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Original Paper

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

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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–

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 ≥ 10 mm) to a standardized cedar pollen extract (Torii Pharmaceutical Co., Tokyo, Japan), and a serum cedar and cypress pollen-specific IgE level score ≥ 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.
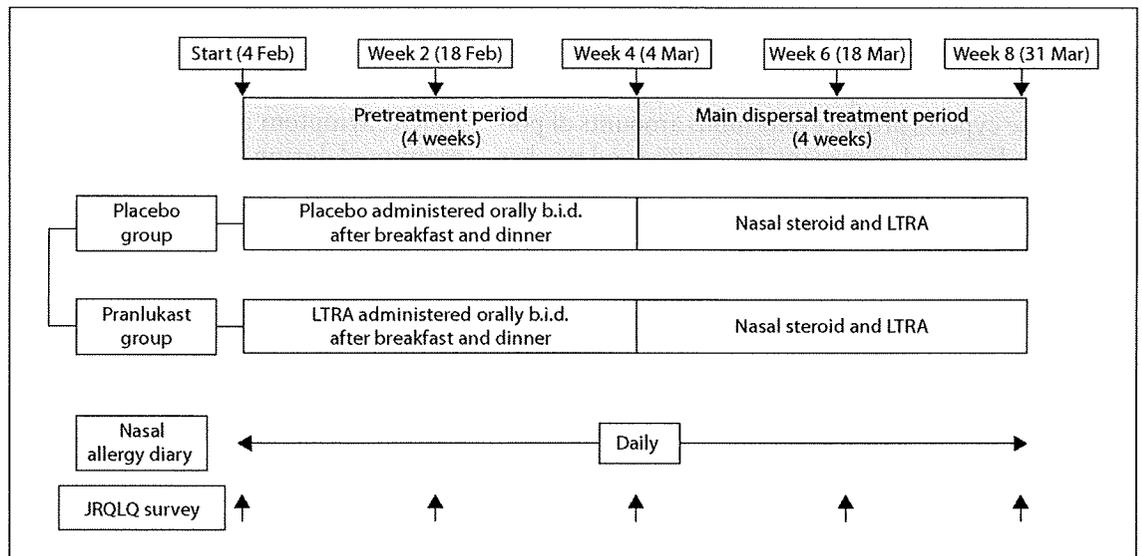
Fig. 1. Study schedule. b.i.d. = Twice daily.

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

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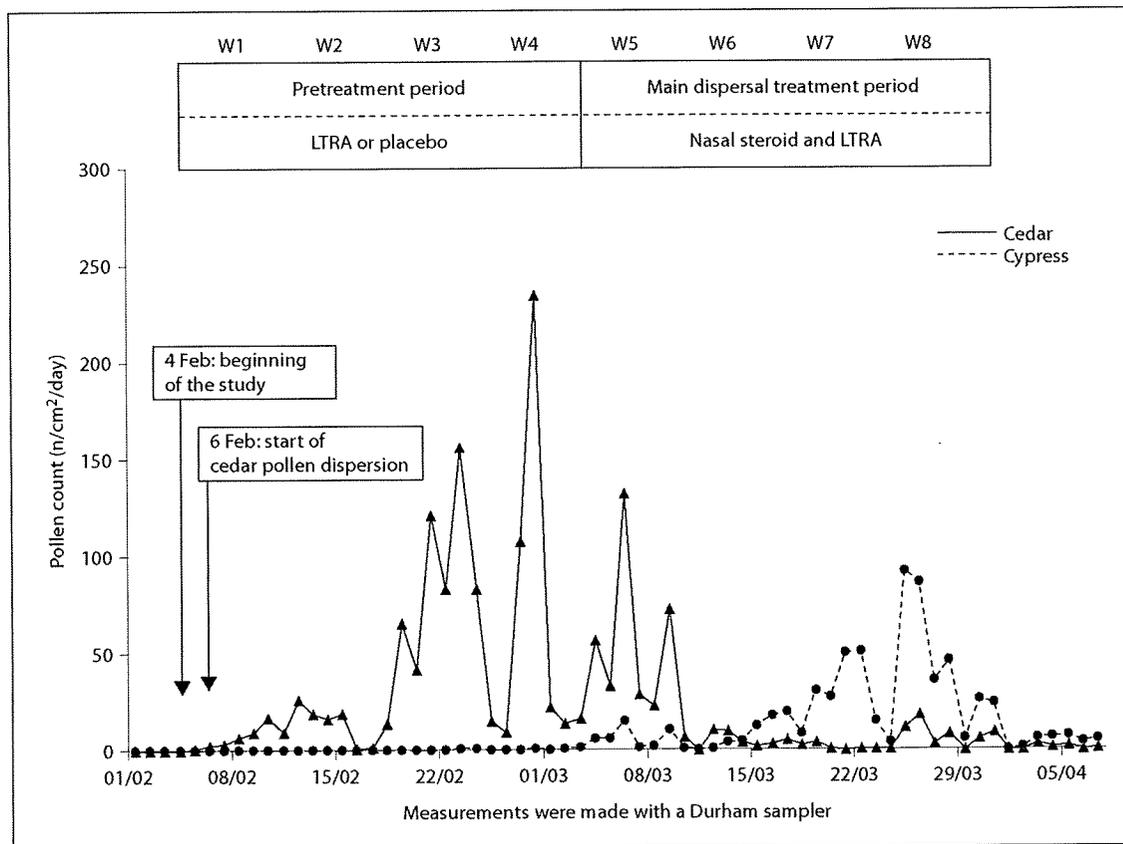
Fig. 2. Dispersal of cedar and Japanese cypress pollen in 2007 and study schedule.

