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ORIGINAL ARTICLE

Evaluating the Effects of Testing Period on Pollinosis Symptoms Using an Allergen Challenge Chamber

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ABSTRACT

Background: We previously built a pollen challenge test unit (allergen challenge chamber: ACC) to collect objective data about Japanese cedar pollinosis. In this study, we investigated adequate conditions for pollen challenge using the ACC.

Methods: The study consisted of two parts. The first part was conducted in November, which is not in pollen season. Subjects were exposed to Japanese cedar pollen at a concentration of 50,000 grains/m^3 in the chamber for 120 min each day over the course of three consecutive days. The second part was conducted in April, which is just after pollen season. Subjects were exposed to Japanese cedar pollen at the same concentration (50,000 grains/m^3) in the chamber for 90 min on a single day. Subjects recorded nasal and ocular symptoms before challenge and every 15 min after challenge initiation. The minimum cross-sectional area in the nasal cavity was measured using acoustic rhinometry before and after challenge as an indicator of nasal obstruction. Inflammatory markers in nasal lavage fluid and serum were also measured before and after challenge.

Results: Nasal and ocular symptoms were significantly exacerbated after challenge on all days of the single and 3-consecutive-day challenge tests, particularly on the third day of the consecutive challenge test. Nasal and ocular symptoms were also quickly induced with challenge immediately after the end of pollen season. No significant changes in inflammatory markers were seen.

Conclusions: Care is needed with regard to pollen challenge conditions in the ACC, including timing of the challenge, to induce pollinosis symptoms that accurately reflect chronic inflammation.

KEY WORDS

allergen challenge chamber, allergic reaction, priming effect, seasonal allergic rhinitis

INTRODUCTION

The prevalence of cedar pollinosis has steadily increased in Japan, reaching 30% in some regions and age groups.[1] Although pollinosis is not life threatening, it markedly reduces quality of life and productivity,[2-5] leading to both economic and social problems.

Many clinical studies evaluating the effects of cedar pollinosis have used placebo-controlled double-blind methodology and field techniques involving cedar pollinosis patients.[6,7] However, in this sort of study, the accuracy of evaluation may be hampered by variations in pollen count and climatic conditions between years and regions.

Accurate induction tests and evaluation of the pharmacological effects of drugs require objective observation and the maintenance of identical conditions during pollen challenge. These requirements can be met using an allergen challenge chamber (ACC). Such controlled chambers, allowing the acquisition of objective data, have been established in Western countries and Japan.[8-10]

We established an ACC in Osaka in 2005, and have conducted pollen exposure trials under various conditions using this chamber. We found that the temporal and spatial variations of the pollen level in the ACC

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were small, facilitating stable pollen challenge, and that pollinosis symptoms could be induced in volunteers with cedar pollinosis.

However, sensitivity to antigens is known to be higher in the latter part of the pollen season as a result of the priming effect from repeated exposure to antigens.[11] Therefore, to induce symptoms that match those occurring in real life, we must also consider the effects of when the trial is actually conducted. In trials that use a pollen challenge chamber during non-pollen seasons, the absence of this priming effect cannot be ignored and appropriate pollen exposure conditions need to be established.

Using volunteers with cedar pollinosis as subjects, we compared the induction of symptoms using the ACC immediately after the end of pollen season and during the non-pollen season.

