and more than 70% of patients who are allergic to cedar pollen also develop cypress pollinosis [5, 6]. Around the Tokyo region, the cedar pollen season usually starts in the middle of February and is followed by the cypress pollen season which lasts until the beginning of May. Consequently, many patients with cedar pollinosis suffer from heavy pollen exposure for almost 12 weeks. In addition, Japanese cedar and cypress pollen can travel more than 100 km from the source, thereby raining down large amounts of pollen on other large cities. This situation is considerably different from that experienced in other countries where the most common allergens are grass pollens and ragweed, which generally travel distances of several hundred meters, and the pollen season lasts for less than 6 weeks [7]; however, it is similar to Northern European countries and North America where birch and other tree pollens are the major contributors.

Subcutaneous allergen-specific immunotherapy (SCIT) has been evaluated and shown to be an effective approach to change the course of allergic rhinitis [8–13], including Japanese cedar pollinosis [14]. However, an alternative method of administration is still required because the SCIT approach has been associated with the risk, albeit very low, of anaphylactic shock [15] and the inconvenience of frequent visits to the physician's office.

A recent review of randomized controlled studies of sublingual immunotherapy (SLIT) conducted outside Japan has strongly suggested its efficacy against a variety of allergens [16–21]. SLIT could be an attractive approach for Japanese cedar pollinosis if efficacy, safety, mechanisms and effective biomarkers can be clearly established.

The present placebo-controlled randomized studies were designed to determine the effects of SLIT on Japanese cedar pollinosis employing recombinant hybrid peptides consisting of 7 HLA class 2 restricted T cell epitopes of Cry J, the major allergen of Japanese cedar pollen [22].

Methods

Subjects

The study population consisted of 67 patients (33 males and 34 females), ranging in age from 20 to 37 years, who were otherwise healthy, but had a clinical history of Japanese cedar pollinosis for at least the last 3 consecutive cedar pollen seasons. The subjects lived in and around the city of Chiba, where a similar amount of pollen spread would be expected. The diagnosis of cedar pollinosis was based on clinical history, positive allergen-specific skin tests (wheal diameter ≥10 mm) to a standardized cedar pollen extract (Torii Pharmaceutical Co. Ltd., Tokyo, Japan) and serum cedar pollen-specific IgE levels of ≥score 2 by the CAP radioallergosorbent test (CAP-RAST; SRL, Tokyo, Japan). The exclusion criteria included a history of severe asthma, use of antiallergic drugs within 4 weeks and a prior history of any allergen-specific immunotherapy, including therapy for cedar pollen. Pregnant women or those at risk of pregnancy were also excluded. The study was conducted at the Chiba University Hospital and the protocol was approved by the Ethics Committee of Chiba University; written informed consent was obtained from each of the patients prior to participation in this study.

Japanese Cedar Pollen Extracts

Standardized Japanese cedar pollen extracts (Torii Pharmaceutical Co. Ltd.) were used [23]. The extract [1,000 Japanese Allergy Units (JAU)/ml] contained 1.5 μg of Cry j 1, which is the major allergen of Japanese cedar pollen. The amount of Cry j 1 was quantitated by an enzyme-linked immunosorbent assay, as reported previously [24].

Study Protocol

The study was placebo controlled and single blinded. The enrolled subjects were randomly divided into 2 groups with a ratio of 2:1 according to the table of random numbers by the Department of Pharmacy at the Chiba University Hospital. A controller who was not directly involved in this study was responsible for group allocation. The patients were divided randomly into the active (treatment) and placebo groups. A group allocation number was given to each patient. To prevent the leakage of information, this number was closely guarded jointly by the controller and a member of the ethical committee who was also not directly involved in the study, until accessed with the key after the completion of the study. The active group consisted of 43 patients who received the pollen extract and the placebo group consisted of 24 patients who received the placebo (inactive) for sublingual administration by the spit method (table 1). The sample size was determined based on previous similar studies [25]. The induction/buildup phase was 1 month, with the administration of an increasing daily number of the extract drops at 3 concentrations. The patients received 1 ml of 1,000 JAU extract or placebo once weekly as shown in table 2. Although the safety of the daily administration of SLIT has been reported recently, the weekly administration was chosen in this study in order to further reduce the possibility of any serious adverse events. No study of SLIT for Japanese cedar pollinosis has been reported to date. However, the development of asthma attacks by exposure to pollen has been observed in some patients [14]. The maintenance dose of the antigen in the present SLIT studies was about 100 times higher than that routinely used in SCIT. Administration was started at the beginning of October 2005 and ended at the end of April 2006. The patients carefully completed a pollen diary regarding their nasal symptoms and the usage of rescue drugs (such as antihistamines). Data were collected and analyzed at the Department of Clinical Testing of the Chiba University Hospital.

The nasal symptoms were evaluated on a scale from 0 to 4 in accordance with the *Practical Guidelines for the Treatment of Allergic Rhinitis, Japan* [26], as follows: 0 = no sensation; 1 = mild; 2 = moderate; 3 = severe; 4 = extremely severe. Daily episodes of sneezing and nose blowing were rated 0–4, as follows: 0 = none; 1 = 1–5 episodes; 2 = 6–10 episodes; 3 = 11–20 episodes; 4 = more than 20 episodes. The medications were also recorded according

to drug characteristics and duration of usage, according to the guidelines as follows: antihistamines, mast cell stabilizers and vasoconstrictors were listed as 1, topical ocular or nasal steroids as 2.

Immunoglobulin Assay

Serum Cry j 1-specific IgG4 antibodies were measured using microtiter plates coated with 100 ng/well of Cry j 1 which was purified as reported previously [27]. Allergen-coated wells with serum samples (diluted 1:50) were incubated for 2 h at 37°C, and then washed with PBS. The plates were incubated with 100 μl of biotinylated monoclonal anti-IgG4 antibody (BD Pharmingen; 500 ng/ml) for 1 h at 37°C, and then overnight at 4°C. After washing, the plates were incubated with 100 μl of streptavidin-γ-D-galactosidase conjugate (Roche Diagnostics) at 1:2,000 dilution for 1 h at 37°C, and washed. Finally, 100 μl of 5 mM o-nitrophenyl-β-D-galactopyranoside was added to the wells and incubated for 1 h at 37°C. After the enzyme reaction was stopped with 100 μl of 0.1 M Na_2CO_3, the absorbance at 415 nm was read using a microplate reader.

The specific IgG4 antibody levels were calculated from control curves with serial dilutions of a reference serum pool, which was prepared from 5 sera with high levels of Cry j 1-specific IgG antibody. The IgG4 antibody levels in the reference pool serum were arbitrarily assigned to be 100 U/ml.

Analysis of Th Cytokines and Cell Clones

Peripheral blood mononuclear cells (PBMCs) were obtained by the Ficoll-Hypaque method and stored at –80°C until analysis, using a cell banker (Nippon Zenyaku Kogyo Co. Ltd., Fukushima, Japan).

Th1/Th2 cytokine profiles were analyzed using FACS analysis. PBMCs (5×10^5) were stimulated with PMA and ionomycin for 4 h in the presence of 2 μM monensin, which inhibited the secretion of protein produced de novo. The cells were stained with anti-CD4 antibody for 15 min on ice. After washing with PBS, the cells were fixed with 4% paraformaldehyde for 10 min at room temperature and permeabilized with 0.5% Triton X-100 for 10 min on ice. After blocking with 3% bovine serum albumin for 10 min, the cells were incubated on ice for 30 min with anti-IFN-γ labeled with fluorescein isothiocyanate and anti-IL-4 labeled with phycoerythrin. A flow-cytometric analysis was performed on FACS Calibur (Becton Dickinson, Irvine, Calif., USA). The antibodies for FACS analysis were purchased from BD Bioscience (San Diego, Calif., USA).