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 $\geq 1/cm^2/day$. After 19 February, a pollen count $\geq 20/cm^2/day$ was obtained on most days, which dropped to $<10/cm^2/day$ 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 $>20/cm^2/day$ 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
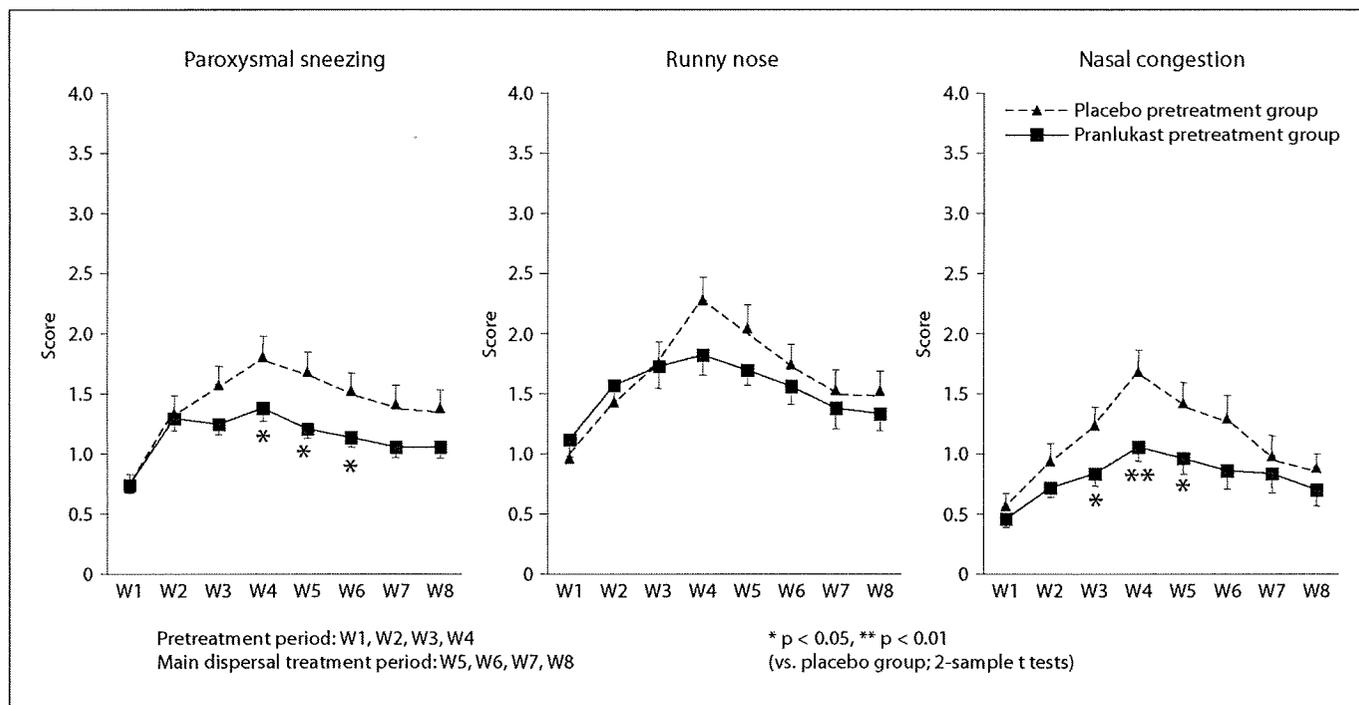
Fig. 3. Mean weekly score for each nasal symptom.

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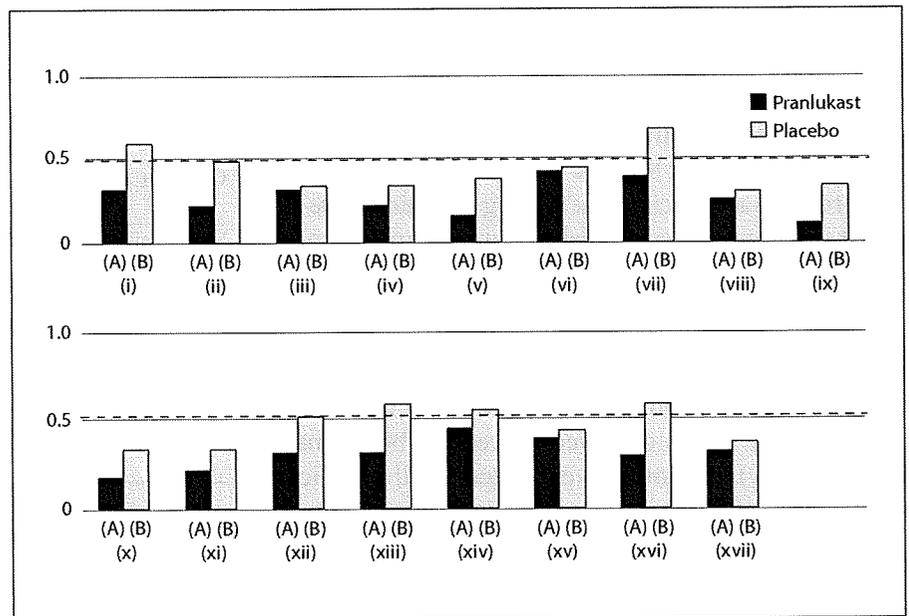
A comparative analysis of the improvement in nasal symptom score in week 8 (at the end of the study) and in week 4 (before the concomitant therapy with nasal steroids) is shown in table 2. The percent improvement in nasal congestion was significantly higher in LTRA-pretreated patients (69.0%) than in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

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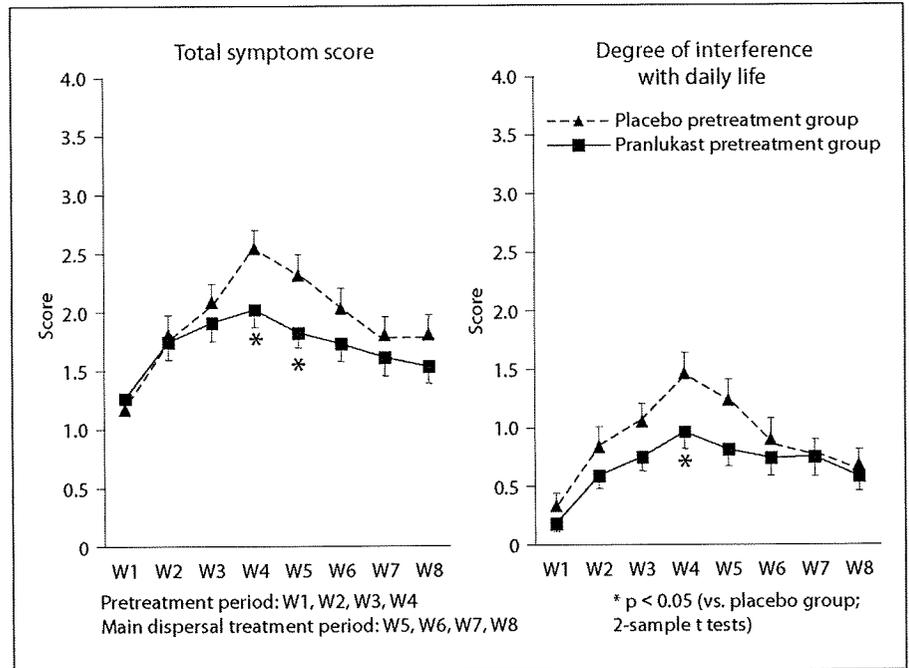
Fig. 4. Symptom scores and the degree of interference with daily life.

