

Characteristics of the Chiba Environmental Challenge Chamber

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ABSTRACT

Background: An environmental challenge chamber (ECC), which we refer to as the α -chamber, was built at Chiba University in 2008. The aim of this study was to validate the functionality of the ECC.

Methods: The stability of the pollen distribution and concentration in the ECC and symptoms of patients with Japanese cedar pollinosis induced by cedar pollen exposure were examined. Carryover effects of symptoms induced by different exposure protocols and correlations between symptoms induced in the ECC and those in the natural cedar pollen season were also determined. All the studies using the α -chamber were conducted out of the cedar pollen season.

Results: The severity of symptoms in the chamber reached a peak about 2 hours after the start of pollen exposure and plateaued thereafter. After subjects left the chamber, the symptoms persisted for several days. There was no significant difference between the severity of symptoms at exposure levels of 8000 and 12000 grains/m³. The symptoms were significantly increased by exposure for 3 consecutive days; however, there were no carryover effects in a study performed with a two-week interval. The total nasal symptom score (TNSS) in the natural pollen season showed a weak correlation with the mean TNSS on the day of exposure and the following 3 days. Symptoms in the ECC also had weak correlations with those in the early natural pollen season.

Conclusions: The ECC under well-controlled conditions is suitable for clinical studies and might accelerate development of treatment for seasonal allergic rhinitis. A complete evaluation requires inclusion of the persistent reaction after subjects leave the ECC.

KEY WORDS

environmental challenge chamber (ECC), environmental exposure unit (EEU), Japanese cedar pollen, Japanese cedar pollinosis, seasonal allergic rhinitis (SAR)

INTRODUCTION

Clinical trials of treatment for seasonal allergic rhinitis (SAR) have to be conducted during the pollen dispersal season. However, performance of a reproducible study is difficult due to annual differences in pollen amounts and weather. The amount of pollen exposure also differs by region and due to the lifestyle of each patient. For these reasons, the environmental challenge chamber (ECC) was developed to make it possible to conduct a study under standardized conditions. A well-designed ECC can be used to investigate

the efficacy of treatment for SAR, including drug dose finding, onset of action, and duration of action.¹⁻⁶ The use of ECCs is becoming more common, including a recent study of the onset of action of sublingual immunotherapy.⁷

Many kinds of pollen cause allergic rhinitis, including cedar, cypress, orchard grass, mugwort and ragweed.⁸ In Japan, Japanese cedar (*Cryptomeria japonica*) pollen is the major allergen and the prevalence of SAR induced by cedar pollen has increased in the last 10 years, with over 26% of the population now thought to be affected.⁹ However, it is difficult to examine the

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exact influence of cedar pollen on nasal symptoms because other pollens such as cypress and birch are scattered in a similar period to that of cedar pollen dispersal.⁸ Use of an ECC can avoid the confounding effects of multiple allergens and allow determination of the effect of each kind of pollen.

An ECC was built in Chiba University in 2008. This ECC, which we refer to as the α -chamber, can accommodate 50 subjects. The α -chamber is the second largest in capacity worldwide, after the ECC in Ontario, Canada.^{10,11} Pollen concentration and distribution can be examined precisely with 56 automatic pollen counters. In this report, we verify the performance of α -chamber using Japanese cedar pollen exposure and examine the nasal symptoms induced in the α -chamber.

METHODS

THE ENVIRONMENTAL CHALLENGE CHAMBER (α -CHAMBER)

The ECC at Chiba University was built in 2008, has an area and ceiling height of 71.8 m² and 2.6 m, respectively, and can accommodate up to 50 subjects. The control room and exposure room are separated by a glass window that allows medical staff to monitor subjects during a clinical study. Pollen (Japan Forest Tree Breeding Association, Tokyo) is supplied from 4 reservoirs outside the exposure room. These reservoirs produce pollen and nine fans above the ceiling agitate the pollen particles. The uniformly agitated pollen particles drop down from holes in the ceiling. There are 50 chairs on a mesh floor above the bottom floor. Pollen that falls through the mesh floor into a catchment area is circulated back into the chamber to maintain pollen dispersal throughout the chamber and to avoid accumulation of pollen on the floor. The chamber unit is controlled automatically at a constant temperature and relative humidity.

The pollen level is monitored during exposure at 56 points (including one on the back of each chair) within the chamber using automatic pollen counters (Shinyei, Kobe, Japan)¹² to check that the concentration of the pollen remains at a constant level (500–16,000 grains/m³). Each patient records the frequencies of induced sneezing and rhinorrhea and subjectively assesses symptoms using mobile communication devices that allow precise evaluation. In the chamber, patients wear clean disposable clothes, including hair caps and shoe covers, to avoid the influence of suspended dust on subjects and transport of pollen out of the chamber unit after exposure (Fig. 1).