The Cry j-specific Th2 clone sizes were determined by an ELISPOT assay using the recombinant hybrid peptide. The hybrid peptide comprised the 7 CD4 T cell determinants of Cry j 1 and Cry j 2, the major Japanese cedar pollen allergens [22]. Almost the entire patient population with Japanese cedar pollinosis respond to this hybrid peptide and the responses are comparable to the individual responses to Cry j 1 and Cry j 2 [22]. The monoclonal antibodies used in the ELISPOT analysis were obtained from Mabtech (Stockholm, Sweden). The anti-human IL-4 or IL-5 monoclonal antibodies were diluted to a concentration of 15 μg/ml in sterile, filtered (0.45 μm) PBS (pH 7.2), and 100 μl per well were added onto nitro-cellulose plates (Millititer; Millipore Corp., Bedford, Mass., USA). The plates were incubated overnight at 4°C and the unbound antibodies were washed with filtered PBS thereafter. After the last wash, PBS was sucked through the membrane under vacuum (Millipore). One hundred microliters of AIM-V medium with or without 20 μM hybrid peptide was added to 5×10^5 cells per well, and the plates were incubated for 10 h at 37°C. All assays were done in duplicate. The cells were subsequently washed before adding 100 μl of the biotinylated monoclonal antibodies (1 μg/ml), and incubated for 2 h at room temperature.

The plates were washed and incubated for 90 min at room temperature with 100 μl of streptavidin alkaline phosphatase (Mabtech) at a dilution of 1:1,000. The unbound conjugate was removed by another series of rinsing before 100 μl of BCIP/NBT substrate solution (Bio-Rad, Richmond, Calif., USA) was added and the plates were incubated at room temperature until dark

Table 1. Baseline characteristics of the patients

	Treatment group (n = 43)	Placebo group (n = 24)
Mean age, years[1]	26.8 ± 5.4	26.4 ± 5.9
Female sex	21 (48.8)	13 (54.2)
Mean duration of cedar pollinosis, years	8.7	9.1
Type of allergic rhinitis		
Cedar pollinosis with perennial	7 (16.3)	3 (17.5)
Cedar pollinosis with other pollinosis	5 (11.6)	4 (16.7)
Cedar pollinosis only	31 (70.5)	17 (70.8)
Additional allergic history		
History of asthma symptoms	2 (4.6)	0
Current asthma symptoms	0	0
History of allergic conjunctivitis	40 (93.0)	19 (75.0)
Cedar pollen RAST score[1]	4.18 ± 1.01	4.14 ± 0.92
Peak of daily total nasal symptoms score in the last cedar pollen season	4.8	4.5

Figures in parentheses are percentages.
[1] Data are means ± SD.

Table 2. Dose and dosing frequency

	Week 1 20 JAU	Week 2 200 JAU	Week 3 2,000 JAU	Week 4 2,000 JAU
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				

The induction phase with an increasing number of extract drops over 5 days a week at 3 concentrations for 3 weeks and the maintenance phase (week 4) with 1 ml of 1,000 JAU extracts once weekly are shown.

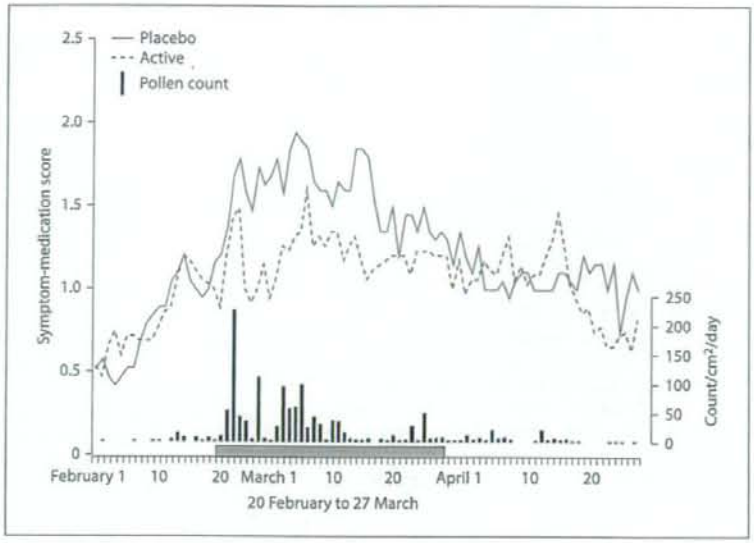
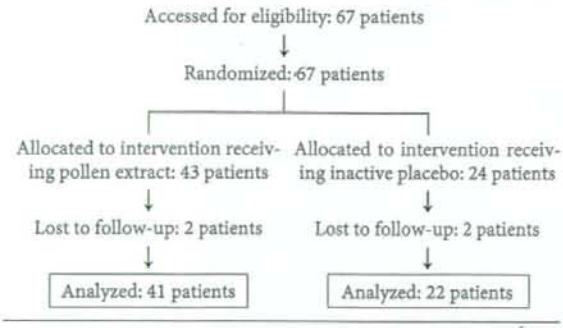


Fig. 1. Daily combined Japanese cedar and cypress pollen counts in 2006 in Chiba measured by the Durham pollen sampler and symptom-medication score (mean values) of patients during the pollen season.

Table 3. Study participation



spots emerged (1 h). The color development was stopped by repeated rinsing with tap water. After drying, the spots were captured photoelectrically and counted by a computed analysis to avoid any visual bias, using an auto counter (ImmunoScan; CTL, Cleveland, Ohio, USA).

Statistical Analysis

After completion of the study, the clinical and laboratory data were analyzed by a biostatistician who was not involved in carrying out the clinical trial. After completing the analysis, the allocation identification numbers for the active and placebo groups were accessed with a key. The Mann-Whitney U test was performed to compare symptom scores as well as symptom-medication scores between placebo and active groups. The Wilcoxon signed rank test was used for paired comparisons of the Cry j 1 specific IgG4 levels before and after SLIT. All statistical analysis was performed using the GraphPad Prism software, version 4.

During the statistical calculations in the present studies, the β error was defined as 0.2, power was 80% and the α error was defined as 0.05. Values of $p < 0.05$ were considered statistically significant.

Results

Four patients were withdrawn from the study for personal reasons, but not due to any adverse effects. All other subjects exhibited full compliance with the study protocol. As a result, 63 patients (41 patients from the active group and 22 patients from the placebo group) were analyzed further for effectiveness of SLIT (table 3).

Adverse Effects

Fifteen adverse effects were reported during the treatment. Of these, 13 subjects were in the active treatment group and 2 in the placebo group. Two patients in the active group complained of mild urticaria of the face or breast. The remaining subjects exhibited mild oral pruritus or oral pain (Common Terminology Criteria for Adverse Event grade 1). All adverse effects were transient and resolved spontaneously. No intervention was necessary.

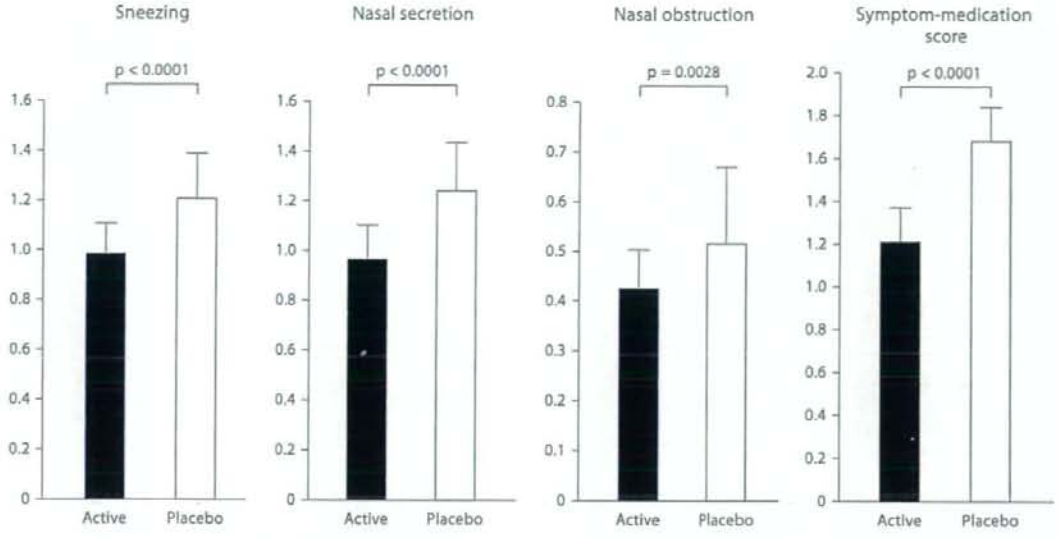


Fig. 2. Average symptom scores of sneezing, nasal secretion, nasal obstruction and symptom-medication score during the high pollen season, from 20 February to 27 March 2006. The average score of the active group was significantly lower than that of the placebo group.