Total symptom score — Degree of interference with daily life

Placebo pretreatment group
Pranlukast pretreatment group

W1 W2 W3 W4 W5 W6 W7 W8
Pretreatment period: W1, W2, W3, W4
Main dispersal treatment period: W5, W6, W7, W8

W1 W2 W3 W4 W5 W6 W7 W8
$p < 0.05$ (vs. placebo group; 2-sample t tests)

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.

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ORIGINAL PAPER | Asthma and Rhinitis

Seasonal changes in antigen–specific T-helper clone sizes in patients with Japanese cedar pollinosis: a 2-year study

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Clinical and Experimental Allergy

Summary

Background Allergic rhinitis (AR) is a typical type I allergic disease that occurs through the induction of allergen-specific effector T cells. Once established, new effector T cells derive mostly from memory T cells that are capable of surviving for extended periods, although the mechanisms by which these memory functions are maintained have not yet been clarified. In particular, the exact life-span of memory T cells is still not well understood.

Objective Pollinosis patients seemed to be suitable subjects to investigate because such patients are exposed to antigens strongly for only a limited period once a year. We compared the seasonal changes in memory T-helper type 2 (Th2) between pollinosis and perennial allergic subjects.

Methods The clone sizes of the Japanese cedar pollen-specific memory Th cells were measured by an ELISPOT assay using specific peptides from the patients with cedar pollinosis, and the seasonal changes were noted. This study was performed for 2 years. The cedar-specific IgE levels in the peripheral blood were also studied. Mite allergy patients were also enrolled in the study.

Results The Japanese cedar-specific IL-4-producing Th2 cells were detected in all patients examined, although the number of cells was low. These Th memory cells 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. The cedar-specific IgE levels exhibited changes similar to the cedar-specific Th cells. On the other hand, there was no drifting of Th memory clone size with the mite allergics, and the IgE levels also did not change.

Conclusions While pollen-specific Th cells decreased after pollen exposure, their memory functions continued. Memory clone size maintenance therefore requires repetitive antigen irritation.

Keywords allergic rhinitis, clone size, IgE, memory T cell, Th2
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Introduction

In recent years, many countries have experienced an increase in the prevalence of allergic rhinitis (AR) [1, 2]. In Japan, Japanese cedar (*Cryptometria japonica*) and Japanese cypress (*Chamaecyparis obtusa*) pollens are considered to be the major unique allergens and their extent of dispersal is quite large, travelling more than 100 km and thus causing serious pollinosis [3, 4].

Pollinosis is thought to be an adaptive immune response that manifests as a type I allergic reaction, and it occurs as a consequence of fundamental allergenic me-chanisms involving the induction of pollen-specific T-helper type 2 (Th2) effector cells from naïve Th0 cells [5]. Most effector T cells are short-lived, but few effector T cells become long-lived memory T cells. Once a memory T cell is established, it retards the induction of new effector T cells from naïve Th0 cells, according to the principle of the 'original antigenic sin'. Hence, most effector T cells are derived from memory T cells [6–10]. This concept describes a phenomenon in which the antibody response elicited in an individual after a secondary viral infection reacts more strongly to the viral variant that originally infected the individual. A similar phenomenon

is likely to be observed in Th cell responses as they play critical roles in promoting antibody responses.

Patients with type I allergy are thought to have allergen-specific memory Th cell clones. Immune-therapeutic intervention directed at diminishing the size of these clone memory Th2 cells and shifting the cytokine type of memory Th clones is thought to play a considerably important role in finding a complete cure.

However, the mechanism by which the antigen-specific Th memory clone is maintained in an allergic patient has not yet been clarified. There are several reports describing the peripheral blood to be re-stimulated by a specific antigen, while the Th2 cytokine response was measured both in season or/and out of season [11–13] as well as before and after immunotherapy [14–16]. In those studies, peripheral blood mononuclear cells (PBMCs) were stimulated by an antigen, and measured Th2 cytokine or its mRNA. However, those cytokine responses that were re-stimulated by whole antigen reflected various kinds of cells such as T cells, B cells, macrophages and antigen-presenting cells and did not precisely reflect the function or the exact number of antigen-specific Th cells. Because the Th cell response is restricted in major hitocompat-ibility complex class II, it is necessary to use the Th cell epitope of class II restrictive to measure the reaction only for Th cell clones respond to allergen. Our purpose is to estimate the duration of life-span of allergen-specific memory T cells. We therefore directly examined the number of specific Th2 cells to respond to class II restric-tive T cell epitope, which matched with Japanese human leukocyte antigen (HLA) variation. These kinds of studies have not yet been carried out. Pollinosis seemed to be a suitable subject to investigate the life-span of Th memory clones because patients are exposed to the antigen for only a limited period. In the present study, we examined the specific memory clone size, which is a population size of memory T cells that recognizes the same specific HLA restrictive epitope and produces isologous cytokine. We tried to detect IL-4 productive cells using only seven T cell epitopes; as a result, the total summation of these seven kinds of clones is the clonal size.

The limited seasonal nature of antigen exposure is useful for elucidating the mechanisms used in the main-tenance of Th memory clone sizes. We examined the Japanese cedar pollen-specific Th clone sizes and the associated seasonal changes in patients with cedar polli-nosis, while also comparing the yearly change in the clone size due to pollinosis with that due to perennial mite allergies that were detected by 14 T cell epitopes.

Materials and methods

A total of 41 patients with Japanese cedar pollinosis were enrolled in this study. The ages of the 20 males and 21 females ranged from 20 to 51, with an average of 31.1 years. The diagnosis of Japanese cedar pollinosis was based on the occurrence of typical nasal symptoms during the cedar pollen season and the detection of Japanese cedar-specific IgE by CAP-RAST (score: 2 or more). All patients had symptoms for at least 3 years. None of the patients received immunotherapy or immunosuppressive drugs (including steroids) within 8 weeks before the start of the study. The study received prior approval from the Ethics Committee of the Chiba University (Chiba, Japan). A written, witnessed informed consent was obtained from all patients. The study design is shown in Table 1. From 2003 to 2004, 23 patients participated in this study, and from 2004 to 2005, another 18 patients were enrolled. Twenty-two patients with perennial AR due to mite were also enrolled in this study. The ages of the 10 males and 11 females with perennial AR were from 20 to 48, with an average of 28.8 years. The diagnosis of mite allergy was based on the occurrence of typical nasal symptoms and the detection of Der f IgE by CAP-RAST (score: 2 or more).

