1). Following a week for washout before treatment (Week 0), the dose was escalated over a period of 3 weeks by administration of an increasing number of extract or placebo drops at three concentrations. Patients received increasing doses from each vial, beginning with 0.2 ml from a 1000-fold diluted vial, and increasing by 0.2 ml per day over 5 days. The vaccine was taken sublingually, kept for 2 min without retention reagent, and then spit out. The procedure was then repeated with each vial until the maximum dose (1.0 ml of a 10-fold diluted vial) was reached, as shown in Table 1. The safety of daily SLIT administration has been shown, but weekly administration was used in this study to minimize the possibility of serious adverse events. The safety of weekly administration was confirmed in our previous study in patients with Japanese cedar pollinosis.[18] The maintenance dose was about 20 times higher than that used in conventional subcutaneous immunotherapy. Administration was started in November/December 2006 and the study was completed at the

**Table 2** Severity of nasal symptoms

| Parameter | Severity | | | | |
|---|---|---|---|---|---|
| | ++++ | +++ | ++ | + | - |
| Paroxysmal sneezing (times/day) | ≥21 | 11-21 | 6-10 | 1-5 | 0 |
| Runny nose (Nose blowing frequency; times/day) | ≥21 | 11-21 | 6-10 | 1-5 | 0 |
| Nasal congestion | Complete congestion, all day | Very severe nasal congestion with frequent oral breathing | Severe nasal congestion with occasional oral-breathing | No oral breathing but nasal congestion | None |

Adapted from the Practical Guideline for the Management of Allergic Rhinitis in Japan, 2009.

**Table 3** Baseline characteristics of the patients

| | Active Group $n = 19$ | Placebo Group $n = 9$ |
|---|---|---|
| Mean age, years (SD) | 9.4 (2.2) | 9.6 (2.5) |
| Female sex, *n* (%) | 6 (31.6) | 2 (22.2) |
| Mite RAST score, mean (SD) | 4.7 (1.2) | 5.3 (0.8) |
| Mean duration of HDM allergic rhinitis, years (SD) | 5.9 (2.9) | 5.3 (2.1) |
| Additional allergic disease, *n* (%) | | |
| Current bronchial asthma | 8 (42.1) | 7 (77.8) |
| Current atopic dermatitis | 5 (26.3) | 2 (22.2) |
| History of atopic dermatitis | 1 (5.3) | 0 (0) |
| Current Japanese cedar pollinosis | 1 (5.3) | 1 (11.1) |

end of October 2007. Patients visited the hospital just after the period of dose escalation and once every three months thereafter. At each visit, physicians checked intranasal findings, nasal allergy diaries and adverse events.

### SYMPTOM SCORING

During the study period the subjects recorded their daily nasal symptoms and use (dose and frequency) of other medications in a nasal allergy diary. Symptom, medication and symptom-medication scores were obtained from the diary records using the following criteria based on a modified Okuda classification (Table 2).[19,20] For nasal symptoms, the severity of paroxysmal sneezing (number of sneezes per day), runny nose (number of times blowing the nose per day) and nasal congestion were evaluated on a five-point scale (0-4): 0, no sensation; 1, mild; 2, moderate; 3, severe; and 4, extremely severe. Episodes of sneezing and nose blowing (rhinorrhea) per day were rated from 0 to 4: 0, none; 1, 1-5 episodes; 2, 6-10 episodes; 3, 11-20 episodes; and 4, >21 episodes. The daily total nasal symptom score was expressed as the highest score for nasal symptoms. Medication was recorded based on drug characteristics and duration of use, using the following codes[21]: 1, anti-histamines, mast cell stabilizers and vasoconstrictors; 2, topical, ocular or nasal steroids. The medication score and symptom-medication score (medication + symptom scores) were calculated for each patient. The symptom and symptom-medication scores were used as the primary outcome parameter and other criteria were used as secondary outcome parameters.

### STATISTICAL ANALYSIS

After completion of the study, clinical and laboratory data were analyzed by a biostatistician who had previously not been involved in the trial. After the analysis was complete, the allocation identification numbers for the active and placebo groups were accessed. Data analysis was performed with two-tailed tests at a significance level of 5%, using a chi-square test, a Mann-Whitney U test and a Wilcoxon signed-rank test in SAS v. 8.02 (Cary, NC, USA).

## *RESULTS*

### SUBJECTS

Three subjects were excluded from analysis because of incomplete allergy diaries. None were excluded due to adverse effects. Data were analyzed for the remaining 28 subjects, who showed full compliance with the study protocol (Table 3). These subjects included 19 in the active group (mean age 9.4 years old, mite RAST score 4.7, duration of HDM allergic rhinitis 5.9 years) and 9 in the placebo group (mean age 9.6 years, mite RAST score 5.3, duration of HDM

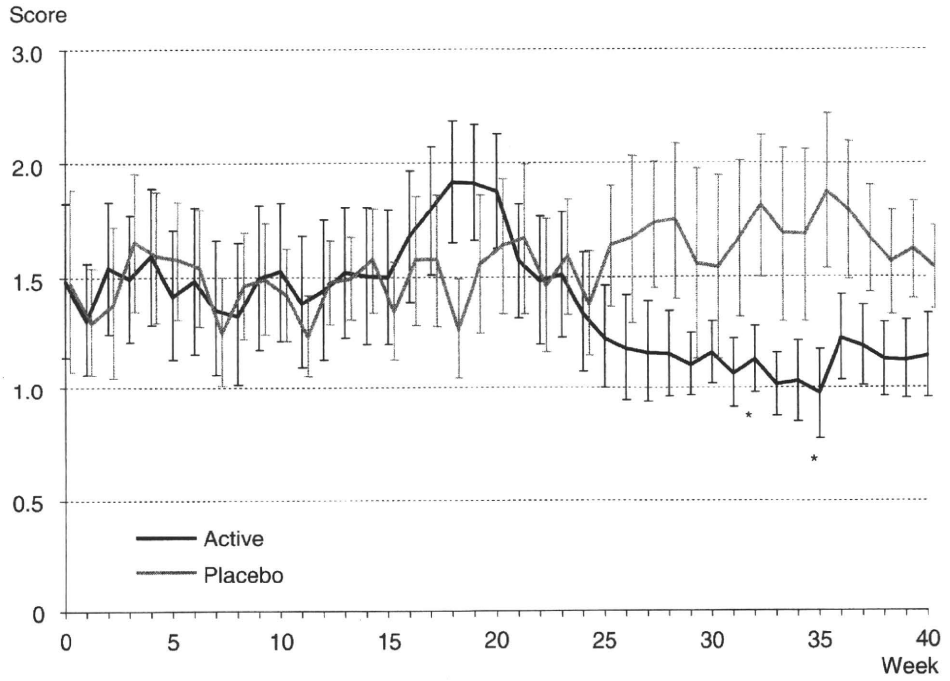


**Fig. 1** The mean scores for paroxysmal sneezing and runny nose in the active group decreased after week 24 and were significantly lower than those in the placebo group in 32 and 35. *$p < 0.05$ (vs. placebo group, Mann-Whitney U test).

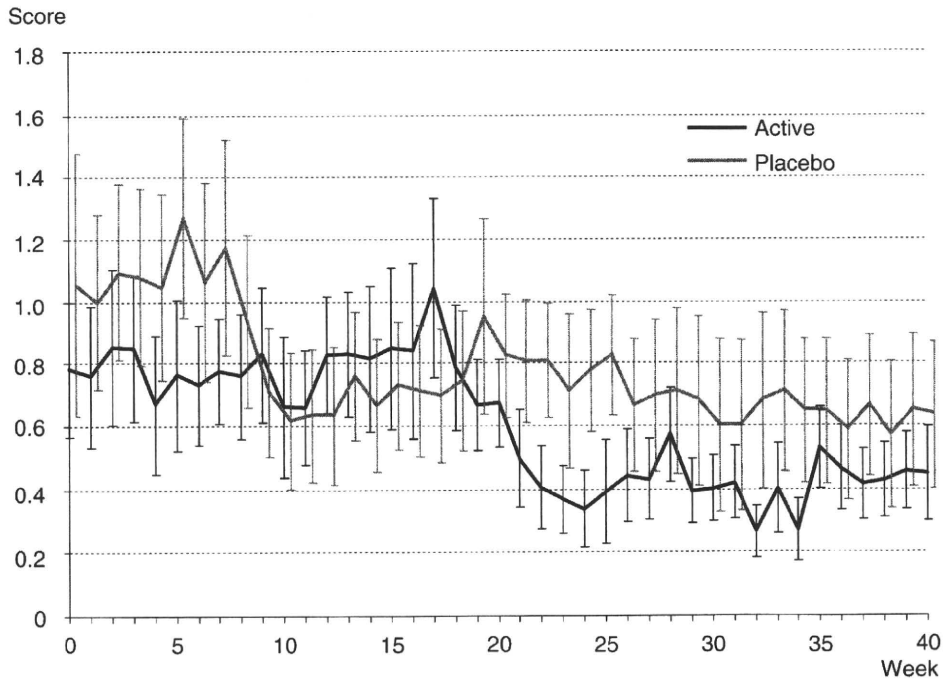


**Fig. 2** The mean score for nasal congestion in the active group decreased after week 20.

allergic rhinitis 5.3 years). There were no significant differences in age, sex, mite RAST score and duration of HDM allergic rhinitis between these two groups. The active group included 8 patients with current asthma, 5 with current atopic dermatitis, and 1 with a history of current atopic dermatitis. The placebo group included 7 patients with current asthma and 2 with current atopic dermatitis. Each group had 1 pa-