METHODS

The ACC system consists of a challenge chamber in which subjects are actually exposed to pollen, a machine room to prepare pollen, an air shower room to remove pollen on leaving the challenge chamber, a consultation room in which a physician examines the patient regarding symptoms, a test room for objective testing, a changing room for patients to change into a clean suit, and a waiting room in which the test is explained to patients. The area and ceiling height of the challenge chamber are approximately 21.8 m^2 and 2.5 m, respectively, with a maximum capacity of 12 people. Patients in the challenge chamber can be observed through a one-way mirror. The challenge chamber is controlled at a constant temperature (summer: 25 ± 3℃, winter: 23 ± 3℃) and relative humidity (summer: 50 ± 10%, winter: 45 ± 10%) using an external air-processing air conditioner, and it can be ventilated with clean air from which dust is removed using a high-performance filter (HEPA filter). Employing a cylinder feeder-type powdering device (Aerosol generator RBG-1000R; Palas Co., NJ, USA), cedar pollen was dispersed during the tests into the compressed air supply while controlling the level, and homogenously sprayed in the challenge chamber via 12 Anemostat-type diffusers installed in the ceiling. The pollen level was determined by the aspiration method, measuring aspirated particles using a light-scattering particle counter for gross particles (KC-20; Rion Co., Ltd., Tokyo, Japan). Pollen in the challenge chamber can be removed through a filter box. The pollen level in the challenge chamber was secondarily diluted by adjusting the ventilation level of the outside air-processing air conditioner. The pollen level was adjusted to a specified level within about 20 minutes, and the maintenance level was adjusted corresponding to the real-time measured level (every 4 minutes). The ceiling and walls are finished with antistatic cloth to avoid the adherence of sprayed pollen.

SUBJECTS AND ASSESSMENTS

Subjects comprised 9 men and 23 women (mean age, 29.7 ± 1.09; range, 20-42 years) with mild or severe cedar pollinosis symptoms. All subjects had a clinical history of Japanese cedar pollinosis and scores of 2 or more for Japanese cedar pollen on the CAP radioallergosorbent test (CAP-RAST), which is the most commonly used test to measure allergen specific IgE levels in sera. Individuals with significant anatomic abnormalities on nasal examination were excluded.

The study consisted of two parts. In the first part, conducted in November, which is not in pollen season, subjects were exposed to Japanese cedar pollen at a concentration of 50,000 grains/m^3 in the chamber for 120 min per day over the course of three consecutive days. In the second part, conducted in April, just after pollen season, subjects were exposed to Japanese cedar pollen at the same concentration (50,000 grains/m^3) for 90 min on a single day. Four men and nine women were enrolled in the first part of study and five men and sixteen women were enrolled in the second part of study. Two subjects participated in both studies.

Nasal and ocular symptoms (sneezing frequency, nose-blowing frequency, nasal obstruction, and ocular itching) were recorded before challenge and every 15 min after challenge initiation. For the evaluation of nasal obstruction and ocular itch, we used visual analog scales (VASs); subjects marked a site on a 10-cm line corresponding to the symptom severity on which absence of symptoms was designated as 0 and worst imaginable symptoms as 10. To objectively evaluate nasal obstruction, the minimum cross-sectional area of the nasal cavity was also measured using acoustic rhinometry (SRE2100 Rhino Metrics, Lynge, Denmark) before and after challenge.

NASAL LAVAGE AND SAMPLE PROCESSING

The nasal lavage was performed by instilling 5 ml of sterile saline to each nostril according to the previous report.[12] To increase cell viability, sample processing was kept at 4℃. The serum was drawn subsequently, and the nasal lavage fluids and sera were separated from cells by a centrifugation at 1,500 x *g* to be stored frozen until analysis.

MEDIATOR ASSAYS

The level of ECP, IFN-γ, total IgE and specific IgE were measured by enzyme immunoassay (EIA; SRL Inc., Tokyo, Japan). IL-4 concentration was measured by chemiluminescent enzyme assay (CLEIA; SRL Inc.). IL-5 concentration was measured by Enzyme-Linked immunosorbent assay (ELISA; SRL Inc.). Anti-allergy medications such as anti-histamines and steroid sprays were prohibited for one week before the trial, and people receiving desensitization therapy

were excluded.

The study was conducted in accordance with Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki. The study protocol was reviewed and approved by the Research Committee at Osaka Medical College. Informed consent was obtained from all subjects prior to study entry. Data were anonymously processed during analysis.

STATISTICAL ANALYSIS

Wilcoxon single-rank test was used to analyze symptom scores and VAS scores in each group. Results were expressed as the change from the score before pollen exposure.