Ten healthy subjects were also enrolled as controls. They were all negative for symptom episodes and allergen IgE.

The blood samples were collected every 3 months after July, and the PBMCs were obtained by the Ficoll–Hypaque method from the patients. The samples were stored in liquid nitrogen until analysis.

Clinical symptoms

The nasal symptoms were evaluated on a scale from 0 to 4 in accordance with the practical guidelines for the treat-ment of AR [17], as follows: 0, no sensation; 1, mild; 2, moderate; 3, severe; and 4, extremely severe. Daily episodes of sneezing and nose blowing were rated 0 to 4, as follows: 0, none; 1, 1–5 episodes; 2, 6–10 episodes; 3, 11–20 episodes; and 4, >20 episodes. The medication was also recorded according to drug characteristics and dura-tion of usage, according to the guidelines, as follows: anti-histamine, mast cell stabilizers and vasoconstrictor were 1, and topical ocular or nasal steroids were 2.

Reagents

Antibodies. The monoclonal antibodies (MoAb) used for the ELISPOT assay were acquired from MABTECH (Stock-holm, Sweden). For coating, MoAb 82-4 and MoAb 1-DIK were used to coat human IL-4 and IFN-γ, respectively. For detection, MoAb 12-1 and 7B6-1 were used to detect human IL-4 and IFN-γ, respectively. For the FACS analy-sis, anti-human Cy5-conjugated CD4, FITC-conjugated IFN-γ and PE-conjugated IL-4 were purchased from Dako (Tokyo, Japan).

Peptides. A recombinant hybrid peptide was used for the ELISPOT assay. This peptide comprised of the seven CD4 T

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Table 1. Time schedule for examination
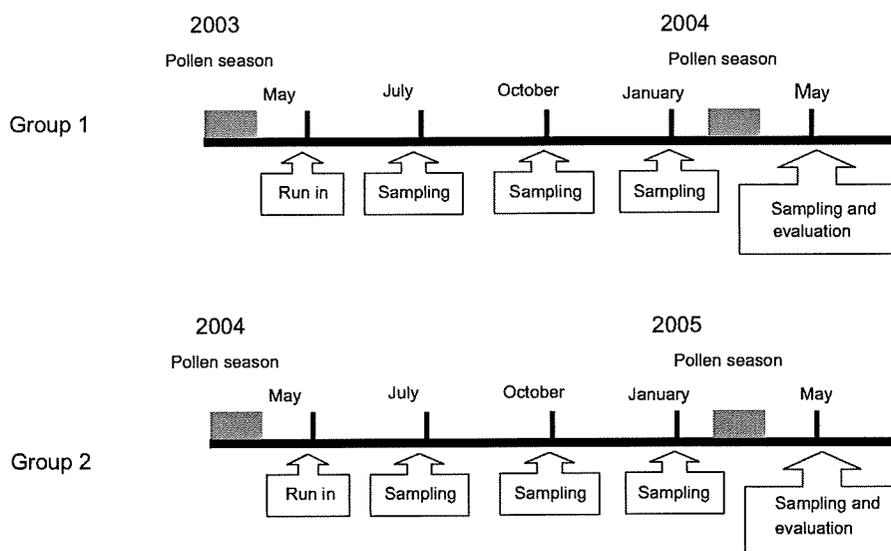
The Group 1 patients were recruited after the pollen season of 2003 and the Group 2 patients were recruited after pollen season of 2004. The blood sampling was performed every 3 months starting in July. The last blood sampling was performed after the pollen season of May, and the samples were analysed simultaneously.

cell determinants of Cry j 1 and Cry j 2, and the major Japanese cedar pollen allergens. Almost all the patient populations responded to this hybrid peptide, which is comparable to the response to Cry j 1 and Cry j 2. Moreover, because the seven peptides do not contain IgE-binding residues of *C. japonica* allergens, the recombinant peptide will not directly influence IgE-bearing cells such as mast cells, basophils and B cells [18]. The recombinant peptides for mite allergy were also prepared. This peptide comprised of the 14 CD4 T cell determinants of Der f 1 [19] and Der f 2 [20], and the major mite allergens. Almost all the patient populations responded to these peptides, which is comparable to the response to Der f 1 and Der f 2. Those peptides were Class II restricted and recognized Th cells only.

ELISPOT

The ELISPOT assay was performed according to the manufacturer's instructions. Briefly, the anti-human IL-4 or IFN-γ MoAbs were diluted to a concentration of 15 µg/mL in sterile, filtered (0.45 µm) PBS (pH 7.2), and 100 µL/well was added onto nitro-cellulose plates (Millititre, Millipore Corp., Bedford, MA, USA). The plates were incubated overnight at 4 °C and the unbound antibodies were washed with filtered PBS thereafter. After the last wash, the PBS was sucked through the membrane under vacuum

(Millipore Corp.). One hundred microlitres of the pre-stimulated cell suspension was added to each well in duplicate, and the plates were incubated for 10 h at 37 °C. The cells were subsequently washed before adding 100 µL of the biotinylated MoAbs (1 µg/mL), and were then incubated for 2 h at room temperature. The plates were then washed and incubated for 90 min at room temperature with 100 µL of streptavidin alkaline phosphatase (Mabtech, Stockholm, Sweden) at a dilution of 1 : 1000. The unbound conjugate was removed by another series of rinsing before 100 µL of BCIP/NBT substrate solution (Bio-Rad, Richmond, CA, USA) was added, and the plates were incubated at room temperature until dark spots emerged (1 h). The colour development was stopped by repeated rinsing with tap water. After drying, the spots were captured photo-electrically and counted by a computed analysis to avoid any visual bias, using Auto Counter (ImmunoScan, CTL, Gmünd, Germany).

Pollen counts

The combined annual cedar and cypress pollen counts were measured using Durham pollen samplers.