BASIC PERFORMANCE OF THE α -CHAMBER

The stability of the pollen density in each area of the ECC and changes in pollen concentration during movement of the subjects were examined.

VALIDATION STUDIES IN PATIENTS

Subjects

The subjects were patients with SAR caused by Japanese cedar pollen. All subjects had a history of rhinitis for at least two consecutive cedar pollen seasons and met the following inclusion criteria: a positive allergen-specific skin test (wheal diameter ≥ 10 mm) to standardized cedar pollen extract (Torii Pharmaceutical, Tokyo, Japan), and a serum cedar pollen-specific IgE score ≥ 2 in a fluorescent enzyme immunoassay (FEIA, SRL, Tokyo, Japan). Exclusion criteria were nasal diseases including AR induced by other allergens that required treatment, severe asthma, use of antiallergic drugs within 4 weeks of the study, pregnant women, women of childbearing potential, and breastfeeding women.

Study Protocol

The following studies were conducted in the α -chamber outside the natural cedar pollen season.

a) Changes of nasal symptoms according to the time course of pollen exposure and the concentration of pollen particles

Fourteen subjects (12 males and 2 females, aged 27–57 years old) with SAR induced by Japanese cedar pollen were exposed to cedar pollen at concentrations of 8,000 and 12,000 grains/m³ for 3 h in the ECC. Symptoms in the chamber and after leaving the chamber were evaluated.

b) Carry-over effects

Exposure to 8000 grains/m³ in these subjects was repeated after an interval of 14 days to examine the carryover effect. The same subjects were also exposed to 8000 grains/m³ in the ECC for 2 h each day over the course of 3 consecutive days and changes of symptoms were evaluated.

c) Comparison of nasal symptoms of patients in the pollen chamber with those in the natural pollen season

Correlations between symptoms induced in the ECC and those in the natural cedar pollen season were analyzed in another 72 subjects (29 males and 43 females, age 19–66 years old) with cedar pollinosis. These subjects were exposed to cedar pollen (8000 grain/m³) for 3 h in the ECC outside of the cedar pollen season. The subjects recorded their symptoms for 4 days, including the day of pollen exposure and the following 3 days, and also recorded symptoms during the natural pollen season in 2009 using an allergy diary. These patients were instructed to avoid drug therapy as much as possible. Pollen counts were determined with a Durham sampler (Nishiseiki, Funabashi, Japan) using the gravimetric method on the roof of the Graduate School of Medicine, Chiba University. All studies were conducted at a university hospital in compliance with the Ethical Guidelines for Clinical Studies and Good Clinical Practice and the Declaration of Helsinki (2008 revision). The protocol



Fig. 1 Equipment used in the α -chamber. (a) Automatic pollen counters on the back of each chair. (b) A mobile communication device. (c) Patients wear clean disposable clothes, hair caps and shoe covers to avoid transport of pollen out of the chamber after exposure.

was approved by the Ethics Committee of Chiba University and Chiba University Hospital Clinical Research Center. Each subject received a detailed explanation of the study and of the possible side effects, and written informed consent was obtained prior to participation in the study.

Assessment of the Severity of Nasal Symptoms

In the ECC, the subjects recorded the frequencies of sneezing and nose blowing and subjective assessments of the severity of symptoms (sneezing, rhinorrhea and nasal congestion) using a mobile communication device during cedar pollen exposure, as reported previously.¹³ The severity of each of the three symptoms was evaluated on a 4-point scale (0-3): 0, none; 1, mild; 2, moderate; 3, severe. The total severity score was defined as the sum of these scores (0-9). The subjective assessment was conducted every 30 min. We also asked the subjects to record the frequency of symptoms at 3 PM, 6 PM and 9 PM after leaving the ECC, using an allergy diary.

Modified criteria based on the Practical Guidelines for the Management of Allergic Rhinitis in Japan (Table 1)⁹ was used for evaluation of nasal symptom scores over the day on which ECC exposure oc-

curred and for 3 days after leaving the ECC. On each day, subjects recorded information for sneezing (number of sneezes per day), rhinorrhea (number of times blowing the nose per day), and nasal congestion. These data were evaluated on 4-point scales (0-3) and the total nasal symptom score (TNSS) was defined as the sum of the three scores (0-9).

Statistical Analysis

Data analysis was performed with two-tailed tests at a significance level of 5%, using a Wilcoxon signed rank test in SAS v. 8.02 (Cary, NC, USA) for comparison of nasal symptoms. Pearson correlation coefficients were used to analyze correlations between symptoms induced in the ECC and those in the natural cedar pollen season.