RESULTS

SYMPTOM INDUCTION TEST IN THE NON-POLLEN SEASON

Changes in symptoms were investigated before and after challenges on three consecutive days. Changes in the four symptoms (sneezing frequency, nose-blowing frequency, nasal obstruction, and ocular itching) are shown in Figure 1. No significant symptoms were induced on the first day of challenge in the non-pollen season. However, each of the four symptoms became more severe with each day of challenge, and a significant increase was seen in cumulative values by the third day.

SYMPTOM INDUCTION TEST IMMEDIATELY AFTER POLLEN SEASON (Fig. 2)

Immediately after pollen season, significant symptoms were induced quickly, 15 min after the start of exposure.

No adverse events were observed during the course of these studies, and none of the subject used anti-allergy medicine during the test periods.

ACOUSTIC RHINOMETRY ANALYSIS (Fig. 3)

Significant decreases were seen in minimum cross-sectional area in the nasal cavity on the third day of challenge in the non-pollen season and after the single challenge immediately after the pollen season.

CHANGES IN EOSINOPHILIC LEUKOCYTE COUNT IN NASAL LAVAGE FLUID BEFORE AND AFTER CHALLENGE

The eosinophilic leukocyte count in nasal lavage fluid tended to be higher on the third day of exposure than on the first and second days, but this difference was not significant. No changes were seen before and after exposure in terms of eosinophil cationic protein (ECP), interleukin (IL)-4, IL-5, or interferon (IFN)-γ in nasal lavage fluid, total IgE in peripheral blood, or IgE specific to Japanese cedar pollen, ECP, IL-4, or IL-5 (data not shown).

DISCUSSION

Conducting the present study in our ACC offered scheduling advantages independent of pollen exposure season, although the results suggest that priming effect should not be overlooked in ACC studies.

Traditional seasonal allergic rhinitis trials are performed for up to several weeks during the pollen season. However, a number of variables, including differences in pollen levels over the course of the study, subject compliance, and recall bias, reduce the sensitivity of these trials. When trials are conducted in multiple locations, some of these variations may be exaggerated and make adjusting for differences much more difficult. These factors contribute to lack of sensitivity for detecting differences between treatments in terms of onset and duration of action, or differences between various doses of the same treatment. This is one reason why onset of action is an endpoint that is difficult to monitor in traditional studies and why it is rarely measured. Use of an ACC can overcome many of the problems associated with traditional trials.

ACCs were initially established in the 1980s,[13] and the first clinical report using an ACC was published in 1988.[14] There are only limited numbers of effective ACCs worldwide because a system capable of strictly controlling antigen particles is necessary. One condition required is the capability to maintain a specific pollen level for a certain period. It is also necessary to induce moderate to severe nasal allergy symptoms in allergic subjects while inducing no symptoms in non-allergic subjects.[15] To meet the increased needs for such facilities, the first ACC in Japan was developed in Wakayama in April 2005. We built a second ACC in Osaka in September 2005. The pollen level in the challenge chamber is secondarily diluted by adjusting the ventilation level of the outside air-processing air conditioner. The pollen level is adjusted to a specified level within approximately 20 min, and the maintenance level is adjusted corresponding to the actual level measured in real time (every 4 min).

Using an ACC, seasonal allergic rhinitis studies may be conducted both in and out of the pollen season. In-season studies allow control of many factors that cannot be regulated with traditional trial methodology. Additionally, less priming is required than in out-of-season studies, particularly for subjects who are already symptomatic. The reactivity of an allergic individual to a seasonal allergen should increase during the season because of the priming effect.[11] It is argued that the reactivity of participants to allergens in an ACC setting may differ from that observed in a natural environment.[16] In the present trial, we found that symptoms were induced more rapidly after the end of pollen season than with out-of-season exposure. With the 3-consecutive-day challenge in the non-pollen season, the severity of symptoms was