Statistical analysis

Wilcoxon's paired rank sum tests were used to compare the mean values. A Friedman two-way ANOVA was used to

analyse paired data. Peason's tests were used to determine the correlation coefficients.

Results

The cedar and cypress pollen counts determined using the Durham samplers were 470 cm/season in 2004, which was 1/20 of the average for the last 10 years. In 2005, we obtained 7852 cm/season, which was threefold higher than that of the average for the last 10 years. The seasonal symptoms were comparatively mild in 2004, and comparatively serious in 2005. The mean symptom-medication scores during the pollen season in 2004 and 2005 were 1.4 ± 2.1 and 2.8 ± 2.3 (mean $\pm$ SD), respectively.

Before the study, we compared the peptide-specific Th2 clone size of the patients with that of healthy controls in October, which is off pollen season. After stimulation with 10 nmol/L of cedar-specific peptides, the mean values of the IL-4 spot from the healthy controls and patients with cedar pollinosis that were obtained are shown in Fig. 1. Positive spots were obtained only in the samples from the pollinosis patients, but not from controls (A), although spots were equally obtained in the samples from both patients and controls when stimulated by Con A as a pan T cell stimulant (B). We also confirmed peptides for mite. The IL-4 spots were obtained only in the samples from the patients, but not from the controls (Fig. 2a), although spots were equally obtained in the samples from both patients and controls when stimulated by Con A as a pan T cell stimulant (Fig. 2b). Peptide-specific IL-4 spots were detected in all samples examined. Approximately 10–100 peptide-specific IL-4 spots were observed in the wells
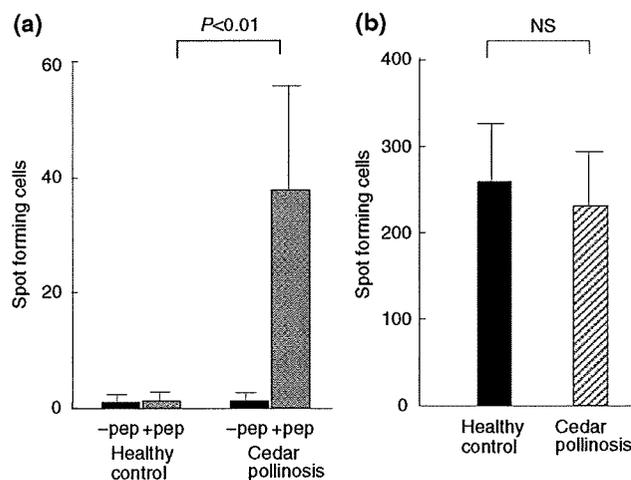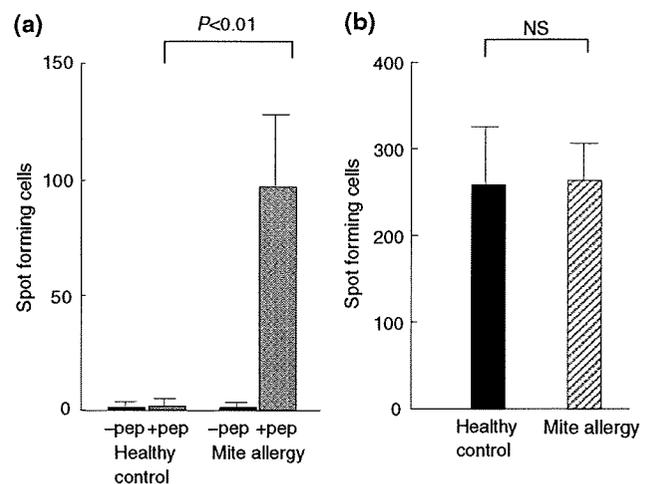

Fig. 2. The mean values of IL-4 spots in the samples from the healthy control subjects and patients with cedar pollinosis are shown. (a) 1×10^5 cells were stimulated by a peptide derived from Der f 1 and Der f 2. The spots were obtained only in the samples from the pollinosis patients, but not from the controls. (b) 1×10^4 cells were stimulated by Con A, a pan T cell stimulant. The spots were equally obtained in the samples from both the patients and the controls. NS, not significant.

incubated with 10^5 PBMC based on the ELISPOT assay. These spots were analysed in triplicate in each of the experiments and exhibited good reproducibility.

We carried out a depletion assay in advance with whole PBMC from three patients with Japanese cedar pollinosis using an antibody-conjugate magnetic bead kit (MACS system, Miltenyi Biotec GmbH, Tokyo, Japan). When CD4 was depleted, the spots of ELISPOT disappeared; however, after the depletion of CD8, the number of spots was equal to that with whole PBMC. The depletion of CD28 also caused the spots to disappear. These results show that the present ELISPOT assay using a hybrid peptide was CD4 restricted and the CD28 expression was indispensable for the detection of the spots (data not shown).

The seasonal changes in peptide-specific IL-4 spots are shown in Fig. 3. The IL-4 spots decreased after July, and were at their lowest in January before the onset of the cedar pollen season, which were almost 60% of those observed in July. The IL-4 spots increased during the cedar pollen season in 2004 despite the small amount of pollen (Fig. 3a). The same trend in seasonal changes was obtained during the 2005 season. The IL-4 spots decreased after July, and were at their lowest in January before the onset of the cedar pollen season, which were almost 60% of those observed in July (Fig. 3b). Interestingly, the clone size on May 2005 was 40% larger than that on July 2004. This phenomenon was not seen during the 2004 season.

The seasonal changes in the cedar-specific IgE levels in the serum of the patients are shown in Fig. 4. While the cedar-specific IgE decreased after the pollen season in 2003, it did not increase during the cedar pollen season in

Fig. 1. The mean values of IL-4 spots in the samples from the healthy control subjects and patients with cedar pollinosis are shown. (a) 1×10^5 cells were stimulated by a peptide derived from Cry j 1 and Cry j 2. The spots were obtained only in the samples from the pollinosis patients, but not from the controls. (b) 1×10^4 cells were stimulated by Con A, a pan T cell stimulant. The spots were equally obtained in the samples from both the patients and the controls. NS, not significant.