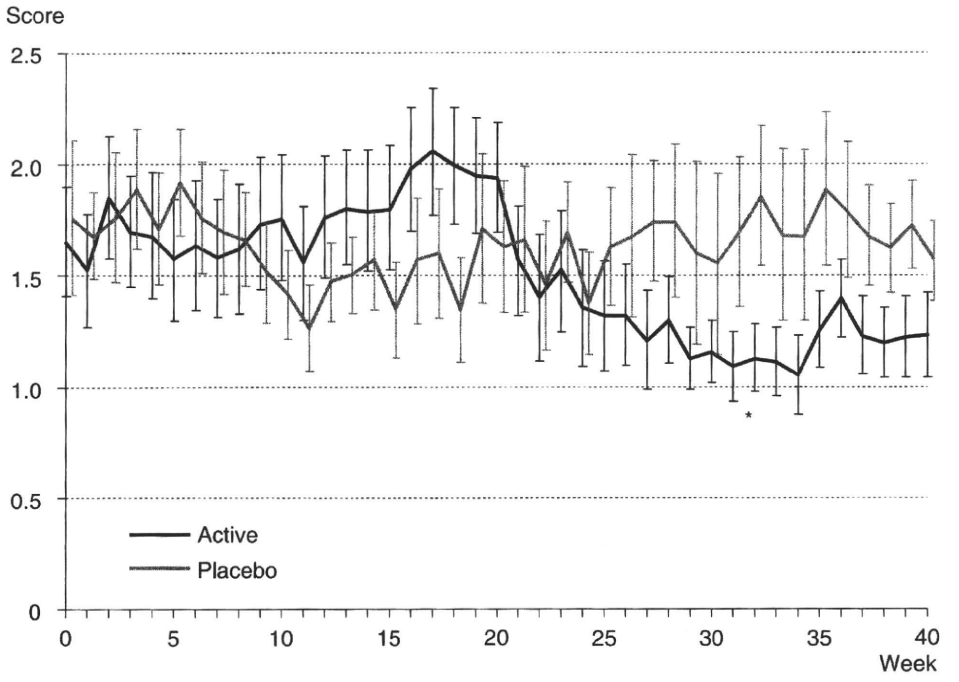


**Fig. 3** Symptom scores in the active group decreased after week 24 and were significantly lower than that in the placebo group in week 32. *$p < 0.05$ (vs. placebo group, Mann-Whitney U test).

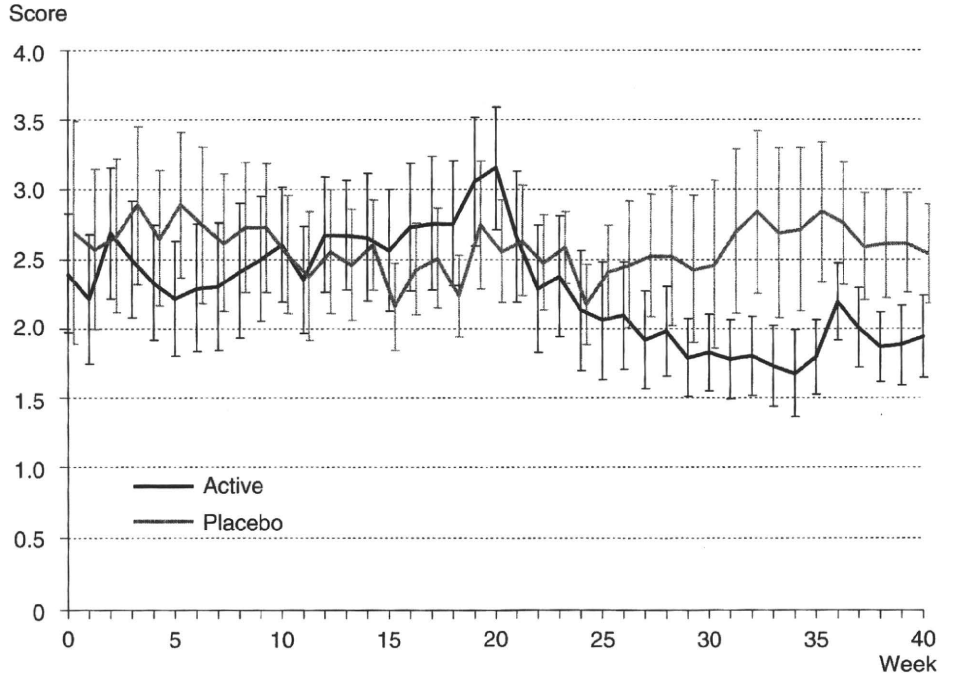


**Fig. 4** The mean symptom-medication score decreased after week 24 in the active group.

tient with current Japanese cedar pollinosis. The rates and severities of the additional allergic diseases did not differ significantly between the two groups.

## NASAL SYMPTOMS SCORES

The mean nasal symptom scores for each week of the study are shown in Figure 1, 2. The mean higher score for paroxysmal sneezing or runny nose (Fig. 1)

did not differ between the active and placebo groups until week 24. After this time, this score decreased for the active group but not for the placebo group, and the mean scores were significantly lower in the active group in weeks 32 and 35. The mean nasal congestion scores (Fig. 2) changed randomly in both groups until week 20, but decreased thereafter in the active group.

### SYMPTOM AND SYMPTOM-MEDICATION SCORES

The mean symptom and symptom-medication scores for each week of the study are shown in Figure 3, 4. These scores showed similar tendencies. Both were almost constant throughout the study in the placebo group, but decreased after week 24 in the active group. The mean symptom scores were significantly lower in the active group in week 32. A comparison of the average scores for week 0-3 (a week of washout before treatment and the first 3 weeks of treatment) with those for weeks 37-40 (the final 4 weeks of treatment) is shown in Figure 5. The average symptom score for weeks 37-40 was significantly lower than that for weeks 0-3 in the active group, while there was no significant difference in the placebo group. The average symptom-medication score for weeks 37-40 was also lower than that for weeks 0-3 in the active group, but the difference was not significant. In the placebo group, the average symptom-medication score did not decrease significantly over the study period.

### IMPROVEMENT OF SYMPTOMS

The average symptom score for weeks 37-40 was compared with the baseline score (the average score for weeks 0-3) in each patient. A decrease of more than 1 point was taken to indicate improvement of symptoms, and this was found in 33% of patients in the active group but in 0% in the placebo group (Fig. 6). Similarly, the symptom scores one year after the end of the trial were compared with those at baseline. This showed improved symptoms in 16% of patients in the active group, but in 0% in the placebo group.

### EFFECT ON OTHER ALLERGIC DISEASES

Of the 8 and 7 patients with asthma in the active and placebo groups, respectively, the frequency of asthma attacks after the trial was reduced in 2 and unchanged in 6 in the active group, and was reduced in 3 and unchanged in 4 in the placebo group. Of the 5 and 2 patients with atopic dermatitis in the active and placebo groups, respectively, improvement of symptoms occurred in 1 patient in the active group. The other 6 patients showed no change through the study. There was no significant difference between the active group and the placebo group in the therapeutic effects on asthma and atopic dermatitis.

### ADVERSE EFFECTS

One patient in the active group reported a bitter taste. There were no other local adverse effects and no severe systemic adverse effects related to the treatment.

## *DISCUSSION*

HDM is the most common allergen in pediatric patients with allergic rhinitis. Natural remission of the disease is rare in childhood and the condition carries over to adulthood in most patients. Allergen-specific SCIT is the only current therapy that can alter the natural course, but the treatment has practical inconveniences. SLIT has been proposed as an effective alternative, but the efficacy of SLIT in pediatric HDM allergic rhinitis has yet to be shown based on recent reviews.[22-24] Therefore, we examined the therapeutic effect of SLIT with house dust extract on pediatric HDM allergic rhinitis over a period of 10 months in a placebo-controlled study. This house dust extract is only available in Japan and contains moth, cockroach and mold antigens, in addition to mite. Except for Der f, the concentrations of these antigens are unknown. The active group had significantly lower symptom scores compared to the placebo group after treatment for 30 weeks, and the mean symptom score in the active group in the last four weeks of the study was significantly lower than that in the first four weeks.