RESULTS

BASIC PERFORMANCE OF THE α -CHAMBER

Cedar pollen concentrations in each of the 9 group areas of the ECC (Fig. 2) are shown in Figure 3. The pollen concentration reached 8,000 grains/m³ within 15 min and was maintained at this concentration thereafter. The difference in concentrations among areas was small and within $\pm 12\%$. Movement of sub-

Table 1 Nasal symptom scores over one day

Parameter	Score			
	3	2	1	0
Sneezing (times/day)	11-20	6-10	1-5	0
Blowing nose (times/day)	11-20	6-10	1-5	0
Nasal congestion	Very severe nasal congestion with frequent oral breathing	Severe nasal congestion with occasional oral breathing	No oral breathing but nasal congestion	None

The evaluation of nasal symptom scores was adapted from the Practical Guideline for the Management of Allergic Rhinitis in Japan.⁸

The most severe symptoms were omitted because such symptoms did not occur in this study.

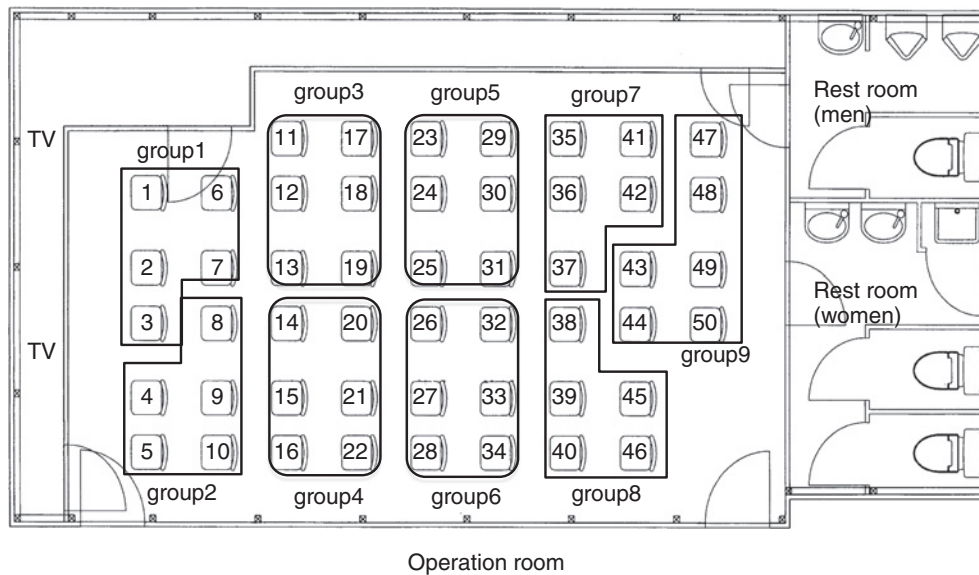


Fig. 2 Design of the α -chamber. There are 9 group areas in the chamber. The pollen concentration in each area was examined.

jects caused the concentration to change, but the target concentration was quickly restored (Fig. 4).

VALIDATION STUDIES IN PATIENTS

a) Changes of Nasal Symptoms according to the Time Course of Pollen Exposure and the Concentration of Pollen Particles

Changes of severity scores in 14 subjects who underwent pollen exposure at 8000 grains/ m^3 for 3 h are shown in Figure 5. Severity of symptoms in the ECC reached a peak about 2 h after the beginning of pollen exposure and plateaued thereafter. The symptoms persisted after the subjects left the ECC, as shown for sneezing and nose blowing in Figure 6. The same subjects were exposed to cedar pollen at 12000 grains/ m^3 for 3 h. There was no significant difference between the severity of symptoms at 8000 and 12000 grains/ m^3 (Fig. 7).

b) Carry-Over Effects

Exposure to 8000 grains/ m^3 in the chamber twice with a two-week interval did not show any carryover

effects (Fig. 8). However, repeated exposure for 2 h per day over the course of 3 consecutive days caused increasingly rapid and marked progression of symptoms (Fig. 9). The severity scores for each symptom on days 2 and 3 were higher than those on day 1 at some time points.

c) Comparison of Nasal Symptoms of Patients in the Pollen Chamber with Those in the Natural Pollen Season

The mean amount of cedar pollen dispersed in the 2009 season was 4372 grain/ cm^2 /day using the Durham method (Fig. 10). The early natural pollen season was defined as the period from February 5 to 28 because the peak of pollen dispersal occurred at the beginning of March. The mean severity scores in the ECC did not have a significant correlation with the mean TNSS over the whole natural pollen season, but did show a moderate correlation ($r = 0.48$) with the mean TNSS in the early natural season (Fig. 11a).