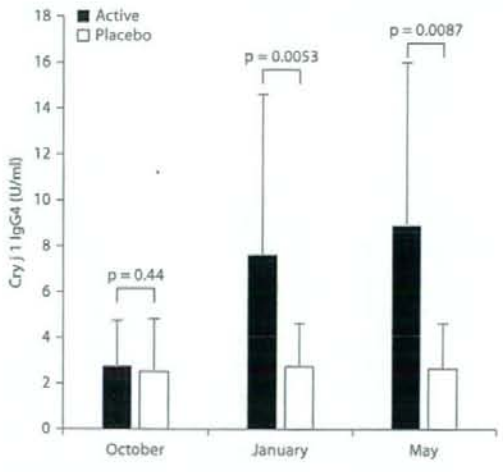


Fig. 3. Levels of serum Cry j 1-specific IgG4 before/after pollen dispersal. Specific IgG4 significantly increased in the active group but not in the placebo group and a significant difference was observed between the groups ($p < 0.05$).

Pollen Counts

In 2006, the Japanese cedar pollen season started in the middle of February and was followed by cypress pollen, which continued until the end of April. The duration of the pollen season extended from 20 February to 27 March. The combined annual amount of cedar and cypress pollen was 1,154/cm^2 according to the Durham pollen sampler in Chiba.

Symptoms

The nasal symptoms and medication scores during the pollen season are shown in figures 1 and 2. The temporal profiles of nasal symptoms and medication scores were in general similar in the active and placebo groups and reflected the pollen counts in the community. However, the scores were lower in the active (treatment) group, especially during the peak of the pollen season as shown in figure 1.

The symptom scores for sneezing, nasal secretion volume, degree of nasal obstruction and medication scores were significantly higher in the placebo group compared to the active (treatment) group ($p < 0.01$) during the peak of the pollen season as shown in figure 2.

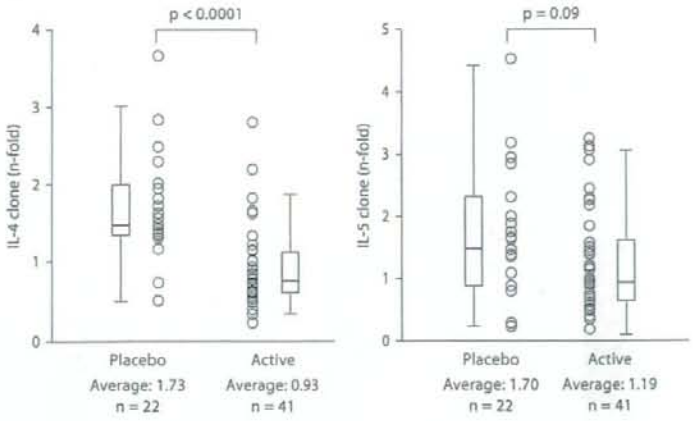


Fig. 4. Relative change of Cry j-specific IL-4 and IL-5 clone sizes in May, after the pollen season, compared with those in January before the season.

Serum Immunoglobulin

There were no significant differences in the 2 study groups for the Japanese cedar pollen-specific IgE and IgG4 levels in the serum samples collected in October, just before SLIT was initiated. On the other hand, after the initiation of immunotherapy, Cry j-specific IgG4 levels exhibited a significant increase in the active group in January before the pollen season. Significantly higher levels of specific IgG4 were observed in the active group for at least 4 months after the initiation of the immunotherapy as shown in figure 3. No significant effects of immunotherapy were detected for the levels of specific IgE (data not shown). No changes in the specific IgE levels were observed relative to the cedar pollen dispersion between January and May. The levels of specific IgG4 also did not exhibit any change after pollen exposure in both the active and placebo groups (fig. 4) and the levels did not correlate with the nasal symptom scores (data not shown).

Th1/Th2 Cytokine Profiles

The number of Th1/Th2 cells in the peripheral blood CD4 T cells did not change significantly and no significant difference was observed between the 2 groups during the study period (data not shown).

Cry j-Specific Th2 Clone Sizes

The number of Cry j-specific IL-4 and IL-5 spots showed a strong correlation. Although the number of spots was similar between the active and placebo groups before the pollen season, a significant increase in IL-4 spots was observed only in the placebo group after the pollen season. The increase in IL-4 clone size during the pollen season in the active and placebo groups was 1.71 $\pm$ 0.71 and 0.70 $\pm$ 0.52 (mean $\pm$ SD), respectively ($p < 0.0001$). On the other hand, the increase in IL-5 clone size between the active and placebo groups was not significant, the power ($1 - \beta$ error) was 0.58 ($p = 0.09$) as shown in figure 4.

Discussion

Although the use of SCIT has been found to be safe for immunotherapy for a variety of pollen allergies, the practical inconvenience associated with its use prompted this study to explore alternative routes of administration. A recent review of randomized controlled studies of SLIT has suggested both its efficacy and safety [16–21]. Although SLIT for Japanese cedar pollinosis is an attractive alternative route, no randomized controlled studies have been carried out to date. The observations of particular importance reported here have shown that the use of SLIT significantly increased the levels of pollen-specific IgG and downregulated the size of pollen-specific Th2 lymphocyte subset clones.

In order to avoid adverse effects, such as local pain and swelling associated with injection and possible anaphylactic reactions, a dose of 40 JAU/month as a maintenance dose has generally been utilized in SCIT for Japanese pol-

linosis. In this study 1,000 JAU/week (4,000 JAU/month) was used as a maintenance dose in SLIT, which was 100 times more than that used in SCIT. The choice for such a dose was somewhat arbitrary and the optimal dose required for effective and safe use of SLIT remains to be determined.

The combined Japanese cedar and cypress pollen counts generally exceed 3,000/cm^2, as measured by the Durham pollen sampler in Chiba and Tokyo. However, in 2006 the pollen counts were 1,154/cm^2, which was one third of the average for the last 5 years. In Japan, the pollen counts and the counts/cm^2 are usually measured by the Durham samplers, which utilize a gravimetric method that is different from the Burkard sampler, a volumetric method that is widely used in European countries. Direct comparison of the counts by the 2 methods can be difficult, because the ratio between the 2 methods depends on the local meteorological conditions and the types of pollen. When these methods were compared in 2005, the counts obtained by the Burkard sampler were about 12 times higher than those obtained by the Durham sampler [28].

During SLIT, no adverse effects greater than Common Terminology Criteria for Adverse Event grade 3 were observed. Three months after SLIT, serum Cry j 1-specific IgG4 was elevated in the active group. However, the specific IgE levels were not significantly different between the groups.

Several previous studies employing SLIT have observed an increase in allergen-specific IgG4 levels and specific IgG4/IgE ratios in the serum [29, 30]. However, the precise role of increased IgG4 in the effectiveness and outcome of such immunotherapy remains to be determined. Lima et al. [31] reported that the IgG levels correlated with the clinical efficacy as a blocking antibody, but other studies have failed to demonstrate such a correlation [32]. The increased levels of Cry j 1-specific IgG4 antibody in this study indicate that SLIT can induce specific antibody responses. However, the role of IgG4 antibody in the mechanisms of clinical effectiveness remains to be defined. No relationship between the IgG4 responses and the clinical efficacy was observed in this study.

In the present studies, the use of SLIT was associated with milder clinical symptoms and lower medication scores during the pollen season and a significant reduction in each symptom was observed. As pointed out earlier, the doses in this SLIT study were much higher than those generally used in SCIT. However, in SLIT with other allergens, the clinical efficacy has been shown to be allergen dose dependant [33]. Although the swallow-SLIT method is currently widely used, we selected the spit-SLIT method to further reduce the possibility of adverse effects, since no SLIT trials with Japanese cedar pollinosis have been carried out to date. Further studies will be needed to assess the dose responses, temporal intervals and vehicles of administration to obtain optimal effectiveness with SLIT.

Cedar pollen-specific IgE and IgG4 did not increase significantly with pollen exposure, which might be explained in part by the relatively small amount of pollen dispersal observed during these studies.