The decrease in the symptom-medication score was not significant, but the medication score may not reflect the exact quantity of rescue medicines for allergic rhinitis, since more than half of the patients had asthma or atopic dermatitis. To evaluate the efficacy of SLIT for asthma and atopic dermatitis, we chose criteria that did not exclude these complications. Of the 15 patients with asthma, 2 in the active group and 3 in the placebo group had a decreased number of attacks in the treatment period. Of the 6 with atopic dermatitis, only 1 patient in the active group showed improvement of symptoms. Therefore, the effects of SLIT on asthma and atopic dermatitis were unclear.

The results for nasal symptoms obtained in this study were comparable with those in randomized studies using standardized mite extracts, based on meta-analysis by Penagos[23] and Calamita.[25] One year after treatment, about half of the patients in the active group, but none in the placebo group, had improved nasal symptoms compared to the start of treatment. To obtain long-term effects, a longer treatment period may be necessary. The doses of allergen extract used in this study were about 20 times higher than those used in conventional SCIT in Japan. However, recent results of SLIT studies for pollinosis have recommended treatment for more than 18 months with doses of allergens of more than 100 μg. Further comparative studies are needed to assess the ideal doses, temporal intervals, and vehicles for administration.

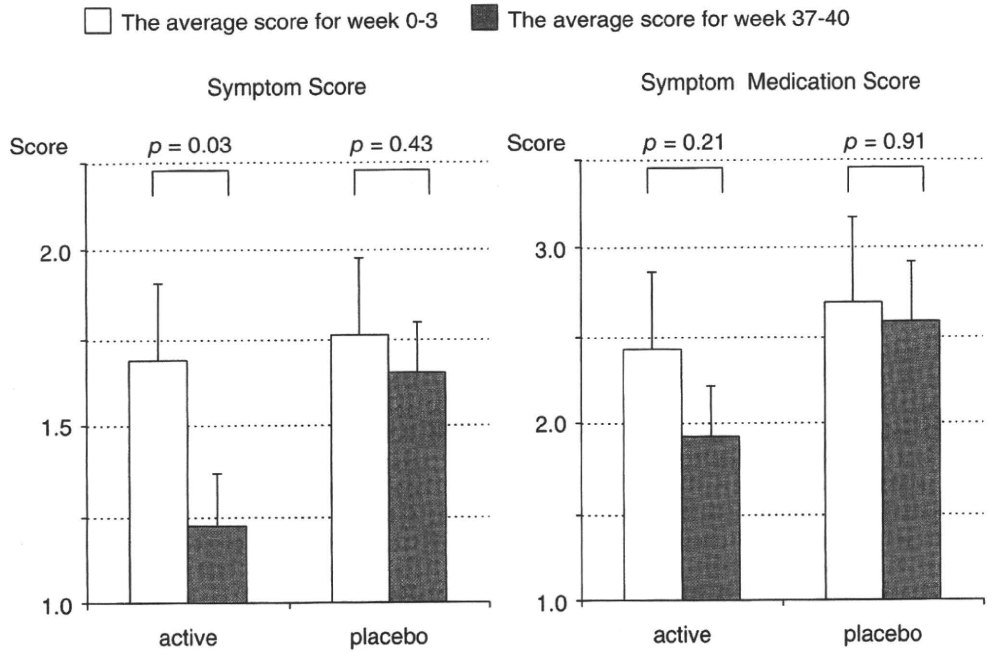


**Fig. 5** The average symptom score for weeks 37-40 (the final 4 weeks of the study) was significantly lower than that for weeks 0-3 (a week of washout before treatment and the first 3 weeks of treatment) in the active group, whereas there was no significant difference in the placebo group. *p*: Wilcoxon signed-rank test.

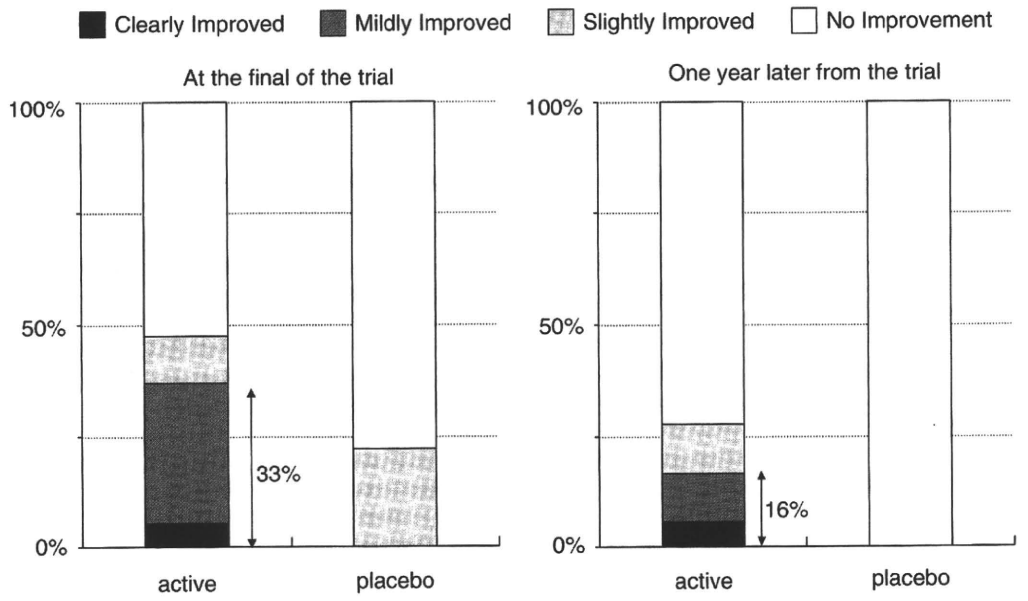


**Fig. 6** Based on changes in symptom scores, improvement of symptoms was found in 33% of patients in the active group but in 0% in the placebo group at the end of the trial. At one year after the end of the trial, improvement was found in 16% of patients in the active group but in 0% in the placebo group.

Although the current study is preliminary, the results are encouraging and suggest a need for a new study that includes a comparison of the effects of house dust extract with those of a standardized mite extract.

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# Increase of regulatory T cells and the ratio of specific IgE to total IgE are candidates for response monitoring or prognostic biomarkers in 2-year sublingual immunotherapy (SLIT) for Japanese cedar pollinosis

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**KEYWORDS**
Allergic rhinitis;
Biomarker;
Immunotherapy;
Japanese cedar pollinosis;
Regulatory T cell;
Sublingual immunotherapy

**Abstract** The aims of this study were to examine the therapeutic effects of sublingual immunotherapy (SLIT) and to identify potential biomarkers that would predict the therapeutic response in a randomized, double-blind, placebo-controlled clinical trial. The trial was carried out over two pollinosis seasons in 2007 and 2008. Carry-over therapeutic effects were analyzed in 2009. SLIT significantly ameliorated the symptoms of pollinosis during the 2008 and 2009 pollen seasons. Cry j 1-specific cytokine production in a subgroup of patients with mild disease in the SLIT group was significantly attenuated. The ratio of specific IgE to total IgE before treatment correlated with the symptom-medication score in the SLIT group in 2008. Patients with increased

*Abbreviations:* DBPC, double-blind, placebo-controlled; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot assay; iTreg, induced regulatory T cells; ITT analysis, intention-to-treat analysis; JAU, Japanese allergy unit; N.S., not significant; OT analysis, on-treatment analysis; PBMCs, peripheral blood mononuclear cells; RAST, radioallergosorbent test; SLIT, sublingual immunotherapy; SMS, symptom-medication score; Treg, regulatory T cells; QOL, quality-of-life

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Cry j 1-iTreg in the SLIT group had significantly improved QOL and QOL-symptom scores. In summary, the specific IgE to total IgE ratio and upregulation of Cry j 1-iTreg are candidates for biomarker of the clinical response to SLIT.


## 1. Introduction

Japanese cedar (*Cryptomeria japonica*) pollinosis is a common allergy in Japan, with a prevalence estimated to be 26.5% in a nationwide survey conducted in 2008 [1].

A 2000 Japanese allergy unit (JAU) sample of standardized extract from Japanese cedar pollen is the only available allergen for subcutaneous and sublingual immunotherapy (SLIT) against pollinosis in Japan. The 2000 JAU extract contains 1.5 to 4.2 μg of the major allergen, Cry j 1 [2]. The common monthly cumulative dose for SLIT is 8000 JAU, which contains approximately 10 μg of Cry j 1. This maintenance dose is 200-fold higher than that used in traditional subcutaneous immunotherapy using 0.2 ml of a 200 JAU/ml extract, which contains approximately 50 ng of Cry j 1. Despite using a low dose of the major allergen compared with that in European trials, positive effects on pollinosis have been shown in randomized double-blind, placebo-controlled (DBPC) studies, in which SLIT significantly ameliorated the symptom score, symptom-medication score (SMS), and quality-of-life (QOL) score [3,4].