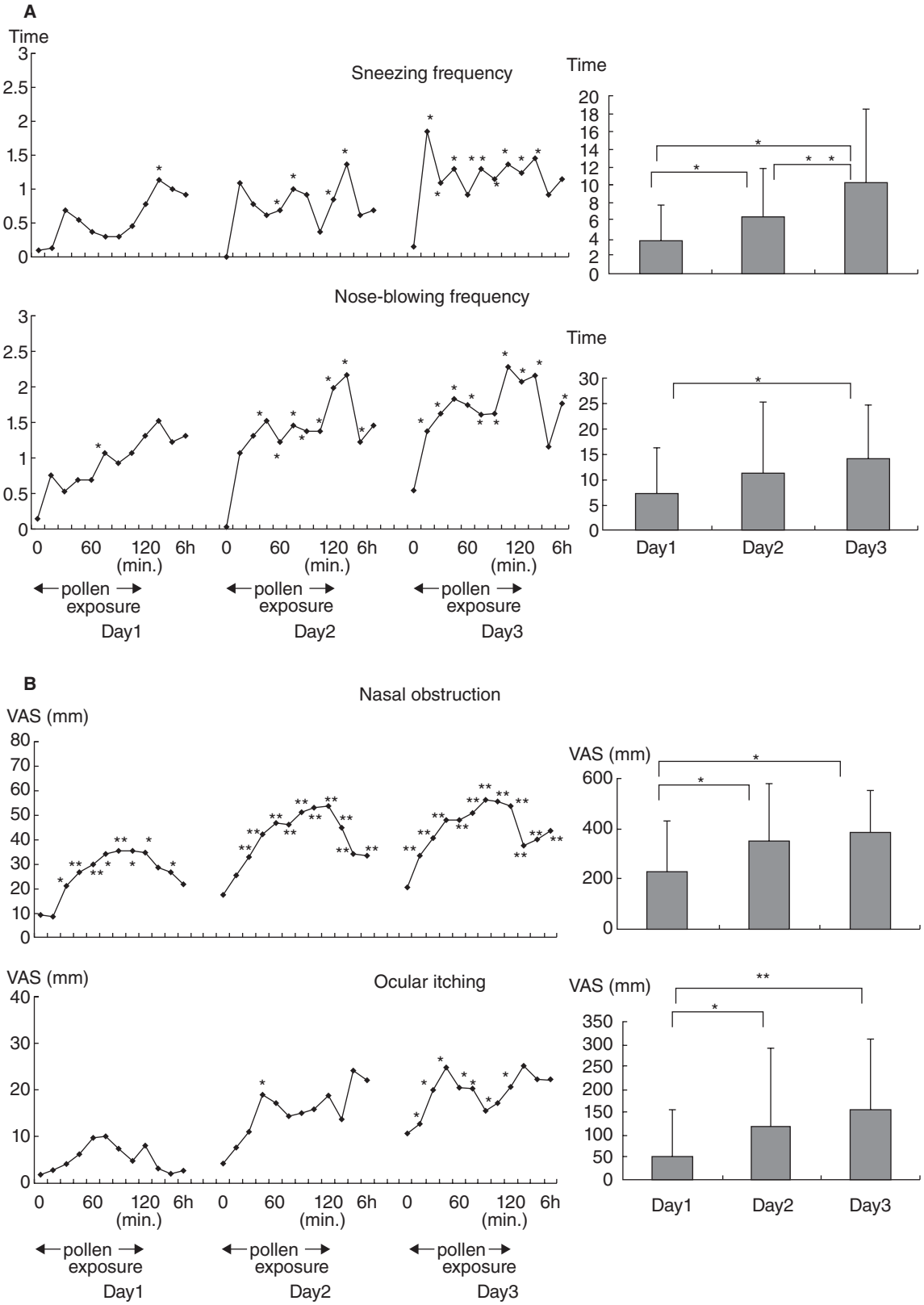

Fig. 1 Changes in symptoms after challenge initiation were investigated in 3-consecutive-day challenge tests. Four items, (**A**) sneezing frequency and nose-blowing frequency, (**B**) nasal obstruction and ocular itching, were significantly exacerbated after challenge on the third day of the consecutive challenge test.