(a)

NS

P<0.01   P<0.01

P<0.05

(b)

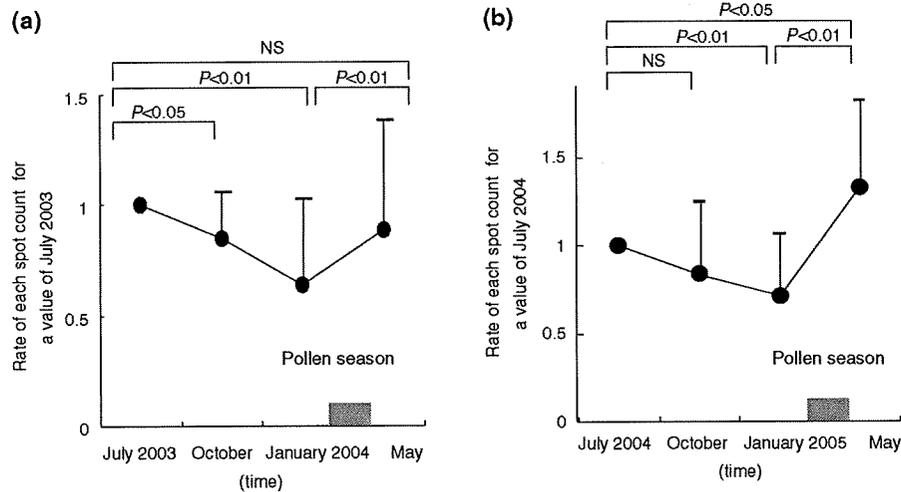P<0.05

P<0.01   P<0.01

NS

Fig. 3. The samples of each time-point were analysed simultaneously. The Cry j 1 and 2 peptide-specific IL-4-producing cells were counted using the ELISPOT technique. The rate of each spot count for the value in July is shown. (a) Twenty-three patients were enrolled during the 2004 season, which was characterized by very little pollen scattering. (b) Eighteen patients were enrolled during the 2005 season, which was characterized by massive pollen scattering. NS, not significant.

(a)

P<0.01

P<0.01   NS

P<0.01
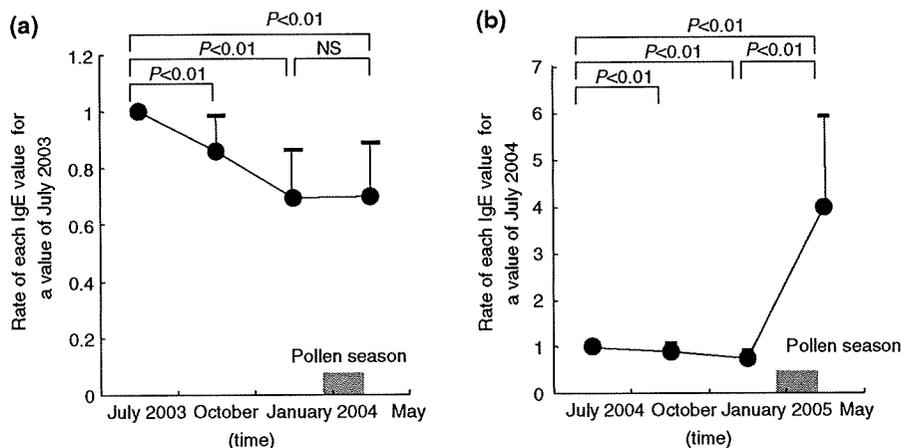

(b)

P<0.01

P<0.01   P<0.01

P<0.01

Fig. 4. The samples of each time-point were analysed simultaneously. Cedar-specific IgE were analysed using the RAST technique. The rate of each IgE for the value in July is shown. (a) Twenty-three patients were enrolled during the 2004 season, which was characterized by very little pollen scattering. (b) Eighteen patients were enrolled during the 2005 season, which was characterized by massive pollen scattering. NS, not significant.

2004 (Fig. 4a). The specific IL-4 spots decreased during the pollen season in some patients, and the cedar-specific IgE levels decreased during the pollen season in all patients. During the 2004–2005 season, the cedar-specific IgE levels decreased after the pollen season in 2004, but increased fivefold during the pollen season in 2005 (Fig. 4b). The number of cedar-specific IL-4 spots did not show a correlation with the cedar-specific IgE levels.

The yearly changes in the mite-specific IgE levels and Der f peptide-specific IL-4 spots were also examined in the samples from 22 patients with mite allergy. The yearly changes in mite-specific IgE and mite-specific memory Th2 clone size could not be clearly obtained (Fig. 5).

The patients with mite AR had persistent nasal symptoms all year around. Although the symptom scores varied among the patients as well as the seasons, the deviation was occasionally large; overall, no significant difference was observed. The pollinosis only demonstrated significant symptoms during the pollen dispersal season; however, the patients' nasal symptom scores during the pollen season were higher than those of the mite AR patients as shown in Fig. 6.

Discussion

We examined the Japanese cedar-specific IL-4-producing memory T cells in the peripheral blood of patients with
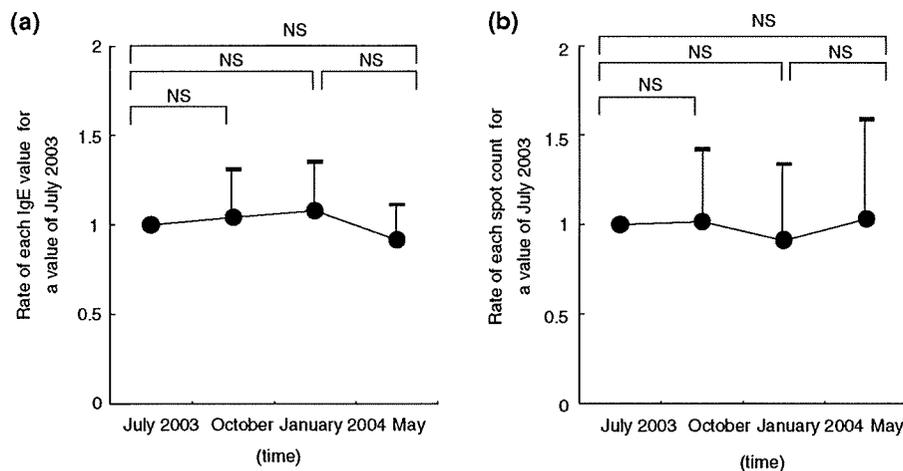
Fig. 5. The samples of each time-point were analysed simultaneously. Mite-specific IgE were analysed using the RAST technique (a). The Der f 1/2 peptide-specific IL-4-producing cells were counted using the ELISPOT technique (b). The rate of each value for the value in July is shown. NS, not significant.
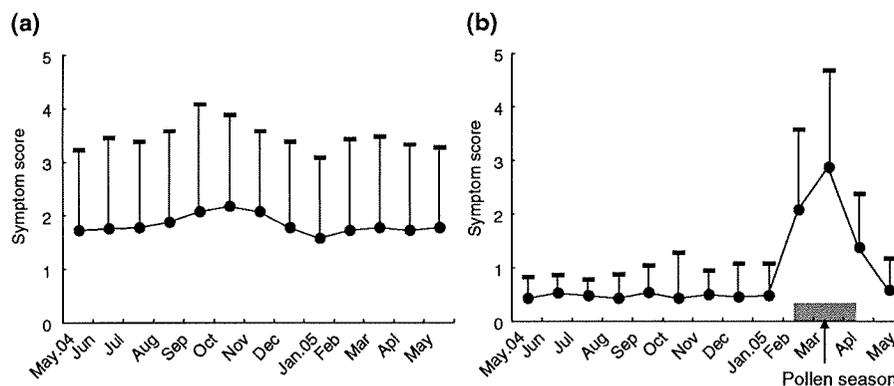