SLIT induces Cry j 1-specific IgG4 production and attenuates the seasonal increase in the number of Th2 cells specific to epitopes from Cry j 1 and Cry j 2 [3]. Involvement of antigen-specific Tr1 cells or regulatory T cells (Treg) in the therapeutic mechanism has also been suggested [5,6]. We previously found that SLIT increased the levels of Cry j 1-specific induced Treg cells (Cry j 1-iTreg; $IL10^+Foxp3^+$ cells in $CD25^+CD4^+$ leukocytes) and that the increase in Cry j 1-iTreg after the pollen season may serve as a response monitoring biomarker that correlates with a positive therapeutic effect based on the QOL-symptom score and distinguishes responders from non-responders after SLIT [6].

In this report, we examined the reproducibility of the positive therapeutic effects and safety of SLIT and upregulation of iTregs as a response monitoring biomarker, with the goal of confirming our previous results in a larger randomized DBPC study. Therefore, the safety and clinical effect of SLIT for Japanese cedar pollinosis were used as the primary endpoint, and carry-over effects, immunological changes, and biomarkers for a positive clinical effect induced by SLIT were secondary endpoints.

## 2. Materials and methods

### 2.1. Study population

The study was conducted as a randomized, DBPC, parallel-group, single center trial in subjects with Japanese cedar pollinosis. This study was performed for two pollen seasons between September 2006 and May 2008, with follow-up in the pollen season in 2009. We recruited 130 participants in September 2006. Diagnosis of Japanese cedar pollinosis was based on clinical history and the presence of IgE specific to Japanese cedar pollen of at least class 2 (CAP-RAST method, Phadia, Tokyo, Japan). Participants with a history of immunotherapy or a diagnosis of asthma, or those who were pregnant, were excluded from the study. Patients who suffered seasonal or chronic rhinitis that required medical treatment were also excluded.

A total of 103 patients were eligible for the study, and all had moderate or severe symptoms in the previous pollen season [7]. We anticipated that some participants in the SLIT group would drop out from the study due to side effects and we planned to evaluate the risk of mild or severe side effects due to the vaccination. Therefore, we randomly divided the patients into treatment (SLIT) and placebo groups with a ratio of 6:4 according to the table of random numbers prepared by the Department of Pharmacy at Chiba University Hospital (Fig. 1). The sample size was determined based on a previous study [3]. Briefly, we planned to have 50 patients in each group with anticipation of dropout. We set 1.0 as a magnitude for the difference of average SMS between that from the SLIT and placebo groups and 1.5 as a standard deviation according to the result of previous study. Therefore, when the power was set to 0.8 and the $\alpha$-error to 0.05, the number of required cases was 35 in each group. A person who was not directly involved in the study was responsible for group allocation. To prevent leakage of information, the allocation table was kept by this person and a member of the ethics committee who was also not directly involved in the study, until accessed with the key after completion of the study. The protocol was approved by the Ethics Committee of Chiba University, and written informed consent was obtained from each patient prior to participation in the study.

### 2.2. Clinical protocols

The SLIT group included 58 patients who received standardized Japanese cedar pollen extract (Torii Pharmaceutical Co. Ltd., Tokyo, Japan) [8], and the placebo group included 45 patients who received an inactive placebo. The protocol consisted of treatment with graded courses of the extract in 50% glycerol, followed by maintenance therapy [6]. Briefly, the extract was graded in three strengths: 20, 200, and 2000 JAU/ml. Patients received increasing doses with each vial, beginning with 0.2 ml from the 20 JAU/ml vial and increasing by 0.2 ml a day for 5 days per week. The vaccine was taken sublingually, kept in place for 2 min without a retention reagent, and then spit out. The procedure was repeated until the maximum dose (1.0 ml of 2000 JAU/ml) was reached. The maintenance dose was 1.0 ml of 2000 JAU/ml given once a week until the end of May 2008. The patients in the placebo group received inactive 50% glycerol in saline. All participants were allowed to take symptom-reducing drugs as needed.

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Cry j 1-iTreg in the SLIT group had significantly improved QOL and QOL-symptom scores. In summary, the specific IgE to total IgE ratio and upregulation of Cry j 1-iTreg are candidates for biomarker of the clinical response to SLIT.


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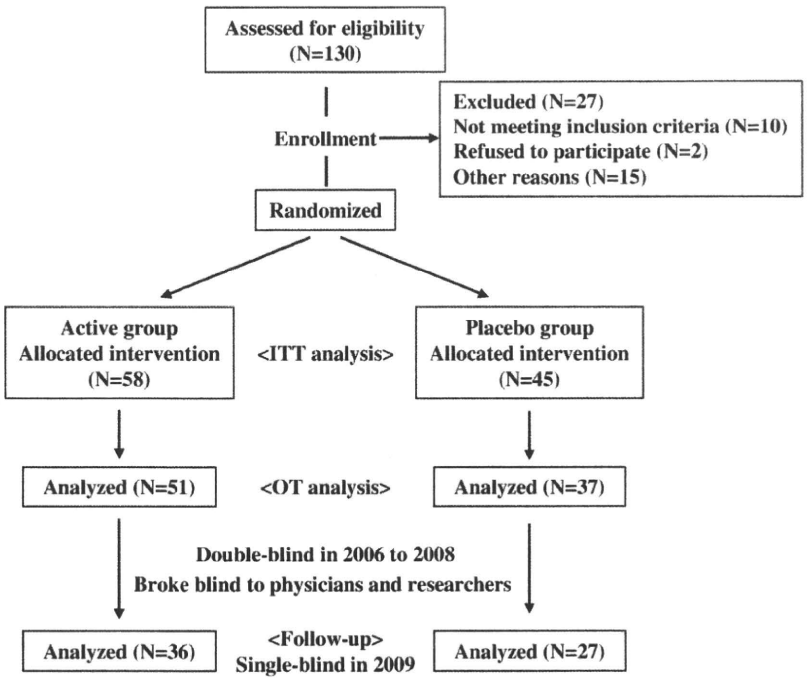


**Figure 1** Flow diagram for groups and individuals in the phases of the randomized trial. Fifteen participants from the SLIT ($N=7$) and placebo ($N=8$) groups were lost to follow-up due to reasons such as moving house and transfer. The double-blind status was maintained until completion of analysis of all clinical and immunological parameters (December 2008). Follow-up analysis in 2009 was undertaken in a single-blind manner.

## 2.3. Clinical symptoms and safety measurements

The patients completed a pollinosis diary to record their nasal symptoms and use of symptom-reducing drugs in the 2007, 2008, and 2009 pollen seasons. The total amounts of pollen scattered from Japanese cedar and Japanese cypress (*Chamaecyparis obtusa*) in Chiba prefecture were 2777, 6596, and 5486 grains/cm$^2$ during the 2007, 2008, and 2009 pollen seasons, respectively, based on measurements with a Durham pollen sampler. The duration and amount of scattered Japanese cedar pollen differed greatly among these years, but the daily amount of scattered pollen typically followed a wide-based bell-shaped curve over the whole pollen season from the middle of January or early February to the middle or end of May. The duration of the peak pollen season was relatively constant in the 3 years, and therefore, we analyzed the SMS during the peak period. The peak pollen season was defined as the period from the first day that the pollen count was $\geq$20 grains/cm$^2$/day for 3 consecutive days until the last day that the pollen count was $\geq$20 grains/cm$^2$/day before a period in which the pollen count was <20 grains/cm$^2$/day for 7 consecutive days.

The daily SMS was calculated as described previously [3]. Briefly, daily episodes of sneezing and nose blowing were rated as 0–4: none, 0; 1–5 episodes, 1; 6–10 episodes, 2; 11–20 episodes, 3; >20 episodes, 4. Daily medication was recorded based on drug types and duration of usage using the following guidelines: antihistamines, mast cell stabilizers, and vasoconstrictors, 1; topical ocular or nasal steroids, 2. Patients with an average daily SMS in the peak pollen season of $\leq$4 were judged to have mild symptoms based on guidelines for allergic rhinitis [7].

In the middle of the 2007 and 2008 pollen seasons, the participants completed the Japanese Allergic Rhinitis QOL Standard Questionnaire No.1 (JRQLQ No.1) for assessment of QOL-symptom and total QOL scores [9]. These scores were calculated as previously described [4,6]. The total QOL-symptom score was calculated as the sum of each component score: none, 0; mild, 1; moderate, 2; severe, 3; and very severe, 4. Nasal and ocular symptoms covered by the questionnaire included runny nose, sneezing, nasal congestion, itchy nose, itchy eyes, and watery eyes. Adverse events were graded using Common Terminology Criteria for Adverse Events (CTCAE) v.3.0 [10]. Briefly, adverse events were graded as mild, grade 1; moderate, grade 2; severe, grade 3; life threatening, grade 4; death, grade 5 according to a category for allergy/immunology in the CTCAE v.3.0 scoring system.

## 2.4. Blood samples

Peripheral blood was obtained from each patient before treatment (September to October 2006) and before and after the pollen seasons in 2007 (December 2006 to January 2007, and May to June 2007, respectively) and 2008 (November to December 2007, and May 2008, respectively). Peripheral blood mononuclear cells (PBMCs) were isolated, frozen, and stored in liquid nitrogen [6]. However, the PBMCs isolated before treatment, and before and after the 2007 pollen season were damaged during storage and we were unable to

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analyze their immunological responses. Therefore, immunological data were obtained only from PBMCs collected before and after the 2008 pollen season.