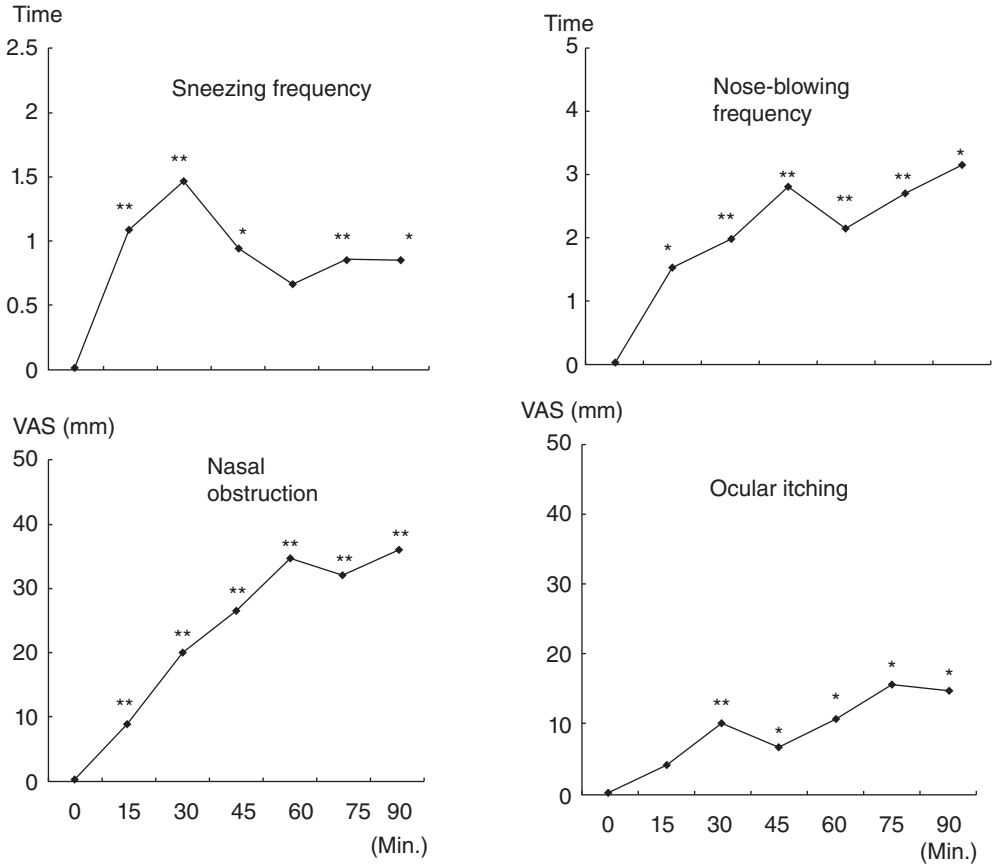

Fig. 2 Changes in the symptoms after challenge initiation were investigated in a single-day challenge test. Nasal obstruction and itch were significantly exacerbated after the challenge test. The four symptoms (sneezing frequency, nose-blowing frequency, nasal obstruction, and ocular itching) increased significantly compared with before the challenge after 15 min of pollen dispersal.