Fig. 6. The mean monthly symptom scores (mean+SD) from May 2004 to May 2005 are shown. (a) Mean nasal symptom scores of 22 patients with mite allergy. (b) Mean nasal symptom scores of 18 patients with Japanese cedar pollinosis.

Japanese cedar pollinosis by an ELISPOT assay using Japanese cedar-specific peptides. Although the number of cedar peptide-specific IL-4 T cells was low, the samples from all the patients examined had 10–100 spots/10^5 PBMC. In contrast, the healthy subjects had no positive IL-4 spot. These IL-4 T cells were thought to be cedar-specific Th2. The cedar peptide-specific IFN-γ T cells, which were thought to be cedar-specific Th1 cells, were detected in several patients. The cedar-specific peptides used in the ELISPOT assay were hybrid peptides that would only react with the T cells of those patients with Japanese cedar pollinosis, but not with the T cells of subjects not suffering from cedar pollinosis. Consequently, these findings could explain the low incidence and detection rates of cedar peptide-specific Th1 cells. The number of cedar peptide-specific IL-4 T cells (Th2 cells) did not correlate with the numbers of specific IFN-γ T cells (Th1 cells). More specifically, no relationship was observed between the cell types, and the cell numbers

appeared to be independent of each other (although cedar-specific serum IgE levels did not correlate with the number of cedar-specific IL-4 T cells, the patients who had specific IFN-γ T cells had lower specific IgE levels than those without specific IFN-γ T cells).

IgE production is likely to be controlled by many factors. IFN-γ T cells inhibit IgE production independently of the number of specific IL-4 T cells [21, 22]. Similarly, pollen-specific IgE is known to exhibit seasonal changes [23, 24], thus increasing during the pollen season and decreasing during the off-season. However, the amounts of cedar and cypress pollen in 2004 were extraordinarily low and the pollen counts, which were less than 1/20 of the annual average for the last 10 years, did not contribute to the enhancement of IgE production in the patients enrolled in this study. Conversely, the cedar peptide-specific IL-4 T cells increased by 30% despite the low levels of pollen exposure. The levels of Th2 clone size increased to levels equivalent to that in July 2003.

Although IL-4 is indispensable for IgE production, the cedar peptide-specific Th cells are thought to be very sensitive to exposure to cedar pollen.

In contrast, because the amount of pollen in 2005 was large, the cedar-specific IgE levels increased to five times after the pollen season. The specific Th2 clone size increased considerably after the pollen season, too, and the clone size increased by 70% after the pollen season. The levels of clone size increased to 1.4 times more than those observed in July 2004. A memory clone size might be affected by allergen exposition considerably. While cedar peptide-specific IL-4 Th2 cells decreased in number during the off-season, 8 months after the cessation of the pollen season, more than 60% of these memory Th2 cells were still found. Such annual drifting was absent in the perennial-allergic subjects. Therefore, pollinosis that undergoes exposition for a very limited period is different from mite perennial allergies.

Indeed, it is difficult to estimate the half-life of allergen-specific Th2. Generally, memory T cells are thought to have a long life-span, considering the duration of the vaccine effect of viral infections. Almost all reports of antigen-specific memory T cell have so far been about infectious diseases, while only a few reports of memory T cell are about type I allergy. The simple theory that an antigen creates a pool of long-lived antigen-specific memory T cells has been surprisingly difficult to prove and it is also still not completely accepted. The issue of longevity is much more controversial for $CD4^+$ than for $CD8^+$ T cells. Regarding $CD4^+$ memory T cells, lymphocytic choriomeningitis virus (LCMV)-specific $CD4^+$ memory T cells [25] and Sendai virus-specific $CD4^+$ memory T cells [26] at first disappeared dramatically, whereas virus-specific $CD8^+$ T cells were fairly stable in numbers over the same period. In humans, however, there have been several reports of vaccinia virus-specific $CD4^+$ memory T cells with a long life-span. These cells were found to decline with a half-life of approximately 10 years, conferring long-term protection [27]. The question remains, however, as to whether these memory Th cells are maintained in the absence of an antigen or by contact with a persistent antigen, bound in the immune complexes on follicular dendritic cells, or by contact with other environmental cross-reactive antigens. Based on our results on memory Th2 cells from cedar pollinosis in comparison with those from perennial mite allergy, the clone size maintenance requires antigen irritation of repetition and clonal mitosis by it. Therefore, the half-life of allergen-specific Th2 might be estimated to be less than 1 year.

The mechanism wherein a daughter Th2 cell discharges Th2 cytokines such as IL-4 in the same way after mitosis from the original Th2 has not been well understood. The opening and closing of chromatin helps to determine the cellular characteristics. It is believed that acetylation and methylation of the chromatin are very stable. Therefore, it is thought that the characteristics of a parent cell are transferred to the daughter cells after mitosis. However, a splitting enzyme for the acetylation and methylation has been discovered in a recent study, and it does not always seem to be stable [28]. It has recently been proposed that the opening and closing of chromatin is induced by an antigen, and that this is due to the Th cells' response [29, 30]. These changes in chromatin may therefore play an important role in retaining the memory functions of Th cells. Further studies need to be conducted on memory retention in order to clarify how these mechanisms could be applied to the development of an effective and fundamental solution for the treatment of allergic diseases.