### 2.5. Total and antigen-specific immunoglobulin titer

The Cry j 1-specific IgE and IgG4 titers in plasma were measured by ELISA [3,11]. Total IgE and specific IgE titers for Japanese cedar, orchard grass, mugwort, and house dust mites were evaluated by the CAP-RAST method (Phadia).

### 2.6. Flow cytometric analysis

The levels of Cry j 1-iTreg were analyzed by flow cytometry [6]. Briefly, PBMCs were cultured with or without Cry j 1 for 3 days, followed by a culture with 10 ng/ml phorbol 12-myristate 13-acetate, 1 μM ionomycin, and 2 μM monensin for 6 h. The PBMCs were stained with PE-Cy7-anti-CD4 antibody, APC-anti-IL10 antibody (BD Biosciences, San Diego, CA, USA), PE-anti-CD25, and FITC-anti-Foxp3 (clone: PCH101) using a Foxp3 staining buffer set (eBioscience, San Diego, CA, USA).

### 2.7. Analysis of the number of IL4-producing cells and the concentration of cytokines

The number of IL4-producing cells stimulated with Cry j 1 was determined by enzyme-linked immunospot (ELISPOT) assay, and the concentrations of IL2, IL5, and IL13 in the culture supernatant were measured using a BD™ Cytometric bead assay (CBA) Flex system (BD Biosciences) [6]. Briefly, a 96-well sterile filter plate (Millipore, Billerica, MA, USA) was coated with monoclonal antibody to human IL4 (Mabtech AB, Nacka Strand, Sweden). The plate was pre-incubated with AIM-V medium at 37 °C for 1 h. The medium was discarded, and then PBMCs ($3\times10^5$ cells/well) were cultured with fresh medium alone or with 10 μg/ml Cry j 1 for 17 h at 37 °C in AIM-V medium containing 5% human AB serum (Sigma-Aldrich, St. Louis, MO, USA). The plates were then incubated with a biotinylated monoclonal antibody to human IL4 for 2 h, and then with streptavidin-conjugated alkaline phosphatase for 1 h at room temperature. After washing with PBS, the plates were incubated with BCIP/NBT$^{PLUS}$ (Mabtech) for 5 min at 37 °C. For the CBA, isolated PBMCs were cultured at $2.5\times10^6$ cells/ml with or without 5 μg/ml Cry j 1 for 3 days at 37 °C in AIM-V medium containing 5% human AB serum (Sigma-Aldrich). After centrifugation at 300×g for 10 min, the supernatant was divided into aliquots and stored at −20 °C until the cytokine assay was performed.

### 2.8. Data representation

The full analysis set ($N=103$) was used for the intention-to-treat (ITT) analysis and per protocol populations ($N=88$) were used for on-treatment (OT) analysis (Fig. 1). Cry j 1-specific cytokine production is shown as the difference between cells stimulated with Cry j 1 and controls stimulated with medium only. Changes after the 2008 pollen season are shown as differences between pre- and post-pollen season values.

### 2.9. Statistical analysis

Two-group comparisons were performed using a Wilcoxon $t$-test or Mann–Whitney $U$-test to determine the significance of differences, or using an unpaired $t$-test as indicated. $P$-values $<0.05$ were considered to be significant.

## 3. Results

### 3.1. Clinical effects and adverse events

A total of 103 patients were included in the overall analysis of efficacy for the 2007 and 2008 pollen seasons. These patients were randomly divided into the SLIT ($N=58$) and placebo ($N=45$) groups at a ratio of 6:4. Diaries and QOL questionnaires for 88 patients were available at the end of the DBPC study. The overall randomized population was considered to be the ITT population. The SMS in the SLIT group did not differ significantly from that in the placebo group in ITT analysis after 2-year SLIT ($P$=N.S.; Student $t$-test, data not shown).

The final sample size included 88 subjects for OT analysis (SLIT; $N=51$, placebo; $N=37$, ratio 4:3). The demographic characteristics of the OT population before treatment are shown in Table 1. The SMS in the SLIT group did not differ significantly from that in the placebo group in the 2007 peak pollen season (February 19 to March 31, $P$=N.S.; Student $t$-test). However, the average SMS in the 2008 peak pollen season (February 29 to April 1) was significantly ameliorated in the SLIT group compared with the placebo group (4.2 vs. 5.3, $P=0.02$; Student $t$-test). The percentages of subjects with mild symptoms (SMS ≤ 4) were 55% and 28% in the SLIT and placebo groups, respectively, in the peak pollen

**Table 1** Clinical data of participants at the start of the study.

| Group | SLIT | Placebo | $P$-value |
|---|---|---|---|
| Number | 51 | 37 | |
| Sex (M/F) | 17/34 | 8/29 | N.S.[a] |
| Mean age | 44.4 | 42.3 | N.S.[b] |
| Range | 16–73 | 19–70 | |
| Total IgE [IU/ml] | 198 | 258 | N.S.[b] |
| Range | 6.8–1480 | 8.6–2090 | |
| Specific IgE[c] | 27 | 29 | N.S.[b] |
| Range | 0.8–100 | 1.5–100 | |
| Class [mean] | 3.5 | 3.8 | N.S.[b] |
| Range | 2–6 | 2–6 | |
| Other allergies[d] (%) | | | |
| Orchard grass | 16 (31%) | 11 (30%) | N.S.[e] |
| Mugwort | 5 (10%) | 3 (8%) | <0.05[f] |
| House dust mite | 24 (47%) | 13 (35%) | N.S.[e] |

[a] Yates2×2 Chi-squared test.
[b] Student $t$-test.
[c] Specific IgE to Japanese cedar pollen; CAP-RAST raw value [kAU/L], mean.
[d] Number of subjects with specific IgE of at least CAP-RAST class 2.
[e] 2×2 Chi-squared test.
[f] Fisher exact probability.

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season (Fig. 2A). QOL-symptom and total QOL scores were also significantly ameliorated in the SLIT group compared to those in the placebo group in the middle of the 2008 pollen season (Fig. 2B).

There were no severe adverse events that required a patient to withdraw from the study; however, some subjects reported adverse events of mild discomfort: six of grade 2 (oral pruritus: 2; gingivostomatitis: 2; asthma: 1; rash in nasal cavity: 1) in the SLIT group (6/51; 11.8%); and one of grade 1 (bitter taste) in the placebo group (1/37; 2.7%).

### 3.2. Immunoglobulin production

There were no significant differences in Cry j 1-specific IgE and IgG4 production between patients in the SLIT and placebo groups before treatment, or before and after the pollen seasons. The SLIT group was divided into subgroups based on the SMS in the 2008 peak pollen season: a mild subgroup with SMS $\leq 4$ (classified as responders; $N=28$) and a severe subgroup with SMS $>4$ (non-responders; $N=23$). IgE and IgG4 production in patients in the mild subgroup were both similar to those in patients in the severe subgroup and in the placebo group at various time points (data not shown).

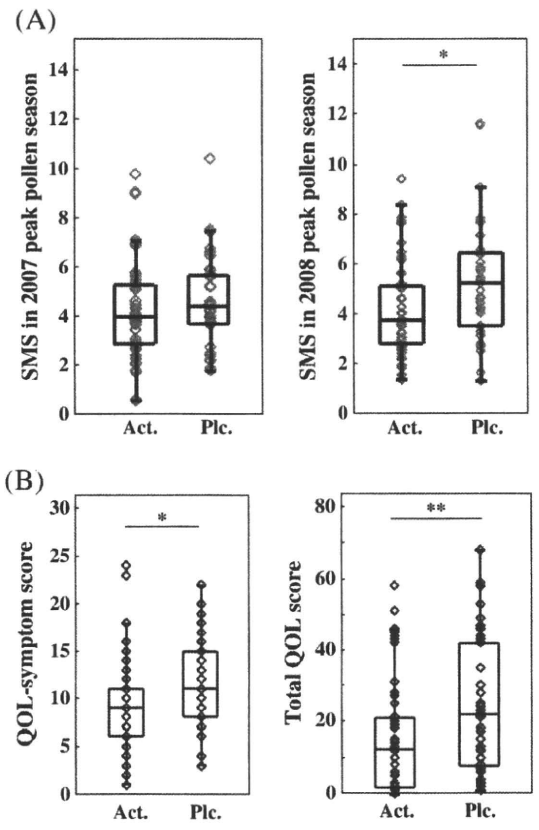


**Figure 2** Clinical scores after 2-year SLIT. (A) Average daily symptom-medication scores (SMS) in the SLIT (Act.; $N=51$) and placebo (Plc.; $N=37$) groups in the 2007 and 2008 peak pollen seasons. (B) QOL-symptom and total QOL scores from the QOL questionnaire were plotted for the SLIT (Act.; $N=51$) and placebo (Plc.; $N=37$) groups in the middle of the 2008 pollen season. Each diamond shows a value for an individual. Two-group comparisons were performed using an unpaired Student $t$-test. $^{*}P<0.05$, $^{**}P<0.01$.