The total number of Th2 cells in the peripheral blood did not increase during the pollen season and Th1/Th2 cytokine profiles did not change throughout the study in either group. However, the profiles of the allergen-specific Th cells were quite different. The patients with cedar pollinosis are thought to have cedar pollen-specific memory Th cell clones and the treatment is aimed at diminishing the size of Th2 clones. Since the Th cell response is restricted in MHC class 2, it is necessary to use a class 2 restrictive T cell epitope to measure the reaction of T cell clones in response to the allergen. Japanese cedar-specific IL-4 and IL-5 producing memory T cell in the peripheral blood were examined by an ELISPOT assay using Japanese cedar pollen-specific peptides. Although the number of cedar peptide IL-4 and IL-5 T cells was low, all patients exhibited specific spots, ranging from 5 to 100 spots/10^5 PBMCs. The number of cedar pollen-specific Th2 cells did not correlate with the cedar pollen-specific serum IgE nor IgG4 levels. This may be related to the possibility that IgE and IgG4 synthesis is controlled by many other factors, including Th1 cells and memory B cells.

The size of the cedar pollen-specific Th2 cell clones was not different between the active and the placebo groups before the pollen season. Interestingly, these Cry j-specific Th2 clone sizes were increased about 1.7-fold during the cedar pollen season by pollen exposure in the placebo group. However, this increase was not observed in the active group and SLIT suppressed the increase in specific Th2 clone sizes. The change in the clone size did not correlate with the levels of allergen-specific IgG4 antibody.

Several recent studies have explored the significance of regulatory T cells in allergic and autoimmune disorders [34–37]. The suppression of allergen-specific Th2 clones observed in this study may be a reflection of such regulatory T cells, although the precise contribution of different T cell subsets remains to be examined.

In summary, this study has demonstrated that SLIT for Japanese cedar pollinosis was safe and is associated with increased cedar pollen-specific IgG4. Such therapy also inhibited the increase in specific Th2 lymphocyte clone sizes induced by the exposure to cedar pollen. It is also suggested that the use of SLIT appears to be an acceptable alternative to SCIT for Japanese cedar pollinosis.

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ORIGINAL ARTICLE

A Randomized Double-Blind Comparative Study of Sublingual Immunotherapy for Cedar Pollinosis

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ABSTRACT

Background: Seasonal allergic rhinitis (SAR) induced by Japanese cedar pollen is a substantial problem in Japan. Sublingual immuno-therapy (SLIT) is safer than conventional antigen-specific immunotherapy, the only treatment modality by which complete cure of the disease can be expected. We investigated the safety and efficacy of SLIT in the treatment of cedar pollinosis patients compared to placebo.

Methods: A randomized, placebo-controlled, double-blind study was conducted in 61 cedar pollinosis patients. Increasing doses of standardized Japanese cedar extract or placebo were administered sublingually in intervals ranging from daily to once a week after six weeks. The primary efficacy variable was the mean of the daily total symptom scores (TSS) during the pollen dispersing period. Secondary efficacy variables included the QOL scores and related variables.

Results: Primary efficacy variable scores were significantly lower for some days in the SLIT group than in the placebo group ($P < .01$ or $P < .05$). Secondary efficacy for the QOL score in SLIT group was almost of half of placebo group. There was no significant difference in the overall incidence of side effects between the SLIT group and the placebo group.

Conclusions: SLIT was effective and safe in the treatment of cedar pollinosis.

KEY WORDS

Japanese cedar, placebo-controlled study, QOL, seasonal allergic rhinitis

INTRODUCTION

In agreement with the results of worldwide epidemiological assessments, the number of patients with allergic rhinitis such as Japanese cedar (JC) pollinosis in Japan is increasing.[1] Okuda considers that the current prevalence of allergic rhinitis is 16%, but many researchers predict that the rate will still increase.[2] Pollinosis is a typical type I allergy in which allergic conjunctivitis and allergic rhinitis develop. In spite of its refractory nature, pollinosis deteriorates patient QOL only in severe cases; however, it greatly affects the patient's life in general in that they must keep working even if the condition is severe.[3] Many of the patients with cedar pollinosis have also been sensitized to cypress pollen which disperses after cedar pollen. Consequently, symptoms of cedar pollinosis are followed by those of cypress pollinosis; patient symptoms last, though they are seasonal, for as long as 4 months (from February to May).

Pharmacological therapy prescribed by general practitioners is common for the treatment of the disease. Both oral medications and topical medication, however, are symptomatic treatment; they do not cure the disease or remain effective until the following year.[4] Antigen-specific subcutaneous immuno-

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therapy (SCIT) is the only treatment modality by which complete cure of the disease can be expected.[5] WHO position paper stipulates the use of standardized antigen and the concentration of the antigen to be maintained.[6] The efficacy of the therapy has been proven in placebo-controlled, double-blind comparative studies using pollen, house dust mite, and animal protein.[6,7] In Japan, it is customary to start the administration of causative antigen extract by subcutaneous injection at the threshold of skin reaction or its 10-fold diluted concentration, and to increase the dose gradually.[4] Treatment with SCIT requires special attention because it may cause, as a side effect, anaphylactic shock, which prevents the therapy from becoming popular in Japan.[8] In order to reduce the possibility of this side effect, immunotherapy is administered by other routes (sublingual, intranasal, oral, and transbronchial) in Europe and the United States, and has achieved desired outcomes.[9-11] Especially, sublingual immunotherapy (SLIT) has become popular in Europe considerably, and there are many reports supporting the effectiveness of the therapy.[11-13] As for side effects due to SLIT, there are no reports of anaphylactic shock, but oral itching, skin reaction (such as urticaria), and mild asthma-like attacks have been reported.[13] Since cedar pollinosis greatly deteriorates patient QOL, many physicians and patients will opt for immunotherapy if it is proven to be safe. We conducted a randomized, placebo-controlled double-blind comparative study to investigate whether SLIT reported in Europe and the United States is effective for the treatment of JC pollinosis and whether it can be performed safely.

STUDY DESIGN

This multi-centre, double-blind, randomized, placebo-controlled, parallel-group study was conducted in six centers across Japan between October 2004 and April 2005. The study protocol was approved by the appropriate local ethics committees, and the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients provided written informed consent prior to participation.

SUBJECTS

Patients with JC pollinosis were enrolled in this study if they had a RAST score of 2 against JC or above and pollinosis symptoms during the cedar pollen dispersal period at least in the past 2 years and if they had visited any of the following medical institutions: Department of Otorhinolaryngology, Nippon Medical School; Department of Otorhinolaryngology, University of Fukui; Department of Otorhinolaryngology, Head and Neck Surgery, Okayama University; Department of Otorhinolaryngology, Dokkyo University School of Medicine; Department of Otorhinolaryngology, University of Yamanashi; and Department of Otorhinolaryngology, Head and Neck Surgery, Chiba University. Patients who had nose diseases (perennial allergic rhinitis, nasal septum deviation, or sinusitis) which may interfere with accurate symptom assessment were excluded from the study. Patients receiving treatment for conditions such as severe cardiac disease and malignant tumor were also excluded. As a result, a total of 61 patients were blindly randomized either to the active group or the placebo group in the ratio of 2 active to 1 placebo.

METHODS

The study was initiated in October, 2004. Patients were assigned and randomized to either the active group or the placebo group. Cedar antigen extract (active group) at concentrations of 2 to 2000 JAU/ml diluted with diluent (made by Torii Pharmaceutical Co., Ltd.) and diluent alone (placebo group) were used in eye drop containers (made by Hirakata Plastic).

Administration of the antigen extract was started at 2 JAU/ml, which is considered a sufficiently safe level, and was increased to the final maintenance concentration of 2000 JAU/ml. Active drug was administered as follows: 1 drop (about 50 μl) to 20 drops (about 1 ml) of prepared extract was dropped onto bits of bread (about 1.5 cm × 1.5 cm × 1.5 cm), which were held sublingually for 2 minutes and then expectorated. The treatment schedule was as follows: antigen extract was administered sublingually daily from Week 1 to Week 4; 20 drops of the antigen extract 2000 JAU/ml were administered two days per week in Week 5, once per week in Week 6 and thereafter throughout the season (Table 1).

Patients experiencing pollinosis symptoms in cedar and cypress pollen dispersal periods received symptomatic treatment with medications such as antihistamines on an as needed basis; such patients were asked to record the date of treatment in their allergy diary.