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特集Ⅱ 花粉症をめぐる現状と将来

自動花粉測定器を用いたリアルタイム花粉測定とその問題点*

岡 本 美 孝**

は じ め に

　正確，詳細な花粉飛散情報の提供は，花粉症患者の花粉曝露の回避，ひいてはよりよいセルフケアーをするために必要である．また，詳細な花粉飛散予報の構築にも不可欠である．これまで飛散花粉の測定はダーラム法やバーカード法により自然落下や吹き付けにより，スライドグラスやテープ上に付着した花粉を染色後，検鏡によるカウントにより行われてきたが，手間がかかり，またリアルタイムでの測定はできなかった．そこで最近，一定量の大気を吸引しながらその中に含まれる花粉を，大きさ，形状，偏光度，あるいは花粉の放つ自然蛍光などを利用して他の空中浮遊物質と鑑別して測定する自動花粉測定器が開発されている．これらの自動花粉測定器はリアルタイムで花粉を測定することが可能で，詳細な飛散情報の提供に有効な手段となることが期待されている[1]~[4]．しかし，これらの測定器の性能について必ずしも十分な検討は行われていない．これまで行った検討結果と問題点について概説する[1]．

各種自動花粉測定器の特徴（表 1）

1．KH3000

　現在もっとも多く設置されている．とくに，環境省は東北から九州まで100台近くを設置してその情報は「はなこさん」としてネット上に公開している．吸引ポンプで大気を吸引し，レーザー照射により花粉などの粒子が存在すると光が散乱するが，その散乱パターンから粒子の大きさを判別し，散乱光の数から粒子の数が算定される．花粉粒子として粒径(20~30μm)，形状から識別する．砂塵除去の手段として砂塵容器を用いて10μm以下の粒子を除去している．大気吸入量はヒトの呼吸量に相当する4.1l/分に設定されている．

2．KP1000

　比較的多く設置されており，環境省は関東，関西に約20台，東京都も採用している．本機種の大きな特徴は，花粉に紫外線を当てると特有の蛍光を発することを利用して検出の特異性を高めただけでなく花粉の種類の識別も可能になったとしている点である．粒子サイズも光散乱信号から測定してより特異性を高めている．砂塵除去にはバーチャルインパクタを使用している．大気吸入量はKH3000と同様に4.1l/分に設定されている．

3．NTT

　やはり，レーザー光による光散乱信号を用いて粒径から花粉を識別している．砂塵は沈降速

表1　各種自動花粉測定器の特徴

	大和（KH3000）	NTT	與和（KP1000）	神栄	NTT－神栄
花粉粒子識別手段	・粒径の識別 ・形状（長・短径）の識別	・粒径の識別	・粒径の識別 ・蛍光による識別	・粒径の識別 ・偏光度による識別	・粒径の識別 ・偏光度による識別
砂塵除去手段	・砂抜き容器により10μm以下の粒子除去	・2段階の分別手段で10μm以下，50μm以上の粒子を分別	・バーチャルインパクターを使用して微粒子を除去	・100μm以上の大粒子フィルターの使用	・100μm以上の大粒子フィルターの使用
吸気吸入量	4.1*l*/分	30*l*/分	4*l*/分	0.9*l*/分	2.2*l*/分

問題点：ノイズが大きい（微粒子をカウントしている），とくに花粉飛散が少ないときにダーラム法との相関が低い（相関係数0.3～0.5）．
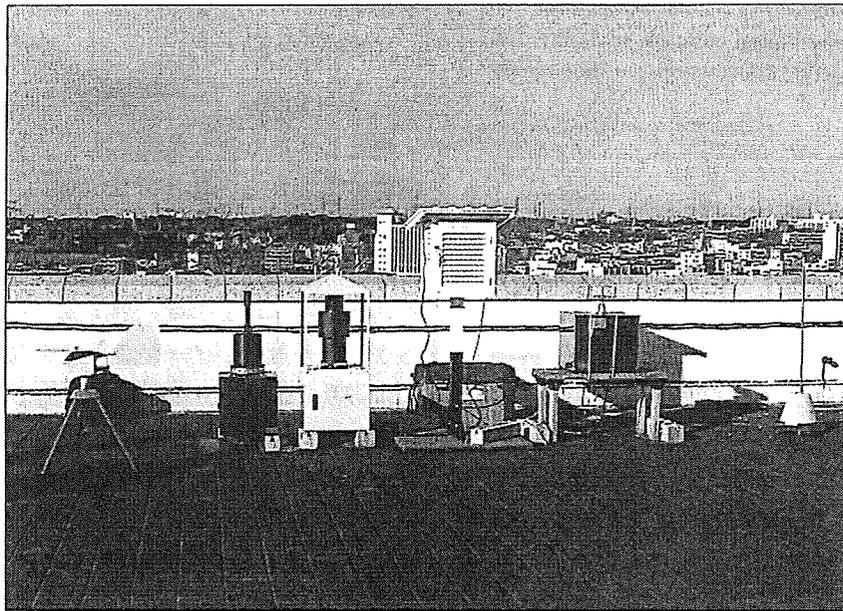
図1　設置の様子（千葉大学医学部屋上）
バーカード，NTT－神栄，NTT，神栄，KP1000，ダーラム，KH3000.

度，信号処理の2段階の分別手段で10μm以下，50μm以下の粒子を除去している．大気吸入量は精度を上げる目的で大きく設定され30*l*/分である．

4．神　栄

偏光フィルターを用いて粒径のみならず偏光度から花粉と浮遊粒子を区別するとされている．フィルターを用いて100μm以上の砂塵を除去している．大気吸入量は0.9*l*/分と少ない．

5．NTT－神栄

神栄を専用筐体に設置し，かつ大気吸入量を2.2*l*/分に増加させたものである．

自動花粉測定器の機能評価

自動花粉測定器の性能については開発メーカー
から検討結果が報告され，またほかにも報告が散見できるが，労力と時間のかかることもあって，詳細な検討は意外に行われていない．そこで，これら自動花粉測定器と従来から花粉測定に用いられているダーラム花粉採集器，バーカード花粉採集器を千葉大学医学部屋上の同一地点に設置し，さらに風速計も併設して詳細な検討を行った（図1）．

1．ノイズの影響

砂塵，空中浮遊粒子によるノイズの処理は自動花粉測定器の精度を高めるために重要である．関連の影響はいずれの機種も少ないとこれまで報告されているが，「日」としての平均でみた結果を検討しており，自動花粉測定器として意義