### 3.3. Cry j 1-specific cytokine production

IL2, IL5, and IL13 levels were analyzed in the culture supernatant. The number of IL4-producing cells was measured by ELISPOT because IL4 was undetectable in the supernatant. There were no significant differences between the SLIT and placebo groups in the production of each cytokine following stimulation with Cry j 1 (Fig. 3A). IL5 was significantly increased after the pollen season in all groups ($P<0.05$; Wilcoxon $t$-test), and the IL2 and IL13 levels and the number of IL4-producing cells were significantly increased after the pollen season in the SLIT and placebo groups and in the severe subgroup ($P<0.05$; Wilcoxon $t$-test). Patients in the mild subgroup (responder to SLIT) did not show significant increase of IL2 and IL13 or of IL4-producing cells after the pollen season ($P$=N.S.; Wilcoxon $t$-test). The increases in the number of IL4-producing cells and IL5 level after the pollen season in the mild subgroup were significantly less than those in the severe subgroup (non-responders) and the placebo group. The increase of IL13 in the mild subgroup was significantly less than that in the severe subgroup and showed a tendency to be attenuated compared with the placebo group ($P$=N.S.; Mann–Whitney $U$-test). The increase of IL2 in the mild subgroup was significantly less than that in the placebo group ($P<0.05$) and showed a tendency to be attenuated compared with the severe subgroup ($P=0.053$; Mann–Whitney $U$-test, Fig. 3B).

### 3.4. Prognostic biomarkers for clinical effects

The average ratio of Japanese cedar pollen-specific IgE to total IgE (sIgE/tIgE ratio) in all patients in the study was 0.193 before treatment. The SLIT group was divided into subgroups with a sIgE/tIgE ratio $\leq 0.19$ (low, $N=28$) and $>0.19$ (high, $N=23$) before treatment. Similar subgroups were established in the placebo group. The SMS in the 2008 peak pollen season for the low subgroup was significantly improved compared to that in the high subgroup in the SLIT group ($P=0.02$; Mann–Whitney $U$-test); however, in the placebo group, the low and high subgroups had comparable SMSs ($P$=N.S.; Mann–Whitney $U$-test, Fig. 4A). Furthermore, the SMS was correlated with the sIgE/tIgE ratio in the SLIT group ($Rs=0.39$, $P<0.01$; Spearman correlation analysis), but not in the placebo group ($Rs=0.08$, $P$=N.S.; Spearman correlation analysis, Fig. 4B).

### 3.5. Upregulation of Cry j 1-iTreg levels as a response monitoring biomarker

A population of IL10$^{+}$Foxp3$^{+}$ cells in CD25$^{+}$CD4$^{+}$ leukocytes was evaluated as a potential marker for iTreg after stimulation with Cry j 1 or medium only before and after the pollen season in 2008. Neither the changes in Cry j 1-iTreg levels after stimulation with and without Cry j 1 nor the upregulation of Cry j 1-iTreg from pre- to post-pollen season differed significantly different between the groups (data not shown).

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We previously reported that upregulation of Cry j 1-iTreg is a candidate biomarker that may distinguish SLIT responders from non-responders based on QOL-symptom scores [6]. Therefore, we divided the SLIT group into subgroups based on an increase ($N=24$) or decrease ($N=27$) in Cry j 1-iTreg levels from before to after the pollen season in 2008. QOL-symptom and total QOL scores in the increased iTreg subgroup significantly improved compared with those in the placebo group. In contrast, the scores in the decreased iTreg subgroup were similar to those in the placebo group (Fig. 4C).

### 3.6. Carry-over effects in the year after treatment

A total of 63 patients completed a pollinosis-symptom diary during the 2009 pollen season; 1 year after the 2-year SLIT treatment (Fig. 1). All participants remained blinded to their treatment with SLIT or a placebo. The SMS in the peak pollen season in 2009 (February 15 to March 6) in the SLIT group ($N=36$) was significantly attenuated compared to the placebo group ($N=27$, $P=0.03$). The average SMSs for the SLIT and placebo groups were 3.5 and 4.5, respectively, in the peak pollen season (Fig. 5).

## 4. Discussion

The primary endpoint of this randomized DBPC trial was the therapeutic effect evaluated in ITT analysis. No significant positive effect was observed between the SLIT and placebo groups after exchanging the perceived improvement of patients who dropped out with each median score from the counter group. In OT analysis, the SMS in the SLIT group was

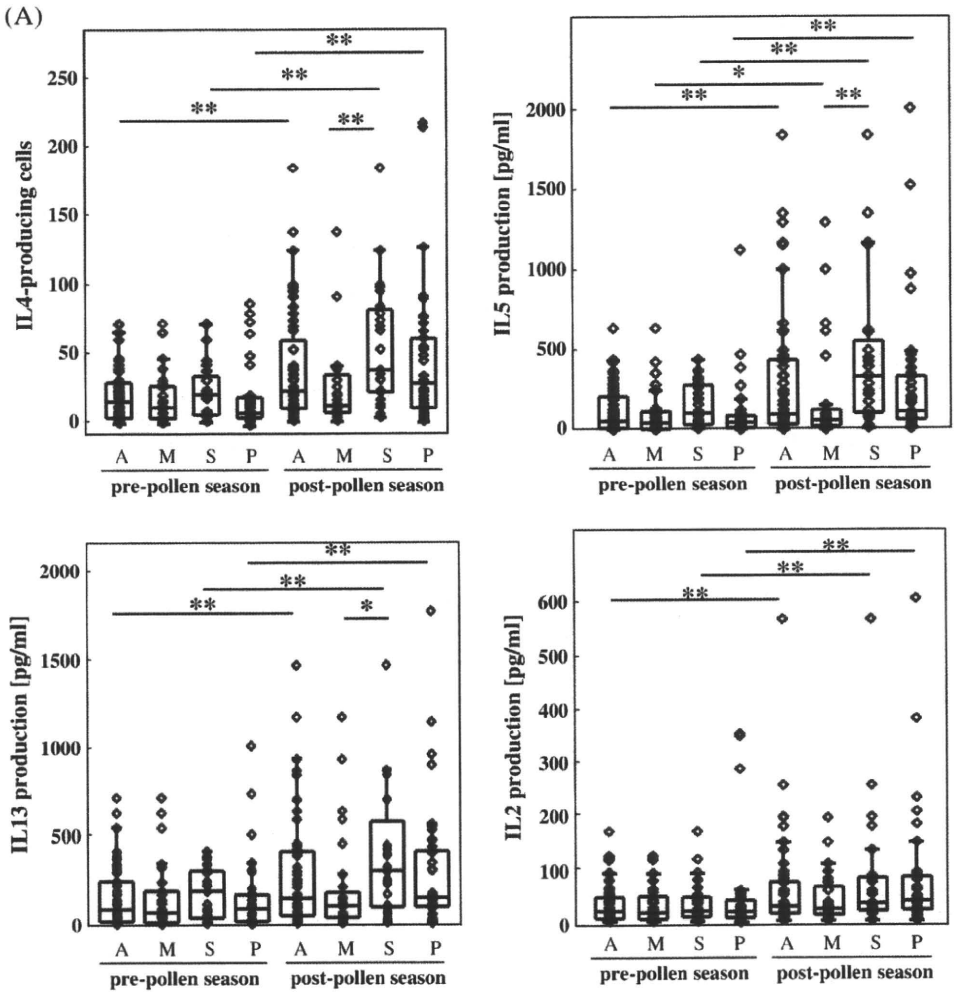


**Figure 3** Cytokine production from PBMCs. (A) Number of Cry j 1-specific IL4-producing cells and Cry j 1-specific cytokine levels in the SLIT group (A; $N=51$), the mild subgroup of the SLIT group (M; $N=28$), the severe subgroup of the SLIT group (S; $N=23$), and the placebo group (P; $N=37$) at before and after the 2008 pollen season. Comparisons with a significant difference are indicated as * and **; otherwise, comparisons are not significantly different ($P=$N.S.). (B) Increases in the number of Cry j 1-specific IL4-producing cells and Cry j 1-specific cytokine levels occurred from before to after the 2008 pollen season in the SLIT group (Act.; $N=51$), the mild subgroup of the SLIT group (Mild; $N=28$), the severe subgroup of the SLIT group (Sev.; $N=23$), and the placebo group (Plc.; $N=37$). Each diamond shows the value for an individual. Two-group comparison was performed using a Mann–Whitney $U$-test. *$P<0.05$, **$P<0.01$.