ENDPOINTS

The patients were instructed to fill in their allergy diary from February 22, 2005 to April 6, 2005, the period when cedar and cypress pollen dispersed in 2005, and they were also asked to fill in QOL questionnaire once a month during the same period. Symptoms recorded in the allergy diary (sneezing, runny nose, nasal congestion, and interference with daily life), the total nasal symptom scores calculated based on each symptom, sneezing, runny nose, nasal congestion (none; 0, mild; 1, moderate; 2, severe; 3), and symptom medication scores (antihistamine; 1, topical steroid; 2, general steroid; 3) were calculated. The Japanese Allergic Rhinitis QOL Standard Questionnaire No.1 (JRQLQ No1) was used for the assessment of the QOL of patients with allergic rhinitis (Fig. 1). Nasal and Ocular symptom scores, QOL-

Table 1 Allergen administration schedule (Increasing dosing)

	Week 1 (2 JAU/ml)	Week 2 (20 JAU/ml)	Week 3 (200 JAU/ml)	Week 4 (2000 JAU/ml)	Week 5 (2000 JAU/ml)
Day 1	1 drop	1 drop	1 drop	1 drop	20 drops
Day 2	2 drops	2 drops	2 drops	2 drops	
Day 3	3 drops	3 drops	3 drops	4 drops	
Day 4	4 drops	4 drops	4 drops	8 drops	
Day 5	6 drops	6 drops	6 drops	12 drops	20 drops
Day 6	8 drops	8 drops	8 drops	18 drops	
Day 7	10 drops	10 drops	10 drops	20 drops	

Initial dose of SLIT for JC pollinosis was 1 drop of 2 JAU/ml of standardized JC allergen, and the administrating dose is increased up to 20 drops of 2000 JAU/ml at 4th week, the maintenance dose.

Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ No.1)

To patients with allergic rhinitis (including pollinosis)
These days, the aim of medical treatment is not just to cure disease but also to give patients a better quality of life. The purpose of this survey is to determine to what extent your rhinitis interferes with your life and whether it would be improved by treatment. As with all medical treatment, the information you provide in this survey will remain strictly confidential.

You may find some of the following questions difficult to answer, but just answer to the best of your ability.

/ Tick the box that best describes the severity of the worst nasal and eye symptoms you have experienced in the past 1–2weeks.

Nasal and eye symptoms	0. No	1. Mild	2. Moderate	3. Severe	4.Very severe
Runny nose	☐	☐	☐	☐	☐
Sneezing	☐	☐	☐	☐	☐
Blocked nose (nasal congestion)	☐	☐	☐	☐	☐
Itchy nose	☐	☐	☐	☐	☐
Itchy eyes	☐	☐	☐	☐	☐
Watery eyes	☐	☐	☐	☐	☐

// Tick the box that best describes the worst extent to which the symptoms in / above have interfered with your quality of life in the past 1–2 weeks. If any of the items listed under Quality of life below definitely do not relate to the symptoms in / (nose, eyes), then there is no need to tick a box for that particular item.

Quality of life	0. No	1.Yes, slightly	2.Yes, moderately	3.Yes, severe	4.Yes, very severe
1. Reduced at work/home	☐	☐	☐	☐	☐
2. Poor moral concentration	☐	☐	☐	☐	☐
3. Reduced thinking power	☐	☐	☐	☐	☐
4. Impaired reading book/newspaper	☐	☐	☐	☐	☐
5. Reduced memory loss	☐	☐	☐	☐	☐
6. Limitation of out of life (e.g. sport, picnics)	☐	☐	☐	☐	☐
7. Limitation going out	☐	☐	☐	☐	☐
8. Hesitation friends or relatives	☐	☐	☐	☐	☐
9. Reduced contact with friends or others by telephone or conversation	☐	☐	☐	☐	☐
10. Not an easy to be around	☐	☐	☐	☐	☐
11. Impaired sleeping	☐	☐	☐	☐	☐
12. Tiredness	☐	☐	☐	☐	☐
13. Fatigue	☐	☐	☐	☐	☐
14. Frustration	☐	☐	☐	☐	☐
15. Irritability	☐	☐	☐	☐	☐
16. Depression	☐	☐	☐	☐	☐
17. Unhappiness	☐	☐	☐	☐	☐

/// Please circle the number of the face that best describes your general state (including your symptoms, life and emotion) in the past 1–2 weeks.

Do not fill out the following

To be completed by physician

Patient's name: Medical record to: Age: yr Sex: M F

Name of medical institution: Physician's name: Date:

Diagnosis:
SAR (Antigen) Treatment [prevention,drug,immunology,therapy,operation]
PAR (Antigen) Treatment [prevention,drug,immunology,therapy,operation]
Non-Allergy: Disease: ( ) Treatment: ( )

QOL score: None 0 , Mild 1 , Moderate 2 , Severe 3 , Very severe 4
Total QOL score
Score by QOL category 1–5 points daily life 6–7 points out-door
8–10 points social 11 points sleep
12–13 points body 14–17 points psyco-life

Please write the names of drugs used it possible

Score: None: 0 points Mild: 1 point Moderate: 2 points
Severe: 3 points Very Severe: 4 points

Fig. 1 Japanese Allergic Rhinitis QOL Standard Questionnaire No.1 (JRQLQ No1).

related questionnaire scores, and the overall face scale were calculated and statistically analyzed. In other words, the QOL deterioration score was calculated by subtracting QOL-related questionnaire scores recorded in February (i.e. at baseline) from the scores recorded in the middle of March to April, when the largest amount of pollen dispersal was observed.

STATISTICAL ANALYSIS

Symptom scores, total symptom scores, and symptom medication scores calculated from the allergy diary in the placebo group and the active group were analyzed by non-paired t-test and the Chi-squared test

Table 2 The background of the subjects

Items	Placebo $n = 22$	Active $n = 37$	p value (*)
Age	40.14±15.30	40.65±15.14	0.901
Sex			
Male	7 (31.8%)	18 (48.6%)	
Female	15 (68.2%)	19 (51.4%)	
Nasal and eye symptoms	0.62±0.54	0.43±0.35	0.169
QOL-related questionnaire	0.25±0.29	0.21±0.25	0.568
Usual daily activities	0.13±0.25	0.11±0.32	0.762
Outdoor activities	0.14±0.48	0.21±0.46	0.630
Social functioning	0.05±0.22	0.08±0.23	0.648
Sleep disturbance	0.14±0.36	0.09±0.29	0.537
Physical problems	0.17±0.33	0.24±0.46	0.510
Emotional function	0.12±0.23	0.11±0.32	0.953
Overall face scale	1.14±0.73	1.09±0.74	0.780

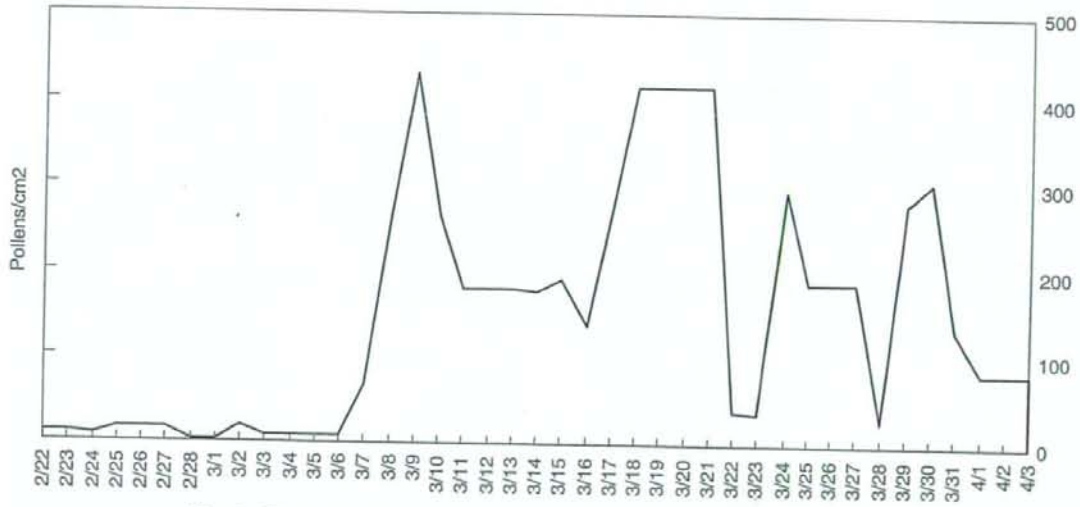


Fig. 2 The changing the number of cedar and cypress pollen dispersals in 2005.

using SPSS 11.0J. QOL-related questionnaire score of the 2 groups were compared using analysis of covariance (ANCOVA).