The nasal symptoms continued for four days after exposure in the ECC, although the symptoms amelio-

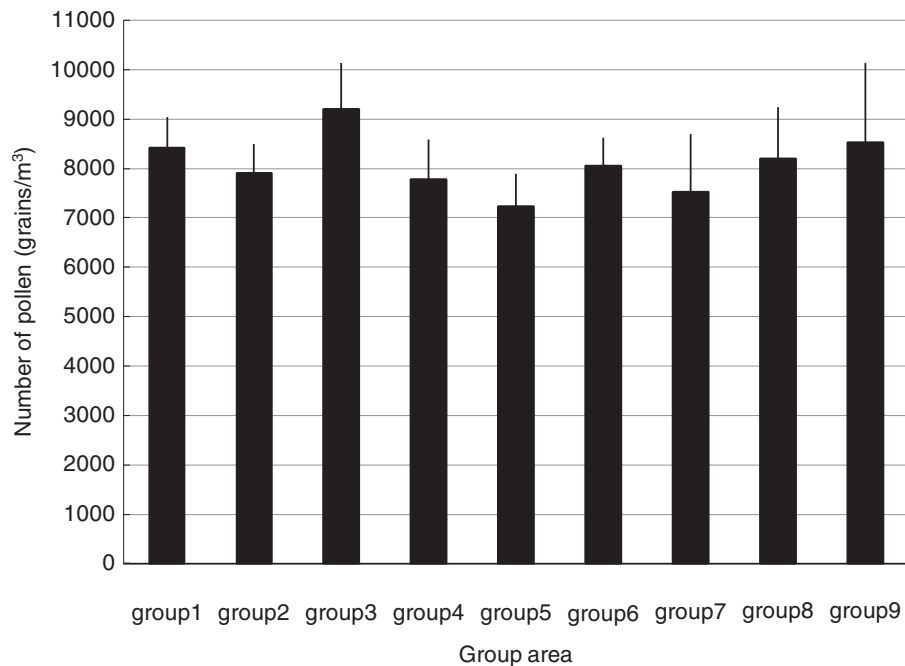


Fig. 3 Change of cedar pollen concentration in each area of the ECC. The average concentration of pollen was 8000 ± 1078 grains in 5 independent experiments. The pollen concentration distribution was within a range of $\pm 12\%$ of the target value of 8000 grains/ m^3 in the chamber. Data are shown as the mean $\pm$ 95%CI.

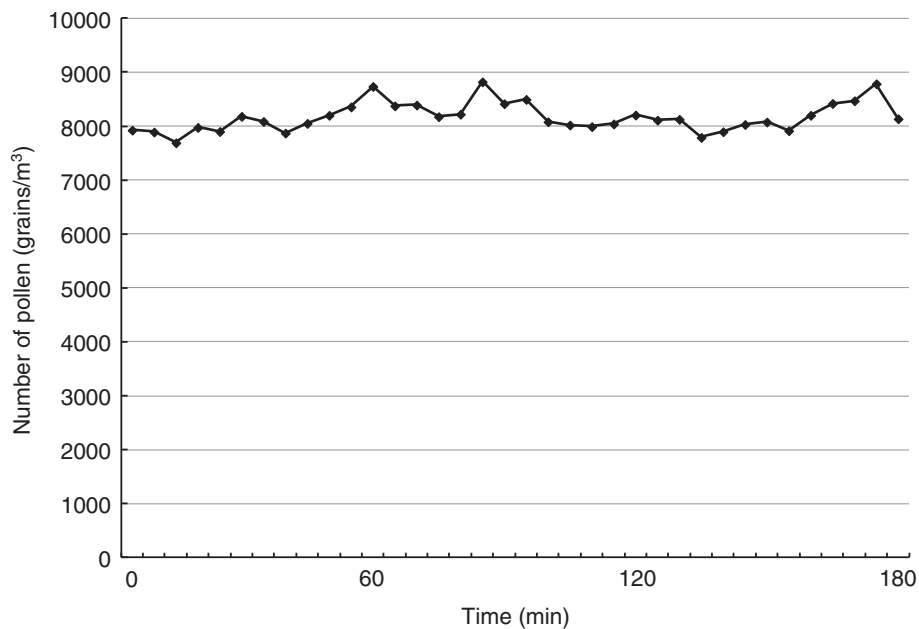


Fig. 4 Change of pollen concentration when subjects moved around. Pollen concentrations were measured every 5 min over 3 h while subjects moved around. The pollen count at each time point is shown as the average value of all sensors over each 5-min period.

rated gradually. The score on day 3 is higher than that of pre-exposure (data not shown). The mean TNSS over the whole natural season was weakly correlated with the mean TNSS for 4 days (day of pollen

exposure and the following 3 days) after exposure in the ECC ($r = 0.33$) (Fig. 11b).