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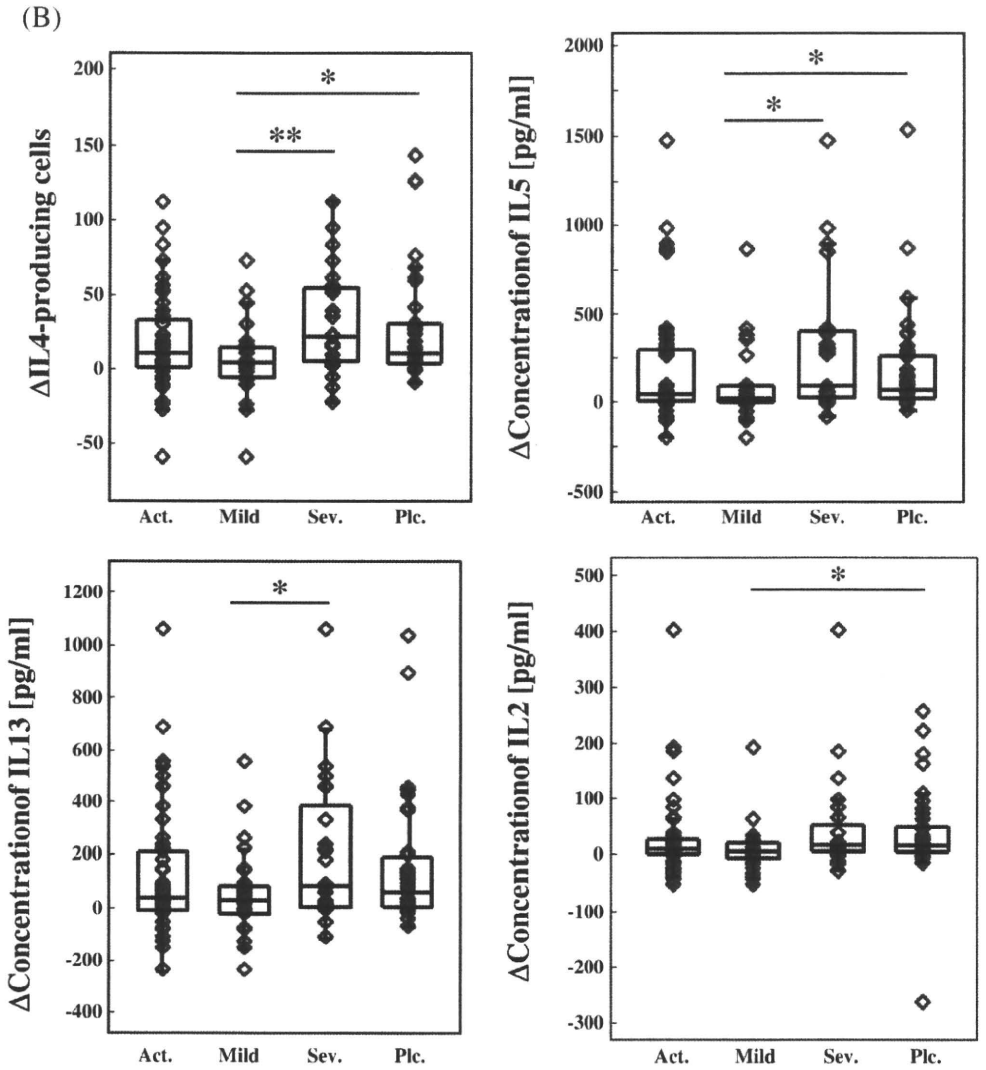


**Figure 3** (*continued*).

significantly ameliorated compared to the placebo group in 2008. The percentage of mild subjects (SMS ≤ 4) in the SLIT group was 28% higher than that in the placebo group (SLIT, 55%; placebo, 27%), and the SMS was reduced by approximately 21% in the SLIT group compared with the placebo group (SLIT, 4.2; placebo, 5.3). This percentage of mild subjects differ significantly between the SLIT and placebo groups ($P=0.009$; 2×2 Chi-squared test). These effects following 2-year treatment were comparable to those in a trial of 1-year daily treatment using grass pollen tablets [12]. The low dose of the extract (about 1/40th of that used in Europe) may be one reason for the poor clinical outcome in the first year [13]. An extract of concentration >2000 JAU is not available for clinical use in Japan, and the clinical effects, safety, and optimum schedule for administration of an extract with a much higher allergen concentration remain unclear.

Positive clinical therapeutic effects were not obtained following 1-year treatment in our study, even in OT analysis (data not shown). In contrast, two previous reports demonstrated positive therapeutic effects after 1-year SLIT for Japanese cedar pollinosis [3,4]. However, in these studies, the annual pollen count (1154 grains/cm$^2$/season) [3] was less than in our study, and daily SMS was significantly attenuated on only 4 days in the pollen season [4]. The severity of SMS is affected by the amount of Japanese cedar pollen in the total and peak pollen season. Natural resolution and tolerance are not usually induced by natural exposure to Japanese cedar pollen, regardless of the amount of pollen [14].

Whether there are detectable alterations in peripheral T-cell responses after specific immunotherapy is still under debate [15–18]. The Cry j 1-specific cytokine profile from the SLIT group did not differ significantly from that in the placebo group. However, the increases in IL2, IL4, IL5, and IL13 production in the mild subgroup in the SLIT group were significantly attenuated (or showed a tendency to be attenuated) compared to the severe subgroup and the placebo

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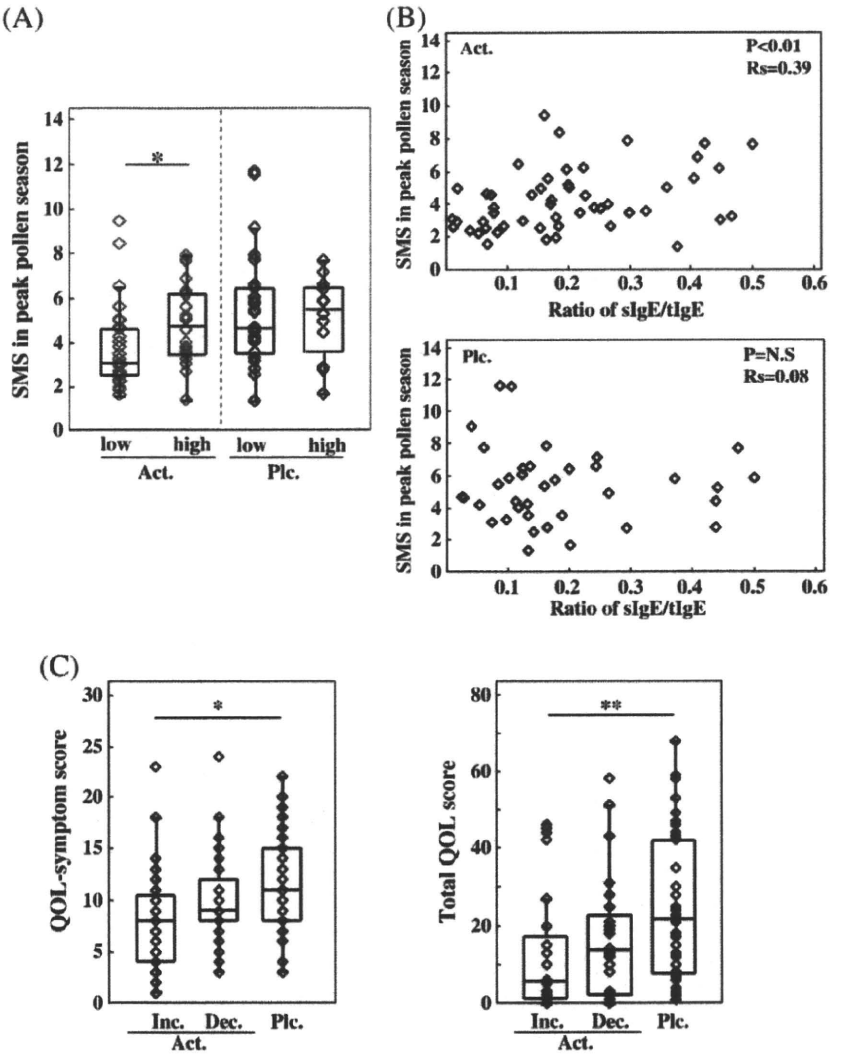


**Figure 4** Biomarkers for positive therapeutic effects following SLIT. (A) SMSs in the 2008 peak pollen season for patients with low (low; $N=28$) and high (high; $N=23$) sIgE/tIgE ratios in the SLIT group (Act.), and for those with low ($N=25$) and high ($N=12$) sIgE/tIgE ratios in the placebo group (Plc.). *$P<0.05$. (B) Correlation between SMSs in the 2008 peak pollen season and sIgE/tIgE ratios before treatment in the SLIT (Act.; $N=51$) and placebo (Plc.; $N=37$) groups. Statistical data were obtained with Spearman correlation analysis. (C) QOL-symptom and total QOL scores from the QOL questionnaire plotted for a subgroup with increased Cry j 1-iTreg in the SLIT group (Inc.; $N=24$), a subgroup with decreased Cry j 1-iTreg in the SLIT group (Dec.; $N=27$), and the placebo group (Plc.; $N=37$) in the middle of the 2008 pollen season. Each diamond shows the value for an individual. *$P<0.05$, **$P<0.01$.

group (Fig. 3B). The SMS in all patients in the study correlated with the seasonal increases in IL4 ($R=0.35$, $P<0.01$), IL5 ($R=0.35$, $P<0.01$), and IL13 ($R=0.36$, $P<0.01$). The discrepancy in our current results and the results of previous studies with regard to downregulation of cytokine production from PBMCs may depend on the extent of the therapeutic effects achieved in each clinical trial.