RESULTS

Of the 61 randomized patients, there were 2 dropouts for those whose treatment was unknown; there were 37 patients in the active group and 22 patients in the placebo group. In the analysis of allergic symptoms, 2 patients whose outcome was available only in the form of a diary were excluded, and the results of 36 patients in the active group and 21 patients in the placebo group were analyzed. In the analysis of QOL, 3 patients were excluded because baseline assessment was unavailable, and the results of 35 patients in the active group and 21 patients in the placebo group were analyzed.

As shown in Table 2, no difference was observed between the two groups in terms of patient characteristics (sex was analyzed by the Chi-squared test, and other items were analyzed by t-test).

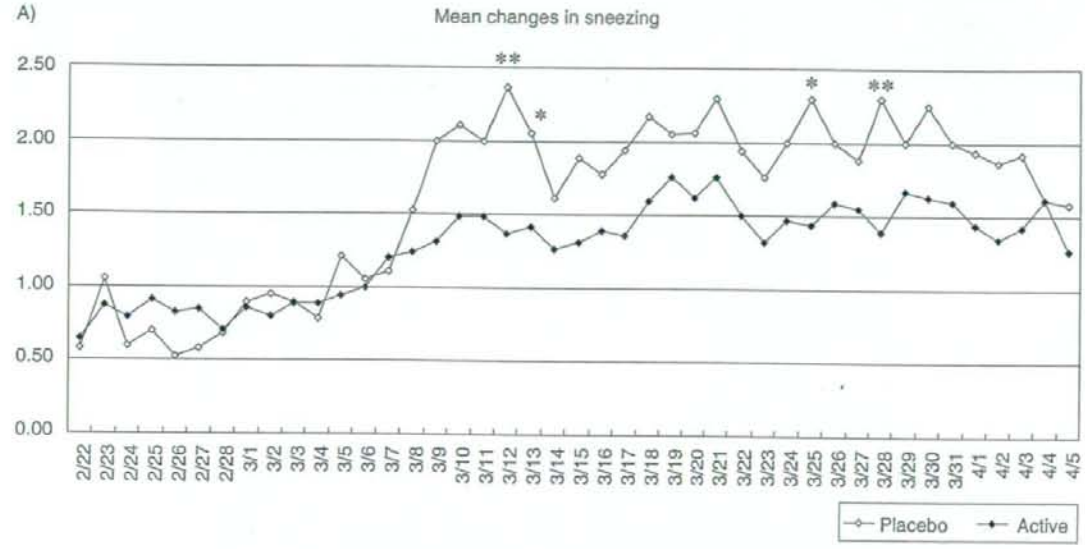
In 2005, the number of cedar and cypress pollen dispersals observed was the largest during the 10-year period since 1995. According to the data of the Chiyoda ward—the area nearest to the Nippon Medical School—announced by the Tokyo Metropolitan Government, the first pollen dispersal was observed on February 22, which was about the same time as in the past years, and an average of 10,625 pollens per square centimeter by the Durham method were ob-

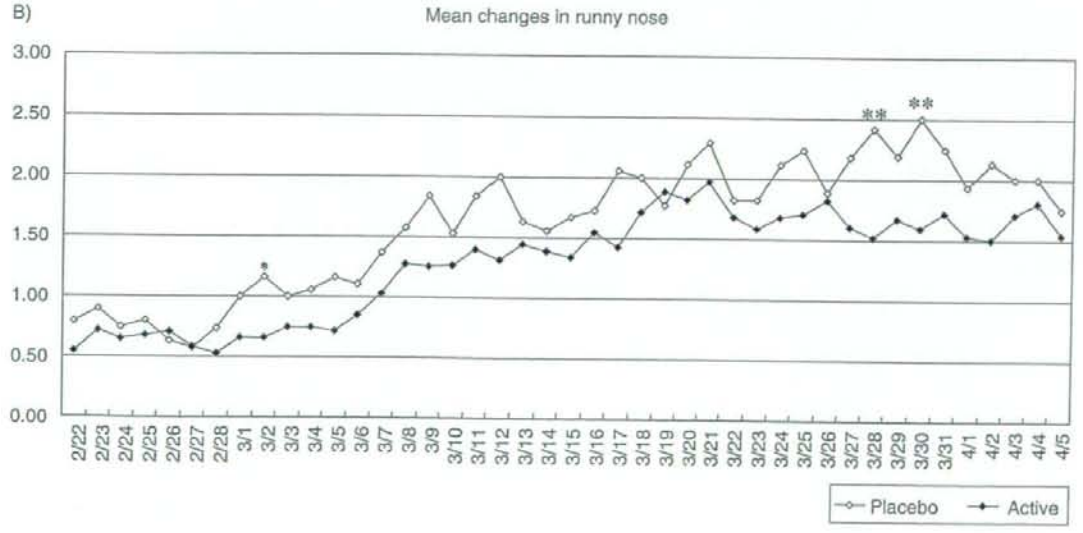
served during the season (Fig. 2). The number of the cedar and cypress pollens by the same method observed in each institution was 3424, 2383, 16002, 5859 and 7752 for University of Fukui, Okayama University, Dokkyo University, University of Yamanashi and Chiba University respectively, and these pollen numbers were also largest dispersing during the last ten years at any place.

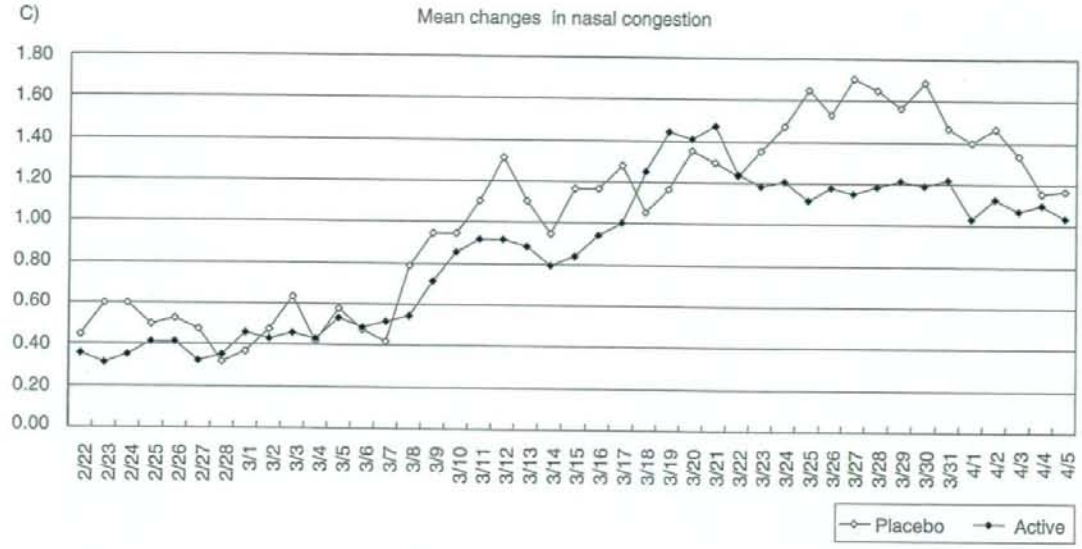
Symptom scores for sneezing (Fig. 3A) and runny nose (Fig. 3B) in the active group were significantly better than those in the placebo group on 4 days and 2 days, respectively, but no difference was observed between the active group and the placebo group in terms of nasal congestion (Fig. 3C). Between the 2 groups, there was no difference in the number of medications used during the season (Fig. 3D).

The active group had a significantly lower total symptom score (Fig. 4A) and symptom medication score (Fig. 4B) on 4 days during the season. Overall, better outcomes were observed in the active group during the latter half of the season (i.e. from the end of March to the beginning of April), which roughly overlaps the period when the largest amount of cedar and cypress pollen was dispersed.