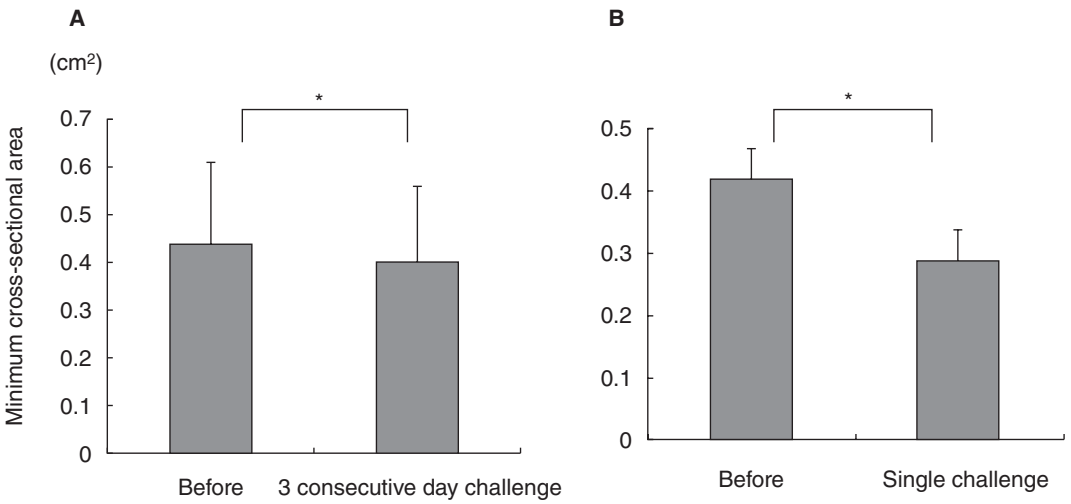

Fig. 3 Changes in minimum cross-sectional area of the nasal cavity measured with acoustic rhinometry. (**A**) Three-consecutive-day challenge during non-pollen season. (**B**) Single-day challenge immediately after the end of pollen season.

seen to increase proportionally with the number of days of challenge. These findings show a priming effect and may be influenced by a late phase reaction. Nasal allergy symptoms from challenge immediately after the end of pollen season are thought to be close to pollinosis symptoms in a natural environment. However, there are also influences from the regional and annual differences in the amount of pollen released into the air, and findings may differ from those with an ACC, which operates on the supposition of inducing symptoms under uniform conditions.

There are several ACCs in Japan. However, it is difficult to accurately compare the results obtained between units, because each chamber uses a different method to count pollen grains. Our ACC uses the KC-20, a laser particle counter which measures the number of particles between 10 and 100 μm in diameter. We attempted measurements with the KC-20 and the Durham method and the pollen counts measured by these two methods were well correlated. The pollen level in the chamber, 50,000 grains/m^3, corresponded to approximately 10 times the maximum count in the pollen-scattering season. There is a difference in the number of pollen that induces nasal symptoms observed in our ACC and in studies conducted in the natural environment. The priming effect of repeated antigen challenge increases sensitivity to the antigen in a natural environment.[16]

In recent years, pollinosis resulting from a single antigen has been decreasing and that from multiple antigens has been on the rise. Because a number of allergens and environmental factors may contribute to developing rhinitis, it has been argued that the single allergen exposure that is typically used in an ACC setting may not reflect the natural pathological process.[17] The degree of exposure to other sensitizing allergens is variable, since the level of previous environmental exposure cannot be controlled. Priming is a complex process that occurs naturally and in the ACC setting, and manifests as typical allergy symptoms.

Clinical trials using an ACC are suitable for scientific evaluations of various treatments for cedar pollinosis, but effective measurements and evaluation items other than clinical symptoms have not yet been identified. In the present study, no significant changes were seen in eosinophilic leukocyte count, ECP, IL-4, IL-5, or IFN-γ in nasal lavage fluid, or in total IgE and cedar pollen specific IgE, ECP, IL-4, or IL-5 in peripheral blood. Although allergy symptoms can be induced, it may not be possible to adequately induce chronic allergic inflammation in an ACC setting. To induce allergic rhinitis symptoms in the form of chronic allergic inflammation without priming from the natural environment, medium- to long-term continuous challenge during the non-pollen season is thought to be necessary, rather than the short-term challenge performed in the present study. This is not easy, however, because of social restrictions on volunteers. In future studies, it may be necessary to induce symptoms with short-term challenge while simultaneously identifying elevated the kind of mRNA in nasal lavage fluid and peripheral blood.

In conclusion, using an allergen challenge chamber, pollinosis symptoms, such as nasal and ocular symptoms, could be induced with good reproducibility even immediately after the end of the pollen season and in the non-pollen season, but differences were seen in the conditions required to induce symptoms. Although somewhat difficult from a practical standpoint, induction of pollinosis symptoms reflecting chronic allergic inflammation is thought to require medium- to long-term continuous challenge during the non-pollen season.

CONFLICT OF INTEREST

None of the authors has any financial interests or conflicts of interest to disclose.

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