Cry j 1-specific IgE production was not changed by treatment, even in the mild subgroup, as also found in our preliminary study [6]. We speculate that more time is required for changing antibody production following the changes of antigen-specific T cell profiles, because the alteration of T cell profiles strongly influences subsequent class switch recombination of B cells and antibody production. Another possibility is that the dose for SLIT used in this study was not high enough to alter the antibody profiles.

The sIgE/tIgE ratio has been found to be significantly higher in responders than in non-responders following 4-year immunotherapy [19]. In our trial, this ratio did not differ significantly between responders and non-responders ($P$=N.S.; Mann–Whitney $U$-test). However, subjects with a low sIgE/tIgE ratio before treatment were more likely to be responders to 2-year SLIT, and the ratio correlated with the SMS only in patients treated with SLIT (Fig. 4A, B). This suggests that SLIT was more effective in patients with a low sIgE/tIgE ratio than in those with a high sIgE/tIgE ratio. The range of total IgE levels for the participants were relatively wide (6.8–2090 IU/ml in all patients); however, the change of the total IgE for each

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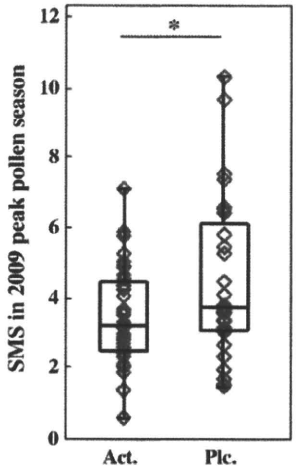


**Figure 5** Carry-over effects following 2-year treatment with SLIT. SMSs in the 2009 peak pollen season were plotted for the SLIT (Act.; $N=36$) and placebo (Plc.; $N=27$) groups. Each diamond shows the value for an individual. Two-group comparisons were performed using an unpaired $t$-test.

individuals after 2-year treatment was not significantly different compared to before treatment ($1.5\pm1.0$ times higher, $P=$N.S.; paired $t$-test). Therefore, the wide range of total IgE levels was due to the variability on the allergic status for individuals, but not on method for measurement. The serum IgE level may affect the surface IgE level on effector cells such as mast cells and basophils, and Tregs can downregulate activation of mast cells and eosinophils [20,21]. We speculate that effector cells with a low specific IgE level are less likely to be activated by antigen crosslinking or are more susceptible to downregulation by Tregs than those with a high specific IgE level. It is also possible that the symptoms of patients with a low sIgE/tIgE ratio may be more readily attenuated by suboptimal potentiation of iTreg induced by SLIT.

We previously reported that an increased count of Cry j 1-iTregs was a candidate biomarker that could be used to distinguish between responders and non-responders to SLIT, as evaluated by the QOL-symptom score. In this report, the subgroup with increased Cry j 1-iTregs showed significant amelioration of the QOL-symptom and total QOL scores compared to the placebo group, while the subgroup with decreased Cry j 1-iTregs did not show this response (Fig. 4C). However, there was no significant difference in Cry j 1-specific cytokine production from PBMCs among patients with increased iTregs and decreased iTregs, and those in the placebo group (data not shown). Foxp3-expressing $CD25^+CD3^+$ cells and IL10-expressing $CD3^+$ cells, which are induced in the nasal mucosa after subcutaneous immunotherapy, have been linked to the clinical efficacy and suppression of seasonal inflammation [22]. Immunotherapy using an Amb a 1-immunostimulatory oligodeoxynucleotide conjugate also induced $CD4^+CD25^+$ T cells and IL10-producing cells in the nasal mucosa after the pollen season [23]. These data suggest that iTregs may downregulate effector cells at local sites of inflammation to suppress clinical symptoms. Induction of iTregs in the nasal mucosa and functional analysis of these cells may be necessary to determine the regulatory mechanisms affected by SLIT. Mucosal biopsy in the peak pollen season is useful for evaluation of local induction of iTregs and downregulation of effector cells. However, nasal biopsy in the pollen season significantly influences the daily SMS in the peak pollen season. Mucosal biopsy outside the pollen season after exposure using an artificial pollen chamber may be a powerful tool for evaluation of local regulatory mechanisms induced by SLIT [24]. Upregulation of iTregs in nasal mucosa may be difficult to determine since the evaluation may be painful for patients. However, upregulation of iTregs in peripheral blood is simple to analyze and may be a useful biomarker because an increase of peripheral Cry j 1-iTregs is correlated with QOL and QOL-symptom scores in the pollen season, as discussed here and elsewhere [6].

Cry j 1-specific IgG4 production was not induced by SLIT in this study to the same extent as that in our previous study [6]. A clinical trial showing that daily 2500 SQ-T (14 μg Phl p 5 per 4 weeks) tablets failed to induce IgG production supports our current results [13]. A change in the immunoglobulin profile may require a higher allergen dose or longer duration of exposure. However, our study suggests that detectable quantitative changes in IgG4 are not essential for the amelioration of clinical symptoms.

In summary, we suggest that the sIgE/tIgE ratio and upregulation of iTregs may be considered as prognostic and response monitoring biomarkers, respectively, for SLIT. However, further investigation of induction of iTregs at local inflammatory sites and downregulation of inflammatory cells is needed. Furthermore, validation studies with larger sample size would be required before either biomarkers should be applied widely in the clinical management of pollinosis patients. Development of a more effective vaccine and better protocols may reveal more significant differences in the Cry j 1-specific cytokine profiles and iTreg induction, and these results may increase our understanding of the roles of iTregs or Tr1 in the therapeutic mechanisms underlying the efficacy of SLIT.

## Acknowledgments

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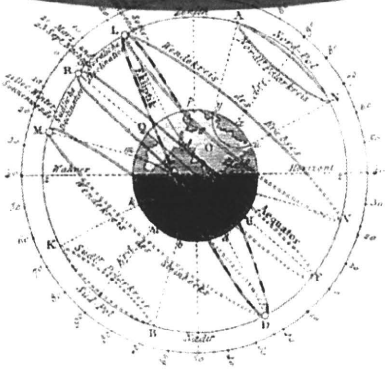
【解説】



# アレルギーワクチンの開発

石井保之

**ワクチンという言葉は普通，感染症の予防用に免疫賦活を目的に投与される弱毒化や不活化されたウイルスなどに用いられる．一方，アレルギー疾患に用いられるワクチンは，様々なアレルゲンに対する過剰な免疫応答を抑制し，最終的には免疫寛容を誘導することを目的とする．アレルギー疾患の現行の治療は症状を抑える対症療法が主体で，根本治療は唯一減感作療法のみであることから，アレルギーワクチンの研究開発に対する期待は大きい．**

現代社会，アレルギー疾患の患者数は増加の一途を辿っているが，未だ決定的な治療法が確立していないのが現状である．喘息，アトピー性皮膚炎，アレルギー性鼻炎や結膜炎などの様々なアレルギー疾患が知られているが，主たる治療法はステロイド薬や抗ヒスタミン薬などで症状を抑える対症療法であることから，病気から完全に寛解することは難しい．しかし，アレルギー疾患の主たる原因抗原（アレルゲン）が特定されている花粉症や一部の喘息疾患に対しては，現在唯一の根本治療である減感作療法が適用可能である．ただ，減感作療法では，天然のアレルゲンを含有する花粉やダニの粗抽出物を皮下注射するため，アナフィラキシーショックを誘発する危険性に留意する必要がある．そこで，低濃度のアレルゲン注射から段階的に投与量を高めていき，最大投与量に到達させることになるが，3年以上にもわたる長期間の通院治療が必要な場合があり，一般には普及していない．今後さらにアレルギー疾患に苦しむ患者さんの数の増大が予想されることから，予防と根本治療を目指した安全かつ有効なアレルギーワクチンの研究開発が急務になっている．

Development of Allergy Vaccines
Yasuyuki ISHII, (独)理化学研究所免疫・アレルギー科学総合研究センター

## アレルギーワクチンの定義

ワクチンという用語は通常，無毒化または不活化した病原体を体内に投与して，あらかじめ抗体価を高めておき，その後の感染症を予防することを意味する．一方，アレルギーワクチンは，病原体ではないものの疾患の原因になっているアレルゲンを使うことから広義のワクチンの範疇に入る．しかしながら，アレルギー症状に関係する免疫応答を抑制することを目的としている点や，予防的というよりはむしろ治療的に使うことが想定されるので，通常の感染症ワクチンとは「逆作用」のワクチンであると定義することができる．

またアレルギーワクチンが医薬品化を目指すからには，感染症ワクチンと同様に作用機序が明らかになって