In the placebo group, the nasal and ocular symptom score was 1.15, the QOL-related questionnaire







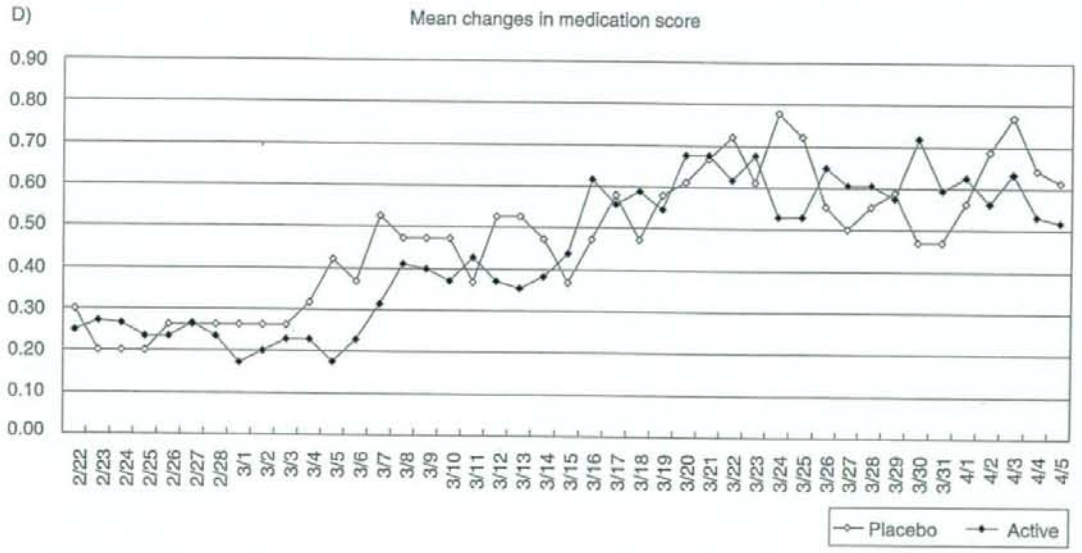


Fig. 3 The mean changes in each symptom in the season of 2005. **A)** Mean changes in sneezing. **B)** Mean changes in runny nose. **C)** Mean changes in nasal congestion. **D)** Mean changes in medication score. The open square indicates the placebo group, the filled square indicates the active group. Significant difference was evaluated as * $p < 0.05$; ** $p < 0.01$.

score was 1.10, and the overall face scale score was 1.24; in the active group, the nasal and ocular symptom score was 0.92, the QOL-related questionnaire score was 0.58, and the overall face scale score was 1.03: the deterioration score in the QOL-related questionnaire in the active group was only about half the score in the placebo group (Fig. 5A). In each domain of QOL question items, deterioration in usual daily activities, outdoor activities, social functioning, sleep problems, general physical problems, and emotional function in the active group was only about half the score in the placebo group as well. The *p*-values for the above domains were 0.089, 0.086, 0.067, 0.060, 0.083 and 0.046; a significant difference was observed only in emotional function (Fig. 5B).

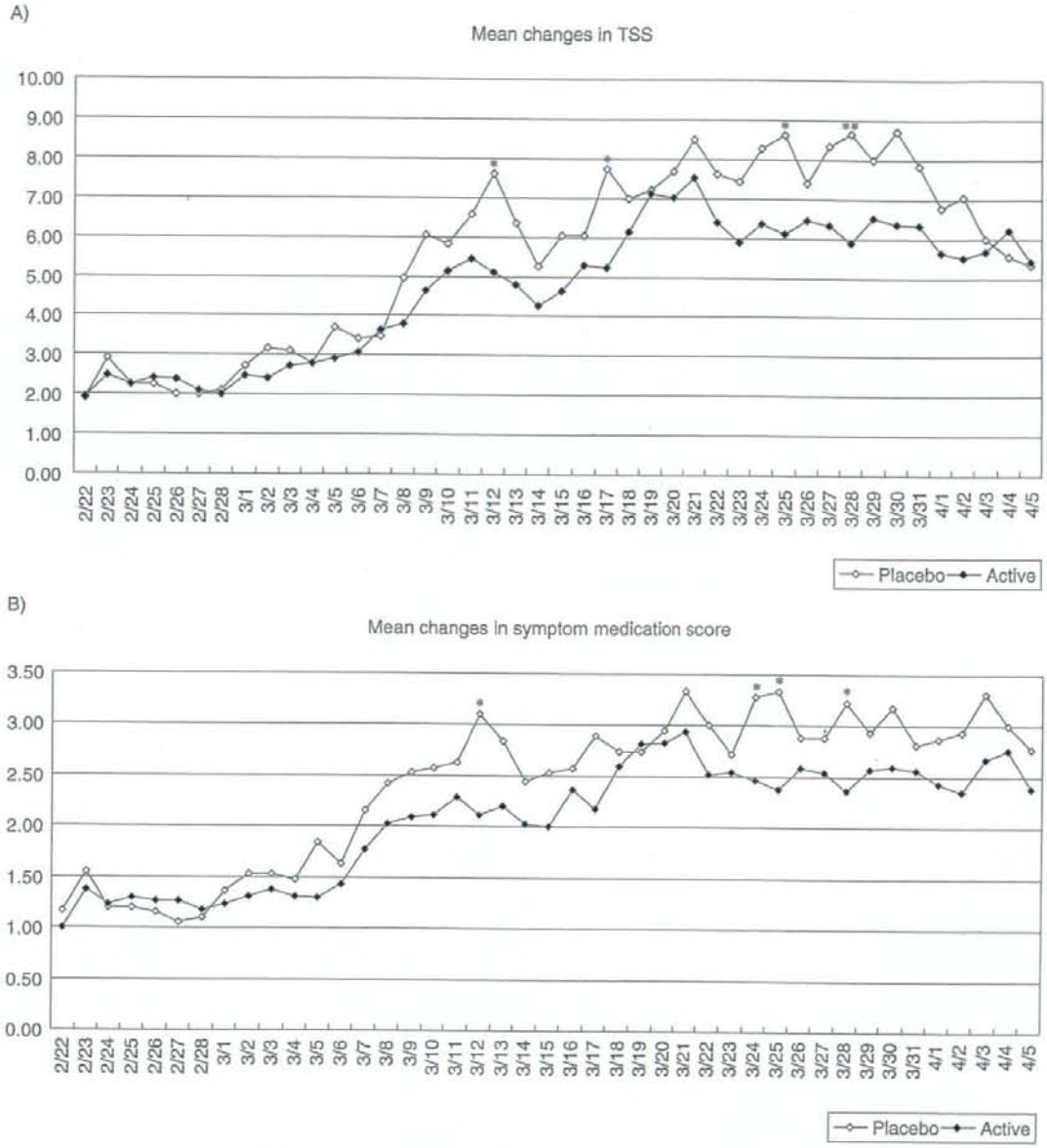


Fig. 4 The mean changes in **A**) the total symptom score (TSS) and **B**) symptom medication score in the season of 2005. Significant difference was evaluated as * $p < 0.05$; ** $p < 0.01$.

SIDE EFFECTS

No systemic side effect occurred during SLIT. Local side effects occurred in six volunteers in the active group. Mild mouth itching was exhibited in all six volunteers in increasing dose up to 2000 JAU 1 ml, however this itching was diminished for two or three times just after allergen administration. All six volunteers finished this study totally without any change of this protocol.

