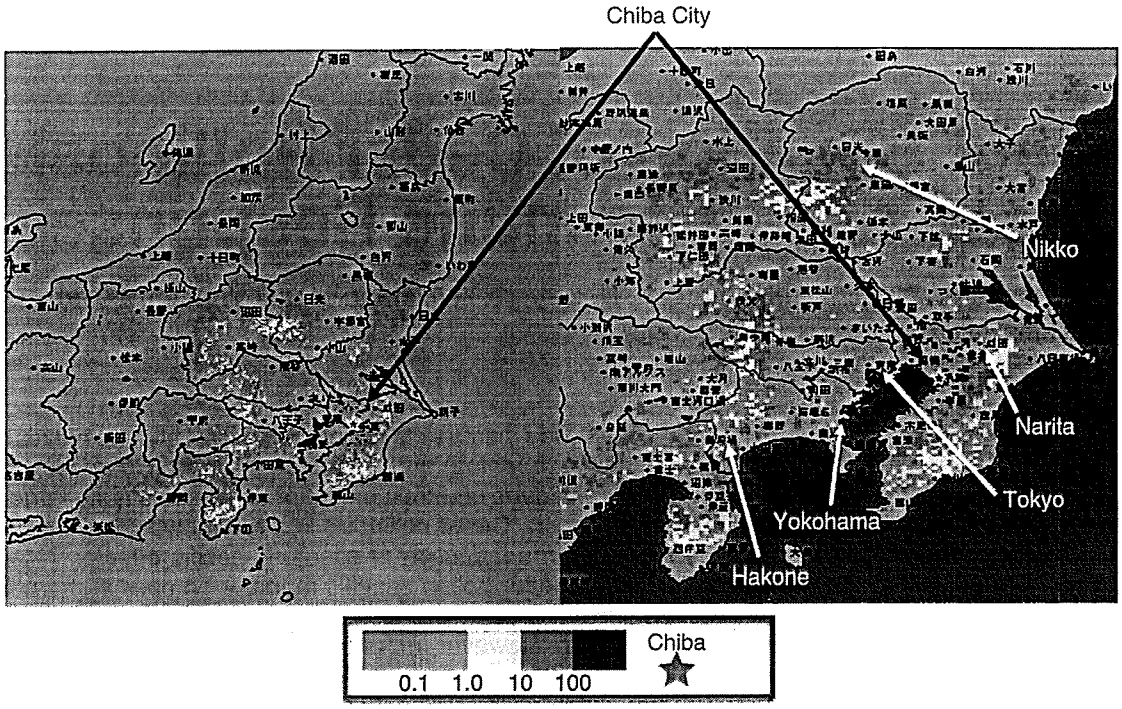

Fig. 1 The source areas from which the cedar pollen detected at Chiba University Hospital spread. This is the computer simulation study done by Mr. Kunihiko Yokota *et al.*, at Weather Service Co.,Ltd..

lowed by dispersal of Japanese cypress pollen, which reaches a peak from late March to early April, with some variation due to changes in the climate each year.[7,8] The pollen dispersal season lasts for more than 10 weeks in and around the Tokyo area.

PREVALENCE OF CEDAR POLLINOSIS IN JAPAN

A survey based only on a questionnaire has the risk of inclusion of a high rate of false-positive cases, because allergic rhinitis is sometimes difficult to distinguish from acute upper respiratory infection and even normal healthy individuals may exhibit mild, nonspecific nasal symptoms, such as sneezing and nasal secretion. In particular, cedar pollen dispersal season is also high flu season. An allergen-specific IgE test is necessary to avoid a high incidence of false positives, but it has been difficult to conduct an epidemiological study in Japan because of laws preventing use of personal information. In 2008, a questionnaire was posed to the Otorhinolaryngologists nationwide to determine whether their families suffered from allergic rhinitis. Although the rate of return of the questionnaire was low, i.e., 40% and the bias of the population could not be ignored, an accurate diagnosis was expected.

According to the analysis of this questionnaire,[9] the prevalence of perennial allergic rhinitis and of cedar pollinosis was 23.4% and 26.5%, respectively. In particular, the prevalence of cedar pollinosis increased more than 10% compared with that observed in a similar questionnaire conducted in 1998. Although the peak of cedar pollinosis is in those in their thirties to forties, the age onset of pollinosis has been decreasing (Fig. 2).

Figure 3 shows the annual amount of cedar pollen dispersal in Japan, which we examined in 2005. The darker brown parts indicate areas where cedar pollen counts were high. We studied the influence of various amounts of pollen exposure on the development of pollinosis and mite allergic rhinitis in elementary school students from schools in rural areas where the movement of students out of or into the school was uncommon. The annual amount of cedar and cypress pollen differed among these five regions. The pollen level was very high in southern Yamanashi: about 7,000/cm^2 on average for the last five years, as determined using Durham pollen samplers. In contrast, the pollen level was low in northern Yamanashi and inland Akita, at about 2,000/cm^2, and very low in coastal Akita, at about 500/cm^2. The pollen level in Chiba was about 4,000/cm^2.

Figure 4 shows the detection rate of cedar- and mite-specific IgE in students in these regions. The positive rate for Japanese cedar was about 60%, except for students in coastal Akita, who had a rate of only 23%. The positive rate for mite IgE was about 50% in each region. These results suggest that the sensitization rate for mite allergen is almost the same nationwide, whereas that for cedar pollen is depend-

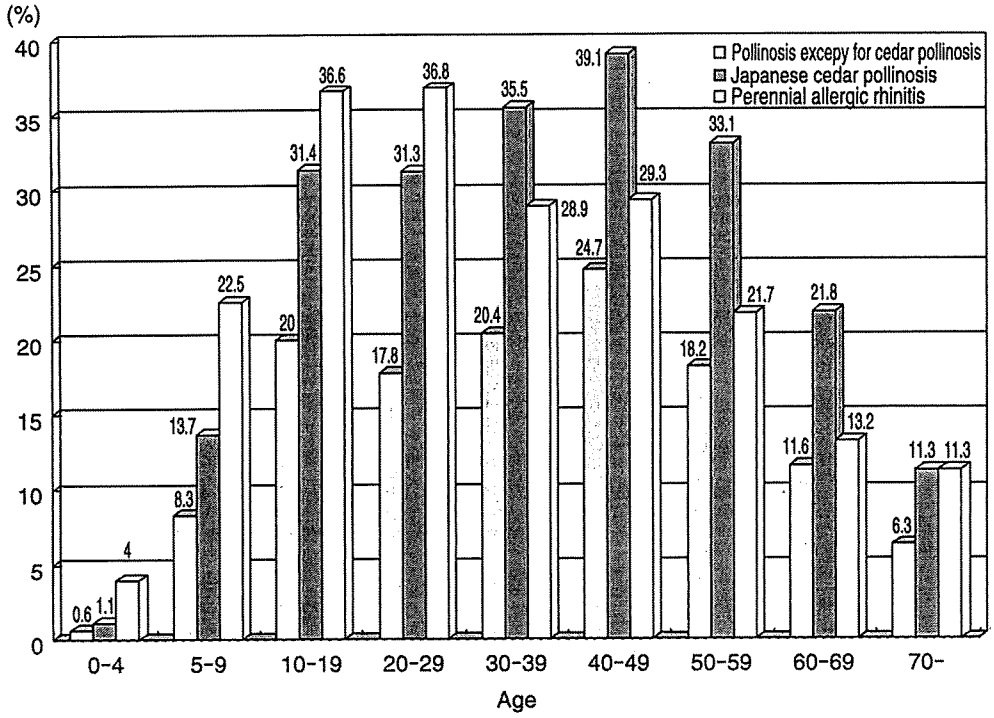

Fig. 2 The prevalence rate of allergic rhinitis in Japan in 2008 (from reference 9).

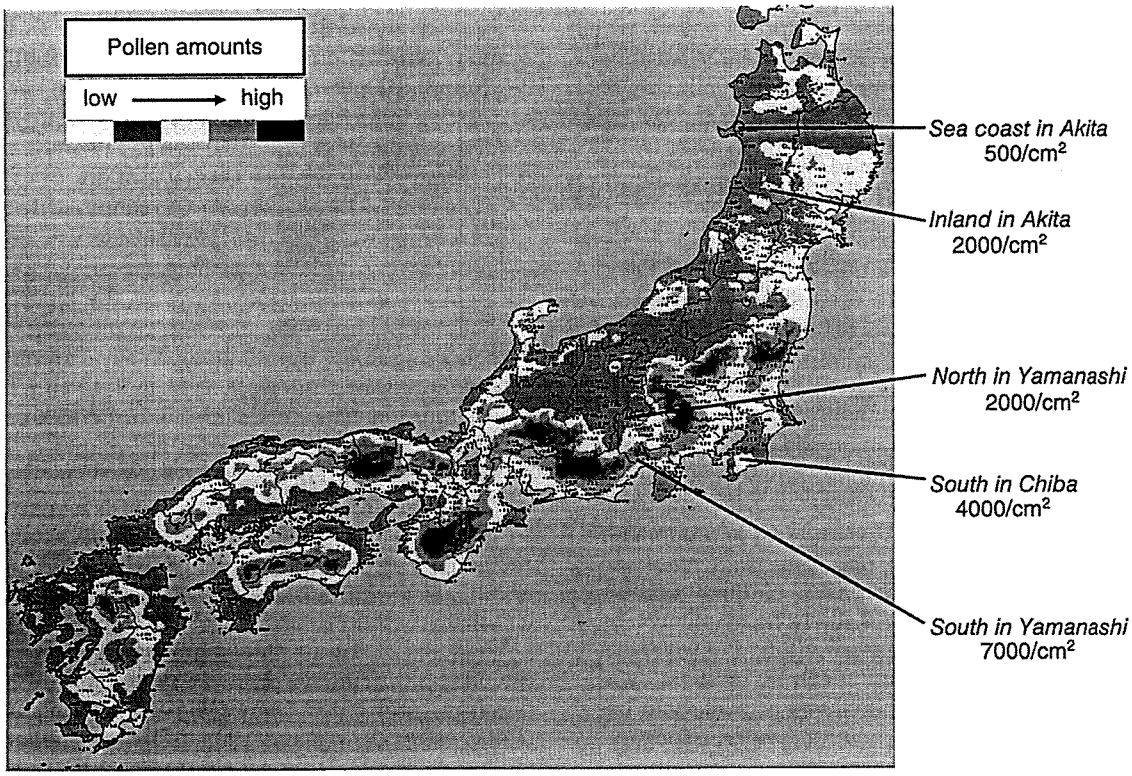

Fig. 3 Annual amount of cedar and cypress pollen dispersal in Japan in 2005.

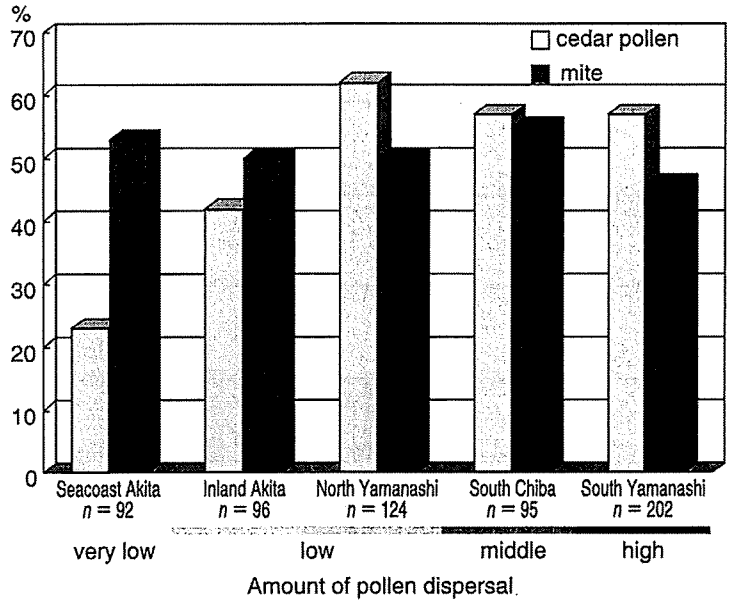

Fig. 4 The detection rate of cedar and cypress pollen-specific IgE in all 4th and 5th grade students in the elementary schools.

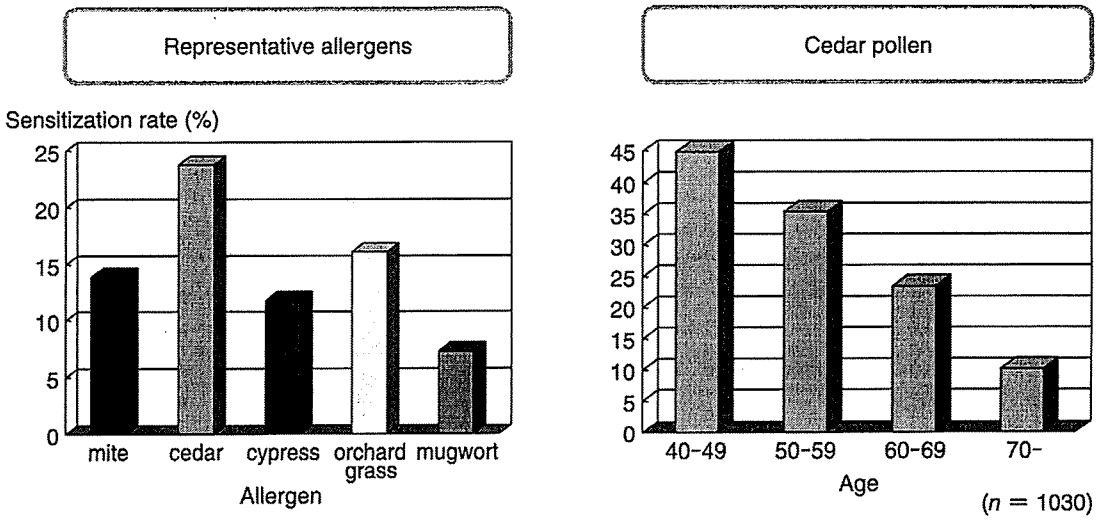

Fig. 5 The sensitization rate to the representative allergen and age distribution of cedar pollen-specific IgE in the adult residents in the forties to seventies in the rural small town in South Chiba.

ent on pollen counts. A very low level of pollen results in a low rate of detection and allergen avoidance is undoubtedly important for prevention. However, a high rate of allergic sensitization can be induced by a relatively small amount of pollen, and it is likely to be very difficult to reduce the amount of pollen exposure to a level that will prevent sensitization. Furthermore, tolerance was not easily induced in students in southern Yamanashi who had been receiving high pollen exposure every year since birth. Interestingly, the incidence of mite allergic rhinitis and pollinosis in these sensitized students was almost the same; about 30 to 35% in each region, respectively.

We have also undertaken medical examination of middle-aged adult residents in their forties to seventies in a rural small town (Maruyama-cho) in South Chiba every year since 1995.[10] The examination includes responses to a questionnaire and testing for specific IgE in serum using a CAP-RAST system. Figure 5 shows the sensitization rate to the representative allergens and the age distribution of cedar pollen-specific IgE. Deterioration of cedar-specific IgE is ob-

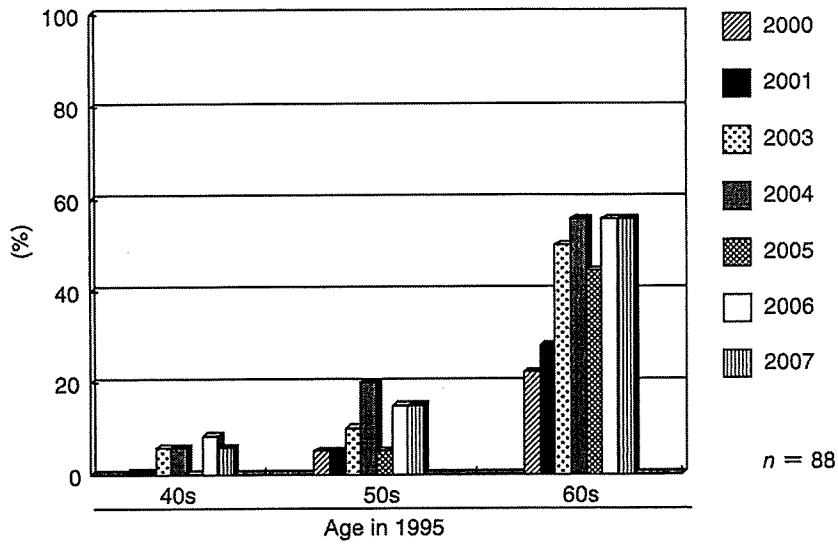

Fig. 6 The rate of change to negative over the last 13 years in cedar pollen-specific IgE in the residents who had tested positive for anti-cedar pollen specific IgE in 1995 and then had received examination every year.

served in elderly subjects. Figure 6 shows the rate of change to negative over the last 13 years in cedar pollen IgE in residents who had tested positive for anti-cedar pollen IgE in 1995. The IgE assays were performed at the end of each cedar pollen season. It appears that the IgE titer is affected by the spread of pollen each year. Interestingly, however, the negative change for 13 years is not commonly observed even in their forties to fifties. The rate of the cedar pollinosis determined by clinical symptoms in combination with positive cedar pollen IgE has also not decreased among these aged subjects.

THE LONG-TERM COURSE OF PATIENTS WITH ALLERGIC RHINITIS

One hundred and seventy-seven patients who were treated in our department from 1970 to 1995 consented to undergo a detailed re-examination. A comparison between the recent symptoms and those observed 10 to 30 years ago showed that 30% of adult patients exhibited some improvements and 10% had resolution. However, only 20% of the pediatric patients exhibited mild improvement of symptoms, whereas the remaining had the same or even worse symptoms as those in childhood (data not shown: in preparation for submitting). Regarding the allergen-specific IgE, a change to negative was not observed in any patients with cedar pollinosis and was seen in only a few of the mite-allergic patients. Thus, natural resolution is not commonly observed in allergic rhinitis and most pediatric patients grow to adulthood without natural improvement of symptoms.

CEDAR POLLEN SPECIFIC MEMORY T CELLS

It has been suggested that dysregulation of cytokine synthesis from Th1 and Th2 cells is fundamental to the pathogenesis of allergic diseases. However, no significant difference was observed between the two groups in the Th1/Th2 cell profile in peripheral blood $CD4^+$ T cells from patients with perennial allergic rhinitis and non-allergic rhinitis by FACS analysis.[11]

Pollinosis is thought to be an adaptive immune response that manifests as a type 1 allergic reaction, and it occurs as a consequence of fundamental allergenic mechanisms involving the induction of pollen-specific T helper type 2 (Th2) effector cells from naïve Th0 cells. Most effector T cells are short-lived, but few effector T cell become long-lived memory T cells. We directly examined the number of allergen-specific Th1/Th2 memory T cells in the peripheral blood of patients of allergic rhinitis by an ELISPOT assay using specific peptides.[12] The Japanese cedar-specific IL-4 producing Th2 cells were detected in all patients examined and increased during the pollen season and decreased during the off-season. However, more than 60% of the cedar-specific memory Th2 cells survived up to 8 months after the pollen season (Fig. 7) .

Allergen-specific immunotherapy is the only current treatment that can change the natural course of allergic rhinitis with long-term effects. However, the conventional immunotherapy with subcutaneous administration is inconvenient because it requires frequent visits to the doctor and also carries the risk of anaphylactic shock.[13] A recent review of randomized

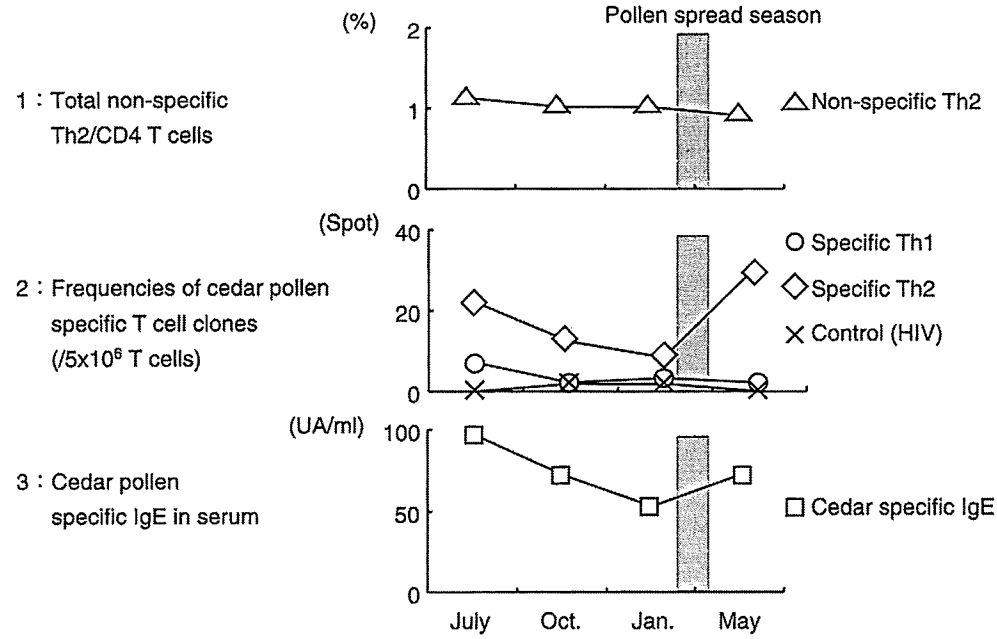

Fig. 7 The seasonal changes of total Th2 cells, frequency of cedar pollen specific T cell clones (spots number) and cedar pollen specific IgE.

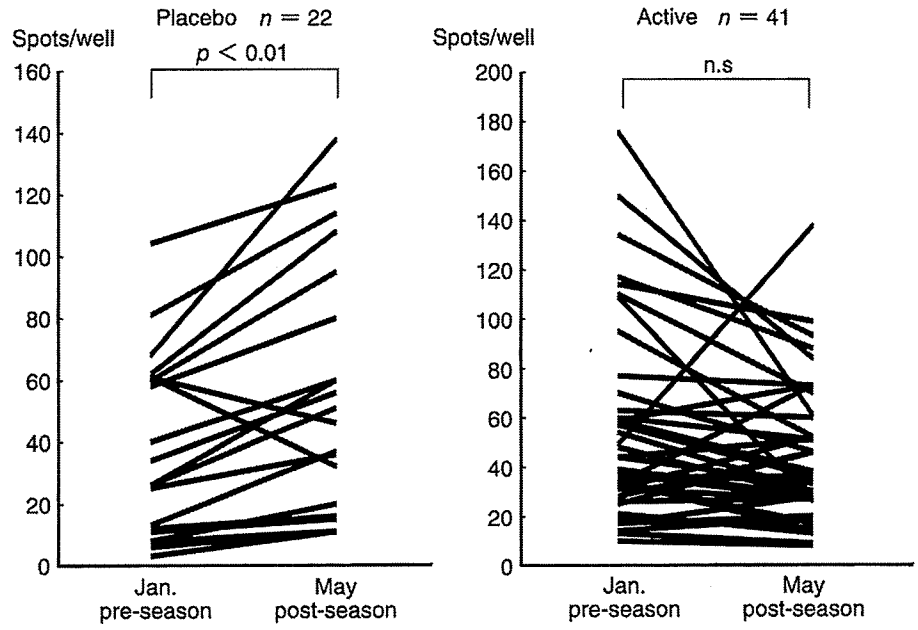

Fig. 8 The number of cedar-specific Th2 cells before and after sublingual immunotherapy.

controlled studies of sublingual immunotherapy suggested that this might be effective as an alternative method of administration.[14-16] To determine the efficacy of sublingual immunotherapy for Japanese cedar pollinosis, we conducted a blinded, randomized, placebo-controlled trial over a period of 6 months (from October 2005 to May 2006).[17] Sixty-seven subjects were enrolled and the nasal symptom scores during the cedar pollen season were evaluated using a symptom diary.

The patients in the active treatment group exhibited significantly lower symptom scores compared to the placebo group. This result suggests that sublingual immunotherapy may offer a safe approach to the management of allergic rhinitis, although the *in vivo* mechanisms of allergen-specific immunotherapy are

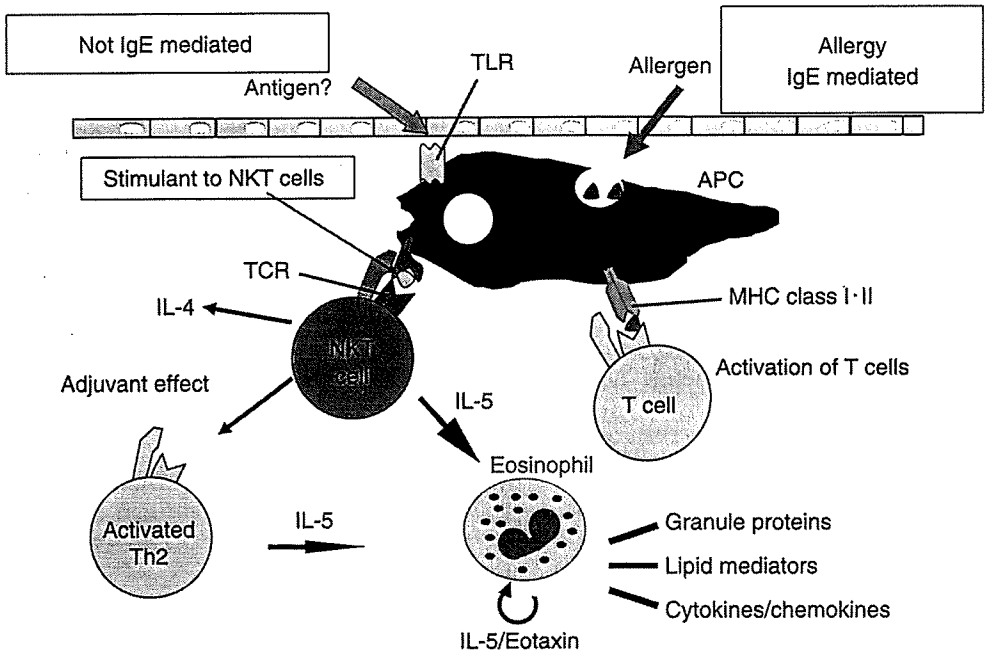

Fig. 9 Mechanism of eosinophil accumulation in respiratory mucosa. Eosinophil accumulation could be observed in MHC class-2 independent.

unknown.

Figure 8 shows the numbers of cedar-specific Th2 cells before and after immunotherapy: the number of Th2 memory cells increased in the placebo group after pollen exposure, but did not increase in the treatment group. Therefore, allergen-specific immunotherapy inhibits an increase in the antigen-specific Th2 memory cell count induced by allergen exposure. Immune-therapeutic intervention might direct at diminishing the size of the clone memory Th2 cells and shifting the cytokine type of memory Th clones.

Natural killer T (NKT) cells represent a unique lymphocyte subpopulation that is characterized by the co-expression of T cells and natural killer receptors.[18,19] Their activity is not restricted to MHC antigens. The relative frequency of NKT cells in the peripheral blood is generally quite low, usually less than 0.1% of PBMCs, and they are not detected in normal peripheral lymph nodes. However, NKT cells play a very important role in innate immunity. Recently, the involvement of NKT cells in the development of airway hypersensitivity in mice and the detection NKT cells in bronchoalveolar-lavage fluid samples from patients with moderate to severe asthma were reported. However, we could not detect the NKT cells in the nasal mucosa of the patients with allergic rhinitis by a polymerase chain reaction. However, NKT cells were detected to varying degrees in the sinus mucosa from asthmatic choronic sinusitis (CS) patients.

These results suggest that NKT cells are not directly related to the development of allergy, but that they may play important roles in the development of sinus disease combined with asthma and in the enhanced Th2 cytokine expression and increased infiltration of Th2 cells and eosinophils observed in the sinus mucosa from asthmatic CS patients via MHC-independent mechanisms (Fig. 9).

SUMMARY

1. The prevalence of allergic rhinitis, in particularly cedar pollinosis, is increasing.

2. Cedar pollen-specific Th1/Th2 dysregulation is observed in patients with pollinosis.

3. Cedar pollen specific memory Th cells increased during the pollen season and decreased during off season, however, morethan 60% of the memory cells survived up to 8 months after the pollen season.

4. NKT cells are not directly related to the development of allergic rhinitis, including pollinosis.

5. Different mechanisms in the accumulation of eosinophilia in the respiratory tract mucosa may exist.

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REFERENCES

1. Bousquet J, Van Cauwenberge P, Khaltaev N; Aria Workshop Group; World Health Organization. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001; **108**:S147-334.
2. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjuctivitis, and atopic eczema: ISAAC. *Lancet* 1998;**351**: 1225-32.
3. Okuda M. Epidemiology of Japanese cedar pollinosis throughout Japan. *Ann Allergy Asthma Immunol* 2003;**91**: 288-96.
4. Kaneko Y, Motohashi Y, Nakamura H, Endo T, Eboshida A. Increasing prevalence of Japanese cedar pollinosis: a meta-regression analysis. *Int Arch Allergy Immunol* 2005; **136**:365-71.
5. Delaunay JJ, Sasajima H, Okamoto Y, Yokota M. Side-by-side comparison of automatic pollen counters for use in pollen information systems. *Ann Allergy Asthma Immunol* 2007;**98**:553-8.
6. Ito Y, Takahashi Y, Fujita T, Fukuyama S. Clinical effects of immunotherapy on Japanese cedar pollinosis in the season of cedar and cypress pollination. *Auris Nasus Larynx* 1997;**24**:163-70.
7. Ito H, Nishimura J, Suzuki M *et al.* Specific IgE to Japanese cypress (*Chamaecyparis obtusa*) in patients with nasal allergy. *Ann Allergy Asthma Immunol* 1995;**74**:299-303.
8. Sasaki K, Okamoto Y, Yonekura S *et al.* Cedar and cypress pollinosis and allergic rhinitis: Quality of life effects of early intervention with Leukotriene receptor antagonists. *Int Arch Allergy Immunol*. In press.
9. [*Practical Guideline for the Management of Allergic Rhinitis in Japan—Perennial Rhinitis and Pollinosis—2009 Edition*], 6th edn. Tokyo: Life Science, 2008(in Japanese).
10. Okawa T, Konno A, Yamakoshi T, Numata T, Terada N, Shima M. Analysis of natural history of Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2003;**131**:39-45.
11. Horiguchi S, Okamoto Y, Chazono H, Sakurai D, Kobayashi K. Expression of membrane-bound CD23 in nasal B cells from patients with perennial allergic rhinitis. *Ann Allergy Asthma Immunol* 2005;**94**:286-91.
12. Horiguchi S, Tanaka Y, Uchida T *et al.* Seasonal changes in antigen-specific Th clone sizes in patients with Japanese cedar pollinosis: A 2-year study. *Clin Exp Allergy* 2008;**38**:405-12.
13. Lockey RF, Nicoara-Kasti GL, Theodoropoulos DS, Bukantz SC. Systemic reactions and fatalities associated with allergen immunotherapy. *Ann Allergy Asthma Immunol* 2001;**87**(Suppl 1):47-55.
14. Cox LS, Linnemann DL, Nolte H, Weldon D, Finegold I, Nelson HS. Sublingual immunotherapy: a comprehensive review. *J Allergy Clin Immunol* 2006;**117**:1021-35.
15. Burastero SE. Sublingual immunotherapy for allergic rhinitis: an update. *Curr Opin Otolaryngol Head Neck Surg* 2006;**14**:197-201.
16. Passalacqua G, Lombardi C, Guerra L, Compalati E, Fumagalli F, Canonica GW. Sublingual immunotherapy: no more doubts. *Eur Ann Allergy Clin Immunol* 2005;**37**: 314-20.
17. Horiguchi S, Okamoto Y, Yonekura S *et al.* A randomized controlled trial of sublingual immunotherapy for Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2008;**146**:76-84.
18. Yamamoto H, Okamoto Y, Horiguchi S, Kunii N, Yonekura S, Nakayama T. Detection of natural killer T cells in the sinus mucosa from asthmatics with chronic sinusitis. *Allergy* 2007;**62**:1451-5.
19. Taniguchi M, Harada M, Kojo S, Nakayama T, Wakao H. The regulatory role of Valpha14 NKT cells in innate and acquired immune response. *Annu Rev Immunol* 2003;**21**: 483-513.
20. Kawano T, Cui J, Koezuka Y *et al.* CDld-restricted and TCR-mediated activation of Valpha14 NKT cells by glycosylceramides. *Science* 1997;**278**:1626-9.
21. Akbari O, Stock P, Meyer E *et al.* Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat Med* 2003;**9**: 582-8.
22. Akbari O, Faul JL, Hoyte EG *et al.* CD4+ invariant T-cell-receptor+ naturel killer T cells in bronchial asthma. *N Engl J Med* 2006;**354**:1117-29.

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Cedar and Cypress Pollinosis and Allergic Rhinitis: Quality of Life Effects of Early Intervention with Leukotriene Receptor Antagonists

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Key Words
Pollinosis · Cedar pollen · Cypress pollen · Leukotriene receptor antagonist

Abstract
Background: Allergic rhinitis involves inflammation of the nasal passages. The use of nasal steroids is generally very effective in providing significant symptom relief. However, compliance for their use is sometimes poor. ***Methods:*** To examine the efficacy of early intervention (before pollen dispersal) with oral cysteinyl leukotriene receptor antagonists (LTRA) on pollinosis in patients with allergy to cedar and Japanese cypress pollens, groups of subjects were treated with LTRA or a placebo for 4 weeks at the beginning of the cedar pollen dispersal season. Subsequently, all patients received nasal steroid therapy concomitantly with LTRA throughout the remaining period of the pollen dispersal season. The effects of such early treatment with LTRA on pollinosis were investigated using symptom scores from an allergy diary and quality of life (QOL) scores. ***Results:*** Sneezing and nasal congestion scores were significantly lower in the LTRA-pretreated subjects than observed in the placebo-pretreated patients between weeks 4 and 6 and weeks 3 and 5, respectively. QOL scores improved significantly in all domains after concomitant therapy with nasal steroids. The percent improvement in the nasal congestion score after the concomitant therapy was significantly higher in the LTRA group (69%) than in the placebo group (41%). ***Conclusion:*** Significant differences observed in symptoms and in QOL effects between LTRA- and placebo-pretreated patients and the absence of major adverse effects noted in these studies suggest that early intervention with LTRA is beneficial and safe and should be considered in the management of pollinosis-associated allergic rhinitis.
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Introduction

Allergic rhinitis is a type I allergic disease mediated by specific IgE antibody responses. The disease develops as inflammation associated with early infiltration with eosinophils and other pro-inflammatory cells into the nasal mucosa. The pathogenesis of later phases of allergic rhinitis exhibits many characteristics similar to bronchial asthma [1, 2]. Dust mite allergens are responsible for at least 90% of cases of perennial allergic rhinitis. Arboreal pollens, including that of cedar and Japanese cypress, are also important causes of rhinitis, especially in Japan [3–

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5]. Cedar forests cover nearly 18% of the total land area of Japan, while Japanese cypress is concentrated in the Kanto region and the western part of the country. Both cedar and Japanese cypress produce enormous amounts of pollen which is dispersed over many kilometers and reaches major cities, including Tokyo and Osaka, causing widespread pollinosis. Cedar pollen dispersal precedes Japanese cypress pollen dispersal, and approximately 70% of patients with cedar pollinosis are also allergic to Japanese cypress pollen. Cedar pollen dispersal starts in early February and reaches a peak between late February and early March, and this is followed by the dispersal of Japanese cypress pollen, which reaches a peak from late March to early April, with some variation due to changes in the climate each year [6]. The pollen dispersal season lasts for more than 10 weeks in and around the area of Chiba.

Outside the pollen dispersal season, patients with cedar and Japanese cypress pollinosis who do not have rhinitis caused by other allergens in general exhibit normal nasal mucosa with few or no symptoms. Repeated exposure to pollen induces allergic inflammation and increases hypersensitivity of the nasal mucosa, and early intervention against mild pollinosis just after the start of the pollen dispersal season may have a significant effect on the severity of symptoms when pollen dispersal is at its peak. In Japan, the Ministry of the Environment makes a detailed prediction of the date around which cedar pollen dispersal is likely to start, making it easy to assess the effects of early intervention in patients with pollinosis due to cedar and Japanese cypress.

Leukotriene receptor antagonists (LTRA) have been shown to be effective in controlling nasal inflammation [7, 8] by their ability to inhibit eosinophil secretion in the airway [9, 10]. Recent studies have shown that LTRA are as effective as antihistamines but less effective than nasal steroids [11–13]. Nasal steroids reduce sneezing, nasal secretion as well as nasal obstruction. However, the compliance for use of nasal steroids is sometimes poor [14] and their market share is much smaller than that for other oral anti-allergic medications in Japan, because patients seem to prefer such non-sensory attribute, painless route of administration [15, 16].

LTRA do not exert any central nervous system depression or adrenal suppressive effects and may be more suitable for early interventional use especially in patients with milder symptoms. In order to determine the effects of early intervention with LTRA on cedar and Japanese cypress pollinosis, we conducted a double-blind, placebo-controlled trial in subjects allergic to both pollens. Either LTRA or placebo was administered to subjects immediately before the start of the pollen dispersal season and continued throughout the pollen season. All subjects received nasal steroids after the initial treatment with LTRA. Symptom and quality of life (QOL) scores were monitored during the pollen and before the dispersal season and after concomitant therapy with nasal steroids and LTRA.

Subjects and Methods

Subjects

The study population comprised 60 subjects (30 males and 30 females), ranging in age from 20 to 65 years, who were otherwise healthy, but who had a clinical history of moderate/severe Japanese cedar and cypress pollinosis for at least 3 consecutive cedar and cypress pollen seasons. The subjects lived in and around Chiba City where the pollen spread would be expected to be consistent. The diagnosis of cedar and cypress pollinosis was based on clinical history, positive allergen-specific skin tests (wheal diameter $\geq$10 mm) to a standardized cedar pollen extract (Torii Pharmaceutical Co., Tokyo, Japan), and a serum cedar and cypress pollen-specific IgE level score $\geq$2 by a CAP radioallergosorbent test (SRL Inc., Tokyo, Japan). Exclusion criteria were complication of moderate/severe perennial allergic rhinitis with a need for treatment, a history of severe asthma, use of anti-allergic drugs within 4 weeks, and a prior history of any allergen-specific immunotherapy, including for cedar pollen. Pregnant women or those at risk of pregnancy were also excluded. The study was conducted at Chiba University Hospital in compliance with the Ethical Guidelines for Clinical Studies and Good Clinical Practice and the Declaration of Helsinki (2000 revision). The Ethics Committee of Chiba University approved the protocol, and written informed consent was obtained from each subject prior to his or her participation in the study.

Methods

Capsules containing 112.5 mg of pranlukast hydrate or placebo were used in the study. The study schedule is shown in figure 1. Prior to the study, patients were interviewed regarding their medical history and underwent the skin test for cedar pollen extract and a CAP radioallergosorbent test in late January 2007 to measure specific serum antibodies against cedar and Japanese cypress pollen. Administration of LTRA or placebo was initiated before the start of the cedar pollen dispersal season, which had been forecast to be in early February. Two capsules were administered orally twice a day after breakfast and dinner for 4 weeks (hereafter referred to as 'pretreatment' period). During the latter 2 weeks of this period, subjects were allowed to use an antihistamine (loratadine, 1 capsule per day), other nasal vasoconstriction drops (tetrahydrozoline hydrochloride, maximum 2 drops to each nasal cavity per day and less than 7 days successively), or disodium cromoglycate eye drops (maximum 4 drops to each eye) at their own discretion, based on the severity of symptoms. Subsequently, all subjects took nasal steroids (fluticasone propionate) and LTRA for 4 weeks (main dispersal treatment period) in accordance with ARIA [2] and the Practical Guidelines for the Management of Allergic Rhinitis in Japan [4], again based on the severity of symptoms.

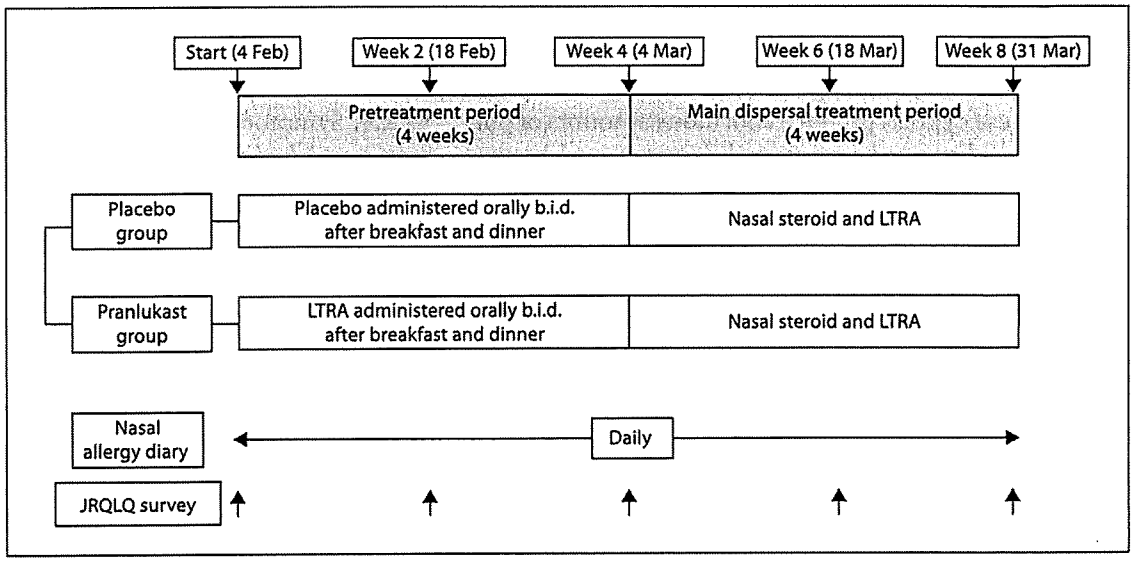

Fig. 1. Study schedule. b.i.d. = Twice daily.

Table 1. 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, 2005 [5].

Some subjects (n = 30) received LTRA throughout the study period (LTRA group). However, a group of other subjects (n = 30) received placebo during the pretreatment period and LTRA during the main treatment period (placebo group). The sample size was determined based on previous studies of LTRA on the change and variance of clinical symptoms [17]. A nasal allergy diary was written daily, and Japan Rhinoconjunctivitis Quality of Life Questionnaire (JRQLQ) survey sheets [18, 19] were completed every 2 weeks until completion of the study. For assignment of subjects to groups, limited randomization was performed in subgroups of 6 age- and sex-matched subjects each, of whom 3 were assigned to the LTRA group and 3 were assigned to the placebo group. A controller who was not directly involved in the study was responsible for group allocation. A group allocation number was given to each subject. This information was closely guarded by the controller and by 1 member of the ethical committee not directly involved in the study.

During the study period, all study subjects recorded their use (dose and frequency) of permitted concomitant medications (listed above) in a nasal allergy diary. Use of other drugs considered unlikely to affect the study was also allowed.

Cedar and Japanese cypress pollen dispersal was measured with a Durham sampler installed on the roof top of one of the buildings in the School of Medicine, Chiba University.

Nasal symptoms, eye symptoms, symptom scores, medication scores and symptom-medication scores were evaluated from the nasal allergy diary using the following criteria. For nasal symptoms, the severity of paroxysmal sneezing (number of sneezes per day), runny nose (number of times of blowing the nose per day), nasal congestion, and the degree of interference with daily life were evaluated on a 5-point scale (0–4) using a modified Okuda classification [4, 20] (table 1). Symptom scores for classification of the severity of nasal symptoms were calculated using the same classification. The daily total nasal symptom score was expressed

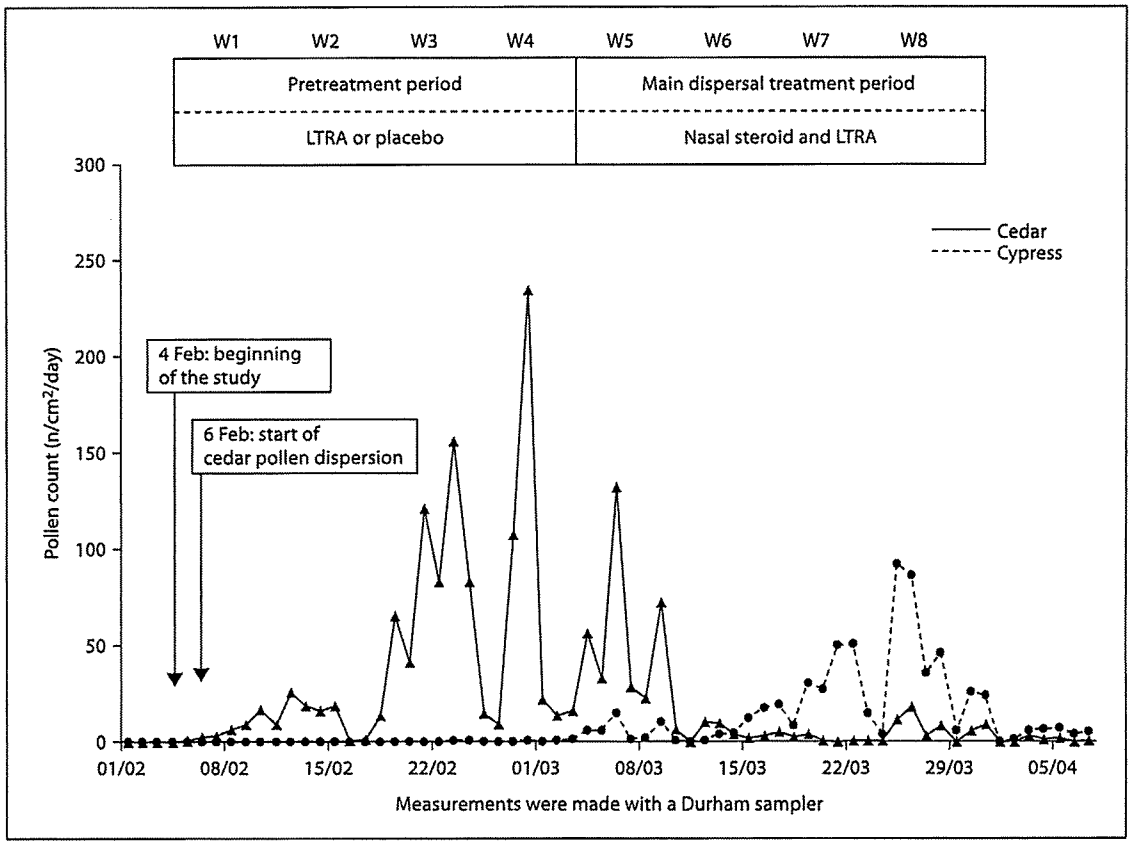

Fig. 2. Dispersal of cedar and Japanese cypress pollen in 2007 and study schedule.

as the highest sore of nasal symptoms. For eye symptoms, itching and watering were evaluated using a 4-point scale. The use of other medications was also scored and recorded according to the characteristics of the drug and the duration of usage, based on the following guidelines: anti-histamines, mast cell stabilizers and vasoconstrictors scored as 1, topical nasal steroids scored as 2, and the symptom-medication score was determined by adding the symptom score and the medication score. The score for each QOL item was also evaluated on a 5-point scale (0–4). In addition, the percent improvement in nasal symptoms was analyzed and expressed as the ratio of the patients who had improved nasal symptoms ≥1 in week 8 at the end of the study compared with week 4 before the concomitant therapy with nasal steroids. The symptom-medication score was used as the primary outcome parameter and other items were used as secondary parameters.

Statistical Analysis

After completion of the study (clinical and laboratory), a biostatistician who had not been involved in carrying out the clinical trial, analyzed the data. After completing the analysis, the allocation identification numbers for the active and placebo groups were accessed. Data comparisons were performed using 2-tailed tests at a significance level of 5%, using a χ^2 test, the Fisher exact test, the Mann-Whitney U test, a 2-sample t test, a paired t test, and the Wilcoxon test in SAS version 8.02 (SAS Inc., Cary, N.C., USA).

Results

Dispersal of Cedar and Japanese Cypress Pollen

Measurements with a Durham sampler (fig. 2) indicated that 6 February was the start of the cedar pollen dispersal season, based on a pollen count of ≥1/cm²/day. After 19 February, a pollen count ≥20/cm²/day was obtained on most days, which dropped to <10/cm²/day after 10 March, marking the end of the dispersal season. Japanese cypress pollen was observed in the middle of March and reached a count of >20/cm²/day on most days after 19 March until dispersal ended in early April.

Subjects

Four subjects withdrew from the study for personal reasons, and not because of any adverse effects. All other subjects exhibited full compliance with the study protocol. Thus, a total of 56 subjects were included for complete evaluation. The LTRA group comprised 29 subjects (mean age 36.1 years and cedar pollen RAST score 3.9). The placebo group comprised 27 subjects (mean age 33

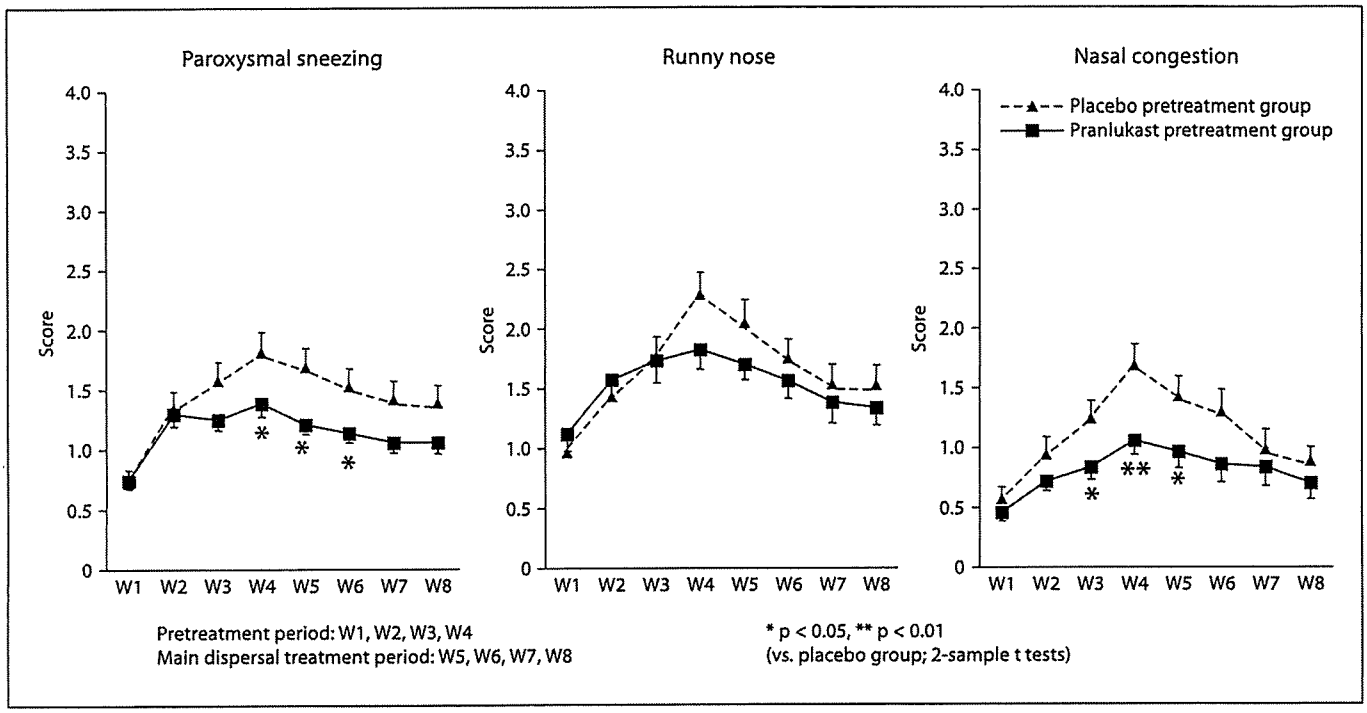

Fig. 3. Mean weekly score for each nasal symptom.

years and cedar pollen RAST score 3.5). There were no significant differences between the 2 study groups for age at disease onset, disease duration, or any subsequent complications.

Treatment Effects

The mean nasal symptom scores for each week of the pollen season are shown in figure 3. In both groups, symptoms worsened as cedar pollen dispersal increased and then improved after week 5, after the start of nasal steroid drops. In the LTRA group, nasal symptoms were mild from week 3 until the end of the study, and sneezing and nasal congestion scores were significantly lower in the LTRA group than in the placebo group between weeks 4 and 6 and weeks 3 and 5, respectively.

The total nasal symptom score increased in both groups from the start of the cedar pollen dispersal season and decreased in week 5, after the use of nasal steroids at the peak of the cedar dispersal season (fig. 4). Mean symptom scores were significantly lower in the LTRA group than in the placebo group in weeks 4 and 5 (fig. 4). Medication and symptom-medication scores also increased following the start of cedar pollen dispersal and decreased in week 5, after the start of nasal steroid therapy. These scores were lower in the LTRA group than in the placebo group during the pretreatment period, although the differences were not significant (data not shown). There were no significant differences in eye itching or watering scores between the groups (data not shown).

The degree of interference with daily life increased in both groups following the onset of cedar pollen dispersal and decreased in week 5 following the start of nasal steroid therapy. The score in the LTRA group was significantly lower than that in the placebo group in week 4 (fig. 4).

A comparative analysis of the improvement in nasal symptom score in week 8 (at the end of the study) and in week 4 (before the concomitant therapy with nasal steroids) is shown in table 2. The percent improvement in nasal congestion was significantly higher in LTRA-pretreated patients (69.0%) than in placebo-pretreated patients (40.7%).

JRQLQ Scores

For 17 QOL items, each mean QOL score generally increased by ≥0.5 points after pollen dispersal (data not shown) and improved for all items after the start of concomitant therapy at week 5 with nasal steroid drops (fig. 5). Although scores for all items in the LTRA group

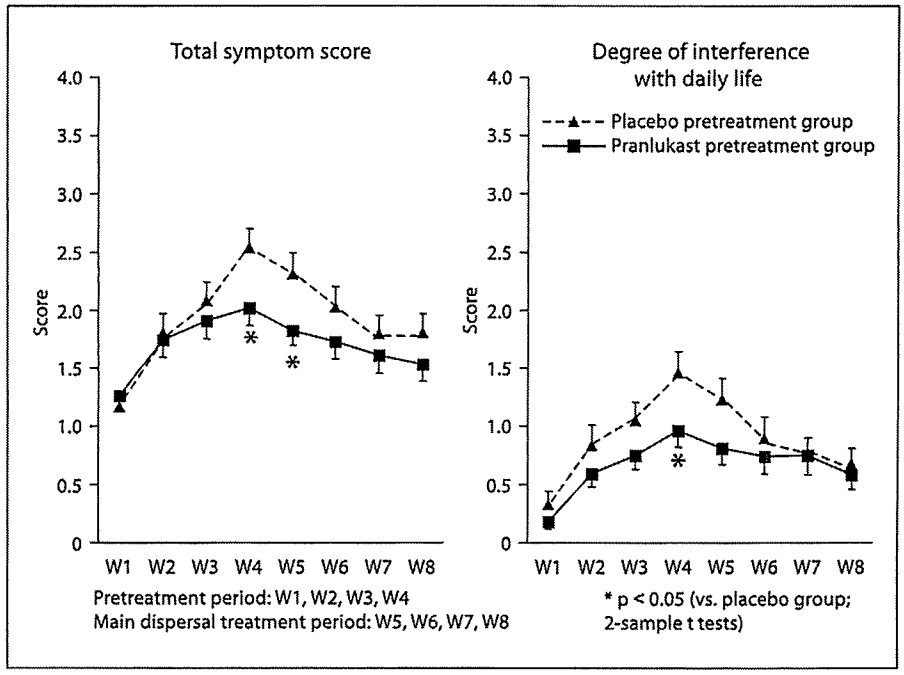

Fig. 4. Symptom scores and the degree of interference with daily life.

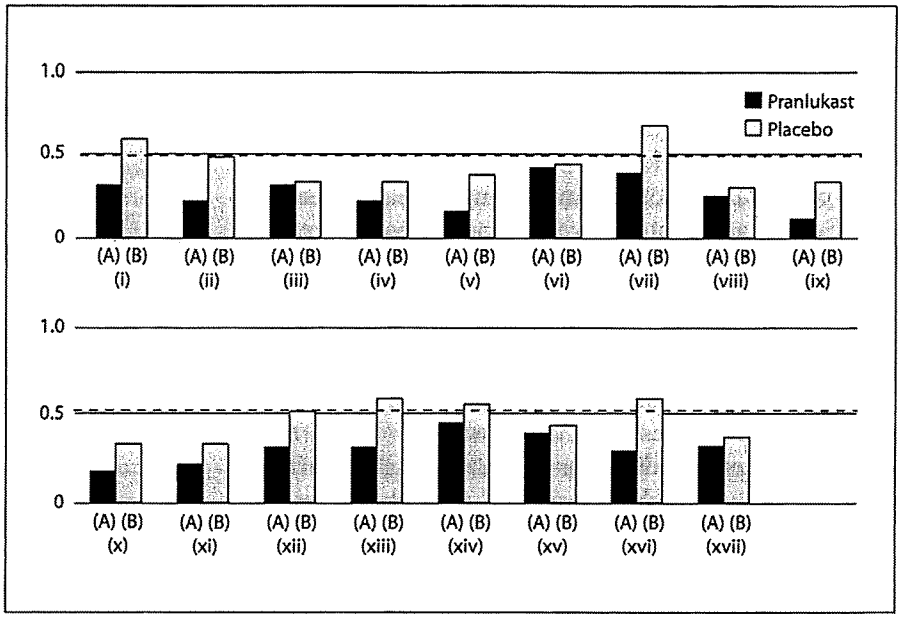

Fig. 5. QOL items at week 8 versus week 0. (i) = Reduced productivity at work/home/school; (ii) = poor mental concentration; (iii) = reduced thinking power; (iv) = impaired reading book/paper; (v) = reduced memory loss; (vi) = limitation of outdoor life (e.g., sports, picnic); (vii) = limitation of going out; (viii) = hesitation visiting friends or relatives; (ix) = reduced contact with friends or others by telephone or conversation; (x) = not an easy person to be around; (xi) = impaired sleeping; (xii) = tiredness; (xiii) = fatigue; (xiv) = frustration; (xv) = irritability; (xvi) = depression; (xvii) = unhappiness.

did not increase by 0.5 points at week 8 compared with week 0 (before pollen dispersal), scores for 7 items (interference with study, work and housework; poor concentration; interference with going out; malaise; fatigue; frustration; depressed feeling) in the placebo group still increased by ≥0.5 points at week 8.

These 17 QOL items were categorized into 6 domains (daily life; outdoor activities; social life; physical exercise; mental life; sleep) and scores were compared among domains (table 3). Mean QOL scores generally increased with cedar pollen dispersal when compared between week 4 and week 0, and scores increased by ≥0.5 points

Table 2. Improvement in nasal symptom score at week 8 compared with week 4

	Improved ≥1 point	Improved <1 point	χ^2 test[1]
Runny nose			
Pranlukast	16 (55.2)	13 (44.8)	0.977
Placebo	15 (55.6)	12 (44.4)	
Sneezing			
Pranlukast	16 (55.2)	13 (44.8)	0.803
Placebo	14 (51.9)	13 (48.1)	
Nasal congestion			
Pranlukast	20 (69.0)	9 (31.0)	0.034
Placebo	11 (40.7)	16 (59.3)	

Data are number of patients, with percentages in parentheses.

[1] The number of the patients who had an improved score of ≥1 point in the pranlukast-pretreated group was compared with that in the placebo-pretreated group.

Table 3. QOL score by JRQLQ (domains)

	Week	Pranlukast mean ± SE	Placebo mean ± SE
Versus week 0			
Usual daily activities	0	0.25 ± 0.08	0.13 ± 0.06
	4	0.97 ± 0.15***	0.87 ± 0.14***
	8	0.48 ± 0.10*	0.56 ± 0.14*
Outdoor activities	0	0.14 ± 0.05	0.19 ± 0.07
	4	1.02 ± 0.19***	1.20 ± 0.19***
	8	0.53 ± 0.14**	0.74 ± 0.18**
Social functioning	0	0.10 ± 0.04	0.06 ± 0.03
	4	0.69 ± 0.14***	0.53 ± 0.13***
	8	0.28 ± 0.09	0.38 ± 0.11**
Sleep problem	0	0.17 ± 0.07	0.19 ± 0.09
	4	0.59 ± 0.14**	0.81 ± 0.15***
	8	0.38 ± 0.10	0.52 ± 0.13*
General physical function	0	0.21 ± 0.08	0.13 ± 0.07
	4	1.00 ± 0.16***	0.96 ± 0.20***
	8	0.52 ± 0.12*	0.69 ± 0.18**
Emotional function	0	0.14 ± 0.05	0.06 ± 0.04
	4	0.78 ± 0.16***	0.74 ± 0.15***
	8	0.05 ± 0.14*	0.55 ± 0.15**
Overall QOL condition	0	1.07 ± 0.19	0.81 ± 0.13
	4	2.48 ± 0.15***	2.26 ± 0.19***
	8	1.45 ± 0.17	1.41 ± 0.16*
Versus week 4			
Usual daily activities	4	0.97 ± 0.15	0.87 ± 0.14
	8	0.48 ± 0.10***	0.56 ± 0.14*
Outdoor activities	4	1.02 ± 0.19	1.20 ± 0.19
	8	0.53 ± 0.14**	0.74 ± 0.18**
Social functioning	4	0.69 ± 0.14	0.53 ± 0.13
	8	0.28 ± 0.09***	0.38 ± 0.11
Sleep problem	4	0.59 ± 0.14	0.81 ± 0.15
	8	0.38 ± 0.10	0.52 ± 0.13
General physical function	4	1.00 ± 0.16	0.96 ± 0.20
	8	0.52 ± 0.12***	0.69 ± 0.18
Emotional function	4	0.78 ± 0.16	0.74 ± 0.15
	8	0.05 ± 0.14*	0.55 ± 0.15

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (2-sample t test).

for 5 of the 6 domains excluding sleeping problem in the LTRA group and for 5 domains excluding social functioning in the placebo group. After the start of nasal steroid therapy at week 5, scores in all 5 domains which had increased by ≥0.5 points at week 4 exhibited significant improvement at week 8 in the LTRA-treated group. The sleep problem and social functioning domains were not significantly aggravated at week 8 under Japanese cypress pollen dispersal when compared with week 0.

In contrast, in the placebo group, scores for 4 domains (social functioning; sleep problem; physical problems; emotional function) did not manifest significant improvement when evaluated during week 8 when compared with the scores observed at week 4 (before nasal steroid therapy). All domains exhibited a still significant increase at week 8 when compared with the scores observed at the beginning of the study (week 0).

Overall QOL condition scores also increased after pollen dispersal in both LTRA- and placebo-treated groups. After the use of nasal steroids, significant improvement was observed at week 8 in the LTRA-treated group but not in the placebo group compared with the scores at week 4.

Use of Concomitant Medications and Safety

Antihistamines and nasal vasoconstrictor drugs were used less frequently by the subjects in the LTRA group in the latter 2 weeks of the pretreatment period than in the placebo group; however, the differences were not significant (data not shown). The compliance for the use of LTRA and nasal steroids during the whole study period did not differ between the groups. However, an adverse event was reported by 1 patient in the LTRA group who experienced abdominal pain on day 16, but this resolved 2 days later and did not prevent continuation of study drug administration.

Discussion

In the present studies, the symptoms of allergic rhinitis increased predictably in the placebo group of subjects. However, many JRQLQ scores also worsened even in the LTRA group at the height of the pollen dispersal season. Therefore, pretreatment with LTRA alone did not appear to result in significant relief of nasal symptoms, and additional nasal steroid therapy in accordance with the standard guidelines was required to induce significant symptom relief [2, 4]. During the later phases of the cedar pollen dispersal season, symptoms and QOL scores exhibited improvement following the initiation of nasal steroid therapy in both LTRA and placebo-pretreated groups. However, differences between the groups in scores for sneezing and nasal congestion were still statistically significant. Subsequently, the symptoms in both groups improved and there were no significant differences in scores between the 2 groups. These findings are considered to reflect the effects of nasal steroid therapy. However, the degree of improvement in nasal congestion scores in week 8 at the end of the study compared with week 4 just before the concomitant therapy with nasal steroids was significantly higher in the LTRA-pretreated group than in the placebo-pretreated group.

QOL scores are considered to be more sensitive markers of clinical improvement than symptom scores derived from an allergy diary [21–23]. All QOL scores improved significantly in the LTRA group after the initiation of concomitant therapy with nasal steroids. The QOL items were categorized into 6 domains (daily life; outdoor activities; social life; physical exercise; mental life; sleep). Scores for the first 5 domains and the overall condition were significantly improved in the LTRA group after the initiation of concomitant nasal steroid therapy. Sleep was not significantly affected in the Japanese cypress pollen dispersal season. In contrast, in the placebo group, domain scores for social life, physical exercise, mental life and sleep (which was disturbed in the Japanese cypress pollen dispersal season in the placebo group) did not improve even after concomitant therapy with nasal steroid, and daily life and overall condition scores demonstrated delayed improvement compared with the LTRA group.

In Japan, pollen counts are typically measured using the gravimetric method with a Durham sampler, in contrast to Western countries in which a Burkard sampler is typically used. In a study in Chiba Prefecture in 2005, the amount of air-borne pollen counted with a Burkard sampler was about 12 times greater than that counted with a Durham sampler [24]. For Durham sampler measurements, a count of 1–10/cm^2/day is defined as low dispersal and >20/cm^2/day is considered high dispersal. In this study (2007), cedar pollen dispersal was detected by a Durham sampler at the beginning of February. The count was >20/cm^2/day on many days after 19 February and then returned to <10/cm^2/day after 10 March, after which dispersal ended. Japanese cypress pollen was detected at the end of February, had a count of >20/cm^2/day on many days after 19 March, with dispersal ending in early April.

Symptoms of allergic rhinitis are generally mild immediately after the start of the pollen dispersal season, but hypersensitivity-induced inflammation of the nasal mucosa is produced by repeated exposure to pollen. Such exposure results in enhanced expression of adhesion molecules, increased infiltration of the nasal mucosa by inflammatory cells, hyperpermeability of epithelial cells, and an increased neural sensory response [1, 2, 25]. Even in the LTRA group, many JRQLQ scores increased by ≥0.5 points at the height of the pollen dispersal season. With standard therapy using nasal steroids for severely affected patients, the QOL scores in the LTRA group were still lower than those in the placebo group. In the placebo group, nasal steroid therapy produced a smaller improvement in QOL scores.

The observations reported here and other earlier studies have suggested that LTRA are extremely safe and do not result in any major adverse effects, such as anticholinergic activity, local irritation or adrenal suppression. In this study, mild abdominal pain was reported by 1 patient, but no causal relationship with LTRA was detected.

Nasal steroids are generally very effective and provide a significant resolution of symptoms. Nasal steroids might be advantageous for early intervention; however, the compliance is sometimes poor, since many patients prefer to use oral medication, particularly in Japan [14, 15].

Although the number of patients enrolled in the study was limited and a comparative study with LTRA and steroids in a large scale will be needed to evaluate the effectiveness, based on the information summarized here, it is proposed that the use of LTRA is safe and might be appropriate for pretreatment before the appearance and establishment of clinical symptoms early in the course of the cedar pollen season.

Acknowledgements

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References

1 Barnes PJ: New directions in allergic diseases: mechanism-based anti-inflammatory therapies. J Allergy Clin Immunol 2000;106: 5–16.

2 Bousquet J, Van Cauwenberge P, Khaltaev N, Aria Workshop Group, World Health Organization: Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol 2001;108: s220–s251.

3 Okuda M: Epidemiology of Japanese cedar pollinosis throughout Japan. Ann Allergy Asthma Immunol 2003;91:288–296.

4 Practical Guideline for the Management of Allergic Rhinitis in Japan – Perennial Rhinitis and Pollinosis, ed 5. Tokyo, Life Science, 2005.

5 Kaneko Y, Motohashi Y, Nakamura H, Endo T, Eboshida A: Increasing prevalence of Japanese cedar pollinosis: a meta-regression analysis. Int Arch Allergy Immunol 2005; 136:365–371.

6 Ito H, Nishimura J, Suzuki M, Mamiya S, Sato K, Takagi I, Baba S: Specific IgE to Japanese cypress *(Chamaecyparis obtusa)* in patients with nasal allergy. Ann Allergy Asthma Immunol 1995;74:299–303.

7 Lipworth BJ: Emerging role of antileukotriene therapy in allergic rhinitis. Clin Exp Allergy 2001;31:1813–1821.

8 Meltzer EO: Clinical evidence for antileukotriene therapy in the management of allergic rhinitis. Ann Allergy Asthma Immunol 2002;88:23–29.

9 Peters-Golden M, Gleason MM, Togias A: Cysteinyl leukotrienes: multi-functional mediators in allergic rhinitis. Clin Exp Allergy 2006;36:689–703.

10 Fukushima C, Matsuse H, Hishikawa Y, Kondo Y, Machida I, Saeki S, Kawano T, Tomari S, Obase Y, Shimoda T, Kohono S: Pranlukast, a leukotriene receptor antagonist, inhibits interleukin-5 production via a mechanism distinct from leukotriene receptor antagonism. Int Arch Allergy Immunol 2005;136:165–172.

11 Wilson AM, O'Byrne PM, Parameswaran K: Leukotriene receptor antagonists for allergic rhinitis: a systematic review and meta-analysis. Am J Med 2004;116:338–344.

12 Meltzer EO, Malmstrom K, Lu S, Prenner BM, Wei LX, Weinstein SF, Wolfe JD, Reiss TF: Concomitant montelukast and loratadine as treatment for seasonal allergic rhinitis: a randomized, placebo-controlled clinical trial. J Allergy Clin Immunol 2000;105: 917–922.

13 Barnes NC, de Jong B, Miyamoto T: Worldwide clinical experience with the first marketed leukotriene receptor antagonist. Chest 1997;111:s52–s60.

14 Borres MP, Brakenhielm G, Irander K: How many teenagers think they have allergic rhinoconjunctivitis and what they do about it. Ann Allergy Asthma Immunol 1997;78:29–34.

15 Okuda M, Ohkubo K, Gotoh M, Ishida Y: Treatment of Japanese cedar pollinosis and its impact on patient satisfaction. Arerugi 2004;53:596–600.

16 Kaliner MA: Patient preferences and satisfaction with prescribed nasal steroids for allergic rhinitis. Allergy Asthma Proc 2001;22: s11–s15.

17 Konno A, Yamagoshi T, Usui S: Clinical assessment of ONO-1078 (pranlukast hydrate) against perennial allergic rhinitis – clinical pharmacological study using airway resistance in the nasal cavity as an indicator – (a double-blind comparative study using placebo as a control drug). J Clin Ther Med 1997;13:1921–1939.

18 Okubo K, Gotoh M, Shimada K, Ristu M, Kobayashi M, Okuda M: Effect of fexofenadine on the quality of life of Japanese cedar pollinosis patients. Allergol Int 2004;53: 245–254.

19 Okubo K, Gotoh M, Shimada K, Ritsu M, Okuda M, Crawford B: Fexofenadine improves the quality of life and work productivity in Japanese patients with seasonal allergic rhinitis during the peak cedar pollinosis season. Int Arch Allergy Immunol 2005;136:148–154.

20 Okuda M: Grading the severity of allergic rhinitis for treatment strategy and drug study purposes. Curr Allergy Asthma Rep 2001;1:235–241.

21 Noonan MJ, Raphael GD, Nayak A, Greos L, Olufade AO, Leidy NK, Champan D, Kramer B: The health-related quality of life effects of once-daily cetirizine HCl in patients with seasonal allergic rhinitis: a randomized double-blind, placebo-controlled trial. Clin Exp Allergy 2003;33:351–358.

22 Kremer B, Klimek L, Bullinger M, Mösges R: Generic or disease-specific quality of life scales to characterize health status in allergic rhinitis? Allergy 2001;56:957–963.

23 Thompson AK, Juniper E, Meltzer EO: Quality of life in patients with allergic rhinitis. Ann Allergy Asthma Immunol 2000;85: 338–348.

24 Delaunay JJ, Sasajima H, Okamoto Y, Yokota M: Side-by-side comparison of automatic pollen counters for use in pollen information systems. Ann Allergy Asthma Immunol 2007;98:553–558.

25 Holgate ST, Peters-Golden M, Panettieri RA, Henderson WR Jr: Roles of cysteinyl leukotrienes in airway inflammation, smooth muscle function, and remodeling. J Allergy Clin Immunol 2003;111:s18–s36.

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REVIEW ARTICLE

Antigen-Specific Immunotherapy against Allergic Rhinitis: The State of the Art

Takashi Fujimura[1,2] and Yoshitaka Okamoto[1]

ABSTRACT

Allergic rhinitis is the most prevalent type I allergy in industrialized countries. Pollen scattering from trees or grasses often induces seasonal allergic rhinitis, which is known as pollinosis or hay fever. The causative pollen differs across different areas and times of the year. Impaired performance due to pollinosis and/or medication used for treating pollinosis is considered to be an important reason for the loss of concentration and productivity in the workplace. Antigen-specific immunotherapy is an only available curative treatment against allergic rhinitis. Subcutaneous injection of allergens with or without adjuvant has been commonly used as an immunotherapy; however, recently, sublingual administration has come to be considered a safer and convenient alternative administration route of allergens. In this review, we focus on the safety and protocol of subcutaneous and sublingual immunotherapy against seasonal allergic rhinitis. We also describe an approach to selecting allergens for the vaccine so as to avoid secondary sensitization and adverse events. The biomarkers and therapeutic mechanisms for immunotherapy are not fully understood. We discuss the therapeutic biomarkers that are correlated with the improvement of clinical symptoms brought about by immunotherapy as well as the involvement of Tr1 and regulatory T cells in the therapeutic mechanisms. Finally, we focus on the current immunotherapeutic approach to treating Japanese cedar pollinosis, the most prevalent pollinosis in Japan, including sublingual immunotherapy with standardized extract, a transgenic rice-based edible vaccine, and an immunoregulatory liposome encapsulating recombinant fusion protein.

KEY WORDS

allergic rhinitis, biomarker, immunotherapy, pollinosis, regulatory T cell

INTRODUCTION

Allergic rhinitis is the most prevalent type I allergy, and pollen grains are one of the most common causes of respiratory allergies. In western Europe, the prevalence of clinically confirmable allergic rhinitis was estimated to be 23%, with more than 50% of the allergic subjects possessing specific IgE against grass pollen.[1] In Japan, the prevalence of allergic rhinitis was estimated to be 39.4% and that of pollinosis was 29.8%.[2]

Pollinosis is induced by the invasion of pollen grains onto the ocular and nasal mucosa. Pollen grains easily access internal binding sites on contact with the aqueous phases of nasal and ocular mucosal membranes. After pollens are hydrated on aqueous membranes, they swell, rupture, and release their cytoplasmic components. It has been reported that grass pollen grains rupture in water and release large amounts of respirable particles, such as starch granules containing allergens.[3] Although pollinosis patients have a low rate of asthma attacks during pollen season, the attacks that do occur may be attributable to these respirable particles bearing allergens from pollen grains.[4] Pollen grains release not only allergen-bearing particles but also immunomodulatory mediators such as pollen-associated lipid mediators (PALMs) and NADPH oxidases. Proinflammatory PALMs such as leukotriene B4-like substances attract and activate human peripheral blood eosino-

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phils and polymorphonuclear granulocytes from both allergic and non-allergic donors.[5,6] Immunomodulatory PALMs, such as phytoprastanes, inhibit IL12 production in dendritic cells and Th1-type cytokine production in antigen-specific T cells, while inducing antigen-specific Th2 responses.[7] NADPH oxidase rapidly increases the level of reactive oxygen species (ROS) in lung epithelium and induces neutrophil recruitment to the airway independent of the adaptive immune responses.[8,9] These reports strongly suggest that pollen grains themselves act primarily as adjuvants to induce pollen-antigen-specific Th2 responses and to enhance inflammatory processes during the elicitation phase of allergic responses.

The most common treatments against pollinosis are medications like antihistamines, leukotriene inhibitors, and corticosteroids. However, these treatments are not curative and sometimes induce impaired performance as a results of their side effects.[10,11] Antigen-specific immunotherapy can change the natural course of allergic rhinitis and is recognized as a curative treatment against type I allergy without impaired performance. In this century, since the first report on subcutaneous immunotherapy (SCIT), SCIT has been developed and improved and has become safer and more effective.[12,13] Recently, sublingual immunotherapy (SLIT) has been developed and has become a safer and more beneficial immunotherapy for patients.

This review focuses on the recent approach of using antigen-specific immunotherapy to treat allergic rhinitis, and focuses especially on the use of SLIT against pollinosis using standardized extract or recombinant allergens. We also discuss the therapeutic mechanisms and therapeutic biomarkers for SLIT. Finally, we discuss the recent immunotherapeutic approach to treat Japanese cedar (*Cryptomeria japonica*) pollinosis, which is the most common pollinosis in Japan.

ANTIGENS FOR IMMUNOTHERAPY

For immunotherapy, extracts from an allergen source, i.e. pollen extract, are widely used after the concentration of their major allergen is adjusted so as to be standardized. To standardize such extracts, it is important to analyze their component allergens and establish a quantification system for major allergens.[14] The World Allergy Organization (WAO) recommends that standardized vaccines be used for immunotherapy if they are available.[15] However, the protocols and methods for the standardization of allergen extract are different among different suppliers, which use their own in-house reference materials and their own unique allergen units. This made it difficult to compare the therapeutic effects and safety among clinical trials involving different products. It has been proposed that vaccines be standardized using a protocol based on mass units of major allergens and that the active ingredients of the treatment be quantified. The CREATE project has been working to select major allergens for use in the standardization of vaccines and to establish a quantification system and recombinant allergens for the standardization.[16]

To improve the safety and clinical therapeutic effects of a vaccine, the selection of allergens for vaccination is an important issue. Extract from pollen may contain many allergens that cross-react with those from fruit, vegetables, and latex. These allergens may cause minor local side effects, especially in SLIT, among patients who suffer from oral allergies and/or latex-fruit syndrome. Latex-fruit syndrome sometimes induces severe systematic reactions such as anaphylactic shock in response to natural rubber and some latex fruits.[17] The cross-reactive allergens may have to be removed from vaccines in order to avoid severe systematic adverse reactions caused by cross-reactivity with latex allergens for safer SLIT. For the elucidation of reactive allergens, protein microarray techniques have recently been applied to allergy diagnosis. Microarray-chip technology using a glass slide with the immobilization of large numbers of proteins on the surface enable us to simultaneously test IgE-binding reactivity against large numbers of allergens from various sources.[18,19] This diagnostic technique is applicable to the diagnosis of allergens from a single allergen source. This component-resolved diagnosis is a powerful tool for selecting components of allergens for immunotherapy vaccines and may improve the safety and clinical therapeutic efficacy of the vaccines in comparison to traditional immunotherapy using crude extract.[20] Such an allergen diagnosis enables us to choose only IgE-binding allergens that are individually sensitized for antigen-specific immunotherapy. This approach, in which only sensitized allergens are used for immunotherapy, avoids secondary additional sensitization against nonreactive proteins that can occur with the use of crude extracts or a mixture of allergens (Fig. 1).

Recombinant technology has been used to construct vaccines for immunotherapy.[21] Immunotherapy clinical trials were performed using a mixture of five recombinant grass allergens (rPhl p 1, rPhl p 2, rPhl p 5a, rPhl p 5b, and rPhl p 6), and the results suggested that a recombinant allergen vaccine can be an effective and safe treatment to ameliorate the symptoms of allergic rhinitis.[22] Immunotherapy using recombinant Bet v 1 was also recently reported to show clinical efficacy, and its therapeutic effects were comparable with those obtained using native Bet v 1 against birch pollen allergy.[23]

Vaccines using allergoids and modified allergens, such as T cell-epitopes, pathogen-related molecular pattern molecule-conjugated allergens, and others, are under development, and some of them are considered to be promising for use as therapeutic vaccines.[13,24]

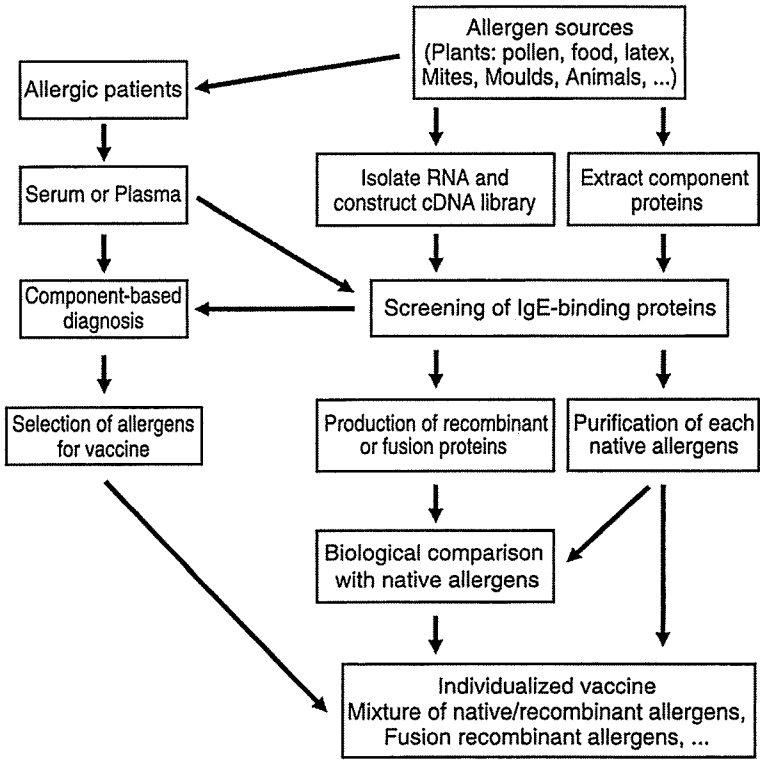

Fig. 1 Schematic procedure of the steps involved in the identification and development of an individualized vaccine using only sensitized antigens for immunotherapy. To identify component allergens which have the capacity to react with serum IgE from allergic patients, it is important to establish individualized vaccines to avoid secondary sensitization. Allergens with which an individual patient reacted can be elucidated by a component-based diagnosis, and an individualized vaccine can be established using a mixture of the purified native or the standardized recombinant allergens to which the patient is sensitized.

ROUTE OF VACCINE ADMINISTRATION FOR IMMUNOTHERAPY AND ITS SAFETY

Immunotherapy vaccines against allergies were originally injected subcutaneously without an adjuvant.[12] However, subcutaneous injection of allergens often induces severe adverse reactions like local allergic reactions, urticaria, asthma, and frequent anaphylaxis. To increase the safety and therapeutic efficacy of immunotherapy vaccines, aqueous allergen extracts absorbed into adjuvants such as aluminum hydroxide have been used in SCIT.[25] Pretreatment with antihistamine or anti-IgE antibody has been used to prevent the adverse events that can be induced after subcutaneous vaccine injection, and the pretreatments also enhance the therapeutic efficacy of SCIT.[26,27]

In this decade, SLIT has been developed as a safer method for immunotherapy and has been used with increasing frequency, especially in Europe and the US. SLIT is noted to be a very safe method without fetal adverse reactions. In most cases, adverse reactions to SLIT have been mild local reactions such as oral pruritus, edema of the mouth, throat irritation, and sneezing.[28] However, a few cases of anaphylaxis have been reported after SLIT using a crude or standardized vaccine.[29-33] These reports suggest that SLIT is not always safe for patients, especially those with severe asthma or who have experienced severe adverse reactions to SCIT. It has been recommended that the first dose of the vaccine is to be administered in a doctor's office under observation.[32]

The administration regimens for SLIT, including dosing, the build-up phase, duration of the treatment, and frequency of the maintenance dose, differ greatly among the clinical trials.[34] The sublingual and supralingual administration methods of oral drops were evaluated by a double-blind, placebo-controlled study using mixed standardized extract in patients allergic to grass pollen. In this report, sublingual administration significantly reduced the nasal, ocular, and bronchial symptoms, as well as the intake of symptom-reducing drugs compared to the placebo. Supralin-

Table 1 Comparison between SLIT and SCIT

	SLIT	SCIT
Administration	Sublingual spitting or sublingual swallowing	Subcutaneous injection with or without adjuvant
Pre-treatment	None	Medication or anti-IgE
Build-up phase	A few weeks, one day for rush protocol, or no up-dosing phase	A few weeks or a few days for rush protocol
Vaccination	Once daily or a few times weekly	A few times weekly or monthly
Adverse event	Local mild reaction in most cases, a few reports of fetal adverse reactions	Sometimes induces fetal adverse reactions

gual treatment also attenuated the symptoms and symptom-reducing drugs intake; however, only the nasal symptom score showed a significant reduction compared to the placebo-control group.[35] Thus, holding the vaccine under the tongue may be an important way to achieve better therapeutic effects with SLIT.

Vaccines for SLIT can also be delivered by two methods: sublingual spitting, in which the vaccine is spat out after being held under the tongue, and sublingual swallowing, in which the vaccine is swallowed after being kept under the tongue. In studies using radiolabeled allergens, most of the allergens remained in the mouth after the vaccine was spat out. However, plasma radioactivity began to increase only after swallowing.[36-38] The author concluded that contact between the allergens and the oral mucosa is a crucial step in the mechanisms of SLIT, and suggested that the more appropriate and advantageous way to administer the allergen sublingually is via the sublingual swallowing procedure.[38]

It has been recommended that the administration of SLIT vaccine be started at least 8 weeks before pollen season for better therapeutic effects.[39] However, an ultra-rush scheme of SLIT treatment for children allergic to grass pollen was reported to significantly improve the symptoms and the medication score compared to the placebo group. In this 2-year randomized, double-blind, placebo-control trial, the authors administered standardized extract of five grass pollen (*Dactylis glomerata, Anthoxanthum odoratum, Lolium perenne, Poa prantensis,* and *Phleum pretense*) beginning 2 weeks before the pollen season started with one day for ultra-rush induction, and followed by daily treatment (120 IR, 10 μg major allergen) for 6 months. It has been reported that SLIT significantly improved the asthma symptom score and reduced the nasal symptom score and the use of rescue medication score compared to the placebo group.[40] The starting point and duration of treatment varied among the clinical trials, and the best procedure for administration remains unclear.[41] (Table 1)

As a novel route to enhance the therapeutic efficacy of the vaccine, direct intralymphatic injection was proposed for the administration of peptide vaccine against viral infection and tumor in the mouse. This paper reported that the direct administration of peptide vaccine into a lymph node induced enhanced immunogenicity compared to subcutaneous and intradermal vaccination.[42] This novel technique was recently applied to patients with hay fever in an open-label, randomized control trial.[43] The authors injected 1,000 SQ-U of aluminum hydroxide-adsorbed grass pollen extract into a superficial inguinal lymph node under ultrasonic guidance. Three intralymphatic injections over 2 months resulted in long-lasting tolerance with the amelioration of hay favor symptoms, reduced skin prick test reactivity, and decreased serum allergen-specific IgE comparable with conventional SCIT. Furthermore, the author reported that there were fewer adverse events than in SCIT, even without premedication with antihistamines, and the injection was less painful than venous puncture.[43] Further clinical trials with a larger population are needed to evaluate the safety, therapeutic efficacy, and duration of tolerance of this treatment.

BIOMARKERS FOR SLIT

The therapeutic effects obtained by antigen-specific immunotherapy are commonly judged on the basis of clinical symptoms according to quality-of-life (QOL) score, symptom diary, and symptom-reducing drugs intake. The biomarkers correlated with the therapeutic effects are still controversial, especially for SLIT.

Antigen-specific IgG4 is considered to be a biomarker for antigen-specific immunotherapy; however, the correlation between the induction of IgG4 production and clinical symptoms is controversial.[44] In a report about the use of SLIT against timothy pollinosis, antigen-specific IgG4 was significantly upregulated in the SLIT group compared to the placebo group, and the authors concluded that the upregulation of IgG4 was correlated with the improvement of symptoms compared with the previous year. However, the clinical score and medication score were not significantly different between the SLIT group and the placebo group.[45] A recent study of dairy administration of grass allergen tablets showed dose-dependent efficacy of the SLIT and the induction of blocking IgG. This report showed that the administration of 75,000 SQ-T (15 μg Phl p 5) dose significantly reduced the symptom and medication