DISCUSSION

Approximately 16% of the Japanese population are affected by Japanese cedar pollinosis[2] and the proportion of severe status patients is higher than with

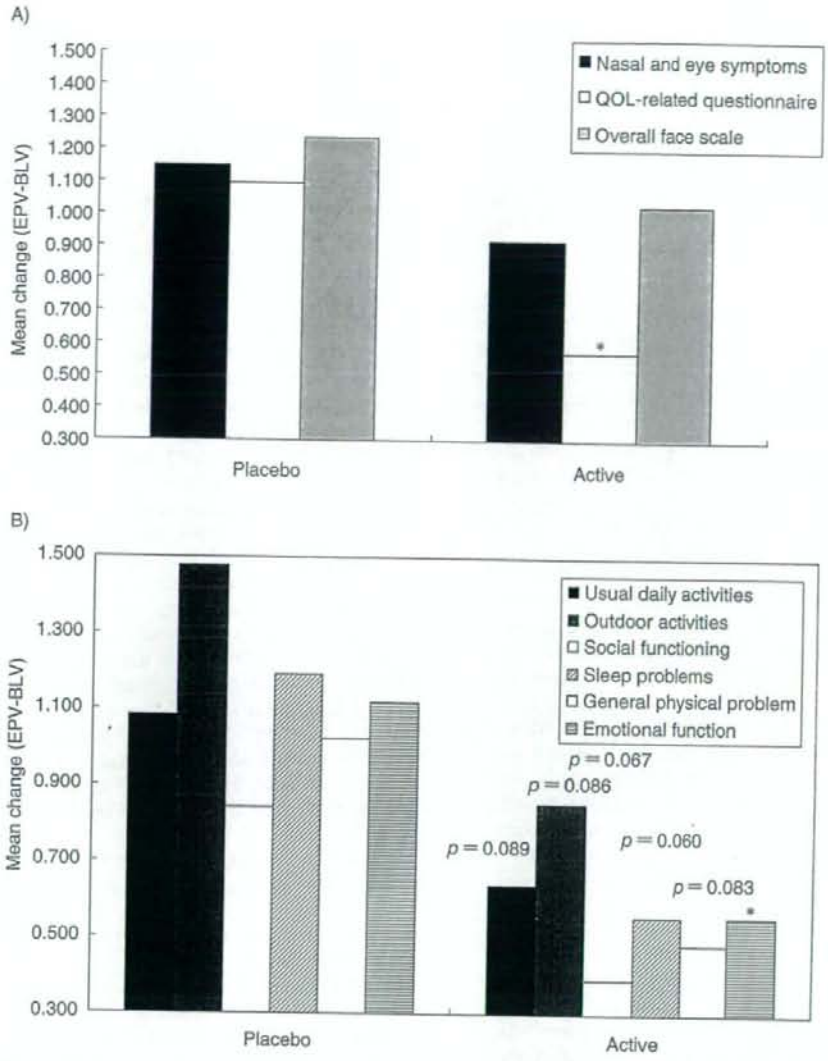


Fig. 5 The mean changes of **A)** QOL scores (nasal and eye symptoms, QOL related questionnaire, overall face scale) and **B)** each domain of QOL question items deterioration (in usual daily activities, outdoor activities, social functioning, sleep problems, general physical problems, and emotional function) from baseline data of February to peak data of peak pollen scattering period. Difference between placebo and active indicates * $p < 0.05$ (analysis of covariance, ANCOVA). Placebo: $n = 21$, Active: $n = 35$.

grass or ragweed pollinosis, which are the representative conditions in other countries, and the symptoms persist for about 3 months, becoming a social issue. When the amount of pollen increases, patients show more severe symptoms, and the number of severe status patients is greatest in mid-March (late season) when the pollen count reaches its peak. Substantial antigen exposure enhances the antigen-antibody reaction in the airways (airway hypersensitivity), which is the mechanism involved in severe pollinosis, and SCIT may control the exacerbation of the symptoms in the latter half of the cedar pollen season by inhibiting antigen-related enhancement of nasal mucosal hypersensitivity.

As shown in the WHO position paper, the effects of immunotherapy in the treatment of pollinosis have

been substantiated in many double-blind comparative studies.[6] However, the therapy tends to be avoided in Japan because of factors such as the current high cost, the complicated procedure involved, and possible side effects. In Japan, owing to these disadvantages and the fact that the department of allergy has not been widely established in medical institutions, pharmacological therapy is the mainstream modality for the treatment of pollinosis. Still, immunotherapy is an important modality for the complete cure of allergic diseases.

The efficacy of our SLIT was not demonstrated based on patient allergy diaries. However, the quality of life (QOL) score was approximately 1/2 of that in the placebo group, with a significant difference. In addition, a *P*-value corresponding to a significant difference was obtained in each QOL domain. In the mental health domain, there was a significant difference. Assessment using the Japanese guidelines differs from that in other countries; even a single sneeze is regarded as (+). In other countries, 4 grades (none, mild, moderate, and severe) are employed for assessment, and the presence or absence of symptoms is not evaluated. For this reason, the usefulness of SLIT may not have been demonstrated based on diaries. However, the QOL is evaluated via self-assessment, which is consistent with the system for the self-reporting of symptoms in other countries (none to severe). Therefore, QOL assessment of SLIT was favorable, and was consistent with the reduction rates in other countries. According to the JRQLQ criteria, the reduction rate for nasal/ocular symptoms was 22%, consistent with the evaluation of SLIT in other countries. In the future, the JRQLQ criteria, which were designed in reference to overseas self-assessment, may be essential for evaluating drug efficacy and such a novel treatment. This finding is suggestive of the fact that the QOL questionnaire developed in Japan is of good quality,[14] and that SLIT is effective for preventing QOL deterioration in patients with pollinosis rather than for lowering their symptom score. Placebo effects of SLIT may be present. However, it was evaluated in 2005, when the amount of scattered pollen was highest over the past 10 years. In addition, considering that the study involved a placebo-controlled design, we can conclude that SLIT was effective for cedar pollinosis in Japan. In evaluating the treatment response, we cannot rule out the influence of Japanese cypress pollen scattering. However, in a study excluding Japanese cypress pollen-positive reacting patients, the efficacy of SLIT and reduction rate for symptoms were also similar (unpublished data). This maybe caused by the combination of a large amount of JC and a small amount of cypress that was dispersed in 2005. These types of pollinosis should be regarded as JC/Japanese cypress pollinosis, as their seasons are sequential in the near future. In addition, a Japanese cypress pollen antigen for immunotherapy must be prepared. It should be considered that symptoms of cedar/Japanese cypress pollinosis in April are associated with cedar pollen scattering-related nasal mucosal/conjunctival inflammation, not with Japanese cypress pollen scattering alone.

Less side effects including problematic anaphylaxis are noted in SLIT although the side effects observed cannot be theoretically complete anaphylactic shock when comparing the therapy administered via injection with sublingual route.[15] Similar to the oral allergy syndrome (OAS), which is the focus of public attention, the development of symptoms such as strange feelings, oral itching, and swelling were feared because the antigen remains in the oral cavity; however, itching was the only reaction observed so far. The results obtained from the study of tentative SLIT, which was performed exclusively in the Department of Otorhinolaryngology, Nippon Medical School, were roughly consistent with the results of similar studies conducted every year thereafter, including the results of the study in 2005,[16] In our study of SLIT for the treatment of cedar pollinosis, symptom medication score was consistently lower than that of the pharmacological therapy group throughout the pollen dispersal season. The finding indicates that patients receiving SLIT tend to use fewer drugs, which is consistent with the results of a double-blind comparative study using a placebo,[17] SLIT, which is as effective as pharmacological therapy and decreases the amount of drug use, is considered advantageous also in the current medical economy in Japan.

The mechanism of action for SLIT, or for conventional SCIT, is still unclear, but for SCIT, reduction of effector cells[18,19] and blocking antibody[20-23] have been the conventional theories. Recently, however, it has become widely accepted that immunotherapy may modify the T cell response to natural allergen because of T cell anergy and/or immune deviation.[24-27] For SLIT in particular, allergen administered to the oral mucosa accumulates in the submandibular lymph node, in which the immune response occurs[28] and peaks at approximately 2 hours after administration.[29] In our investigation, an increase in the Stimulatory Index in PBMC during the early phase of SLIT conducted in 1999 shows at least that systemic immune induction was caused by sublingually administered antigen.[30] In SLIT, it is intended to cause fewer side effects than SCIT injection by decreasing systemic effects. However, it has become clear that the therapy also leads to systemic immune induction, which is greatly different from conventional topical immunotherapy administered intranasally or orally.

In the present study, SLIT both inhibited the exacerbation of symptoms in the latter half of the season and reduced their severity throughout the season. Furthermore, there were neither local nor systemic

side effects, as reported elsewhere for other antigens. SLIT for cedar pollinosis is a new therapy and in the future SLIT may by indicated for patients with nasal allergy caused by other allergens such as house dust mites or animal dander through improvement of the administration schedule and establishing the dose at which the most potent effects are achieved. This study may contribute to the methodology for the future immunotherapy in Japan.

The development of this SLIT in Japan is in progress as a multi-center study conducted as part of the research project on the prevention and treatment of immunological and allergic diseases (H17-immunology-common-001) entitled "Evaluation research of the relationship between the number of dispersed pollen observed by real-time monitoring and QOL achieved by the current treatment modalities, and the development of definitive treatment for pollinosis", which is supported by Health and Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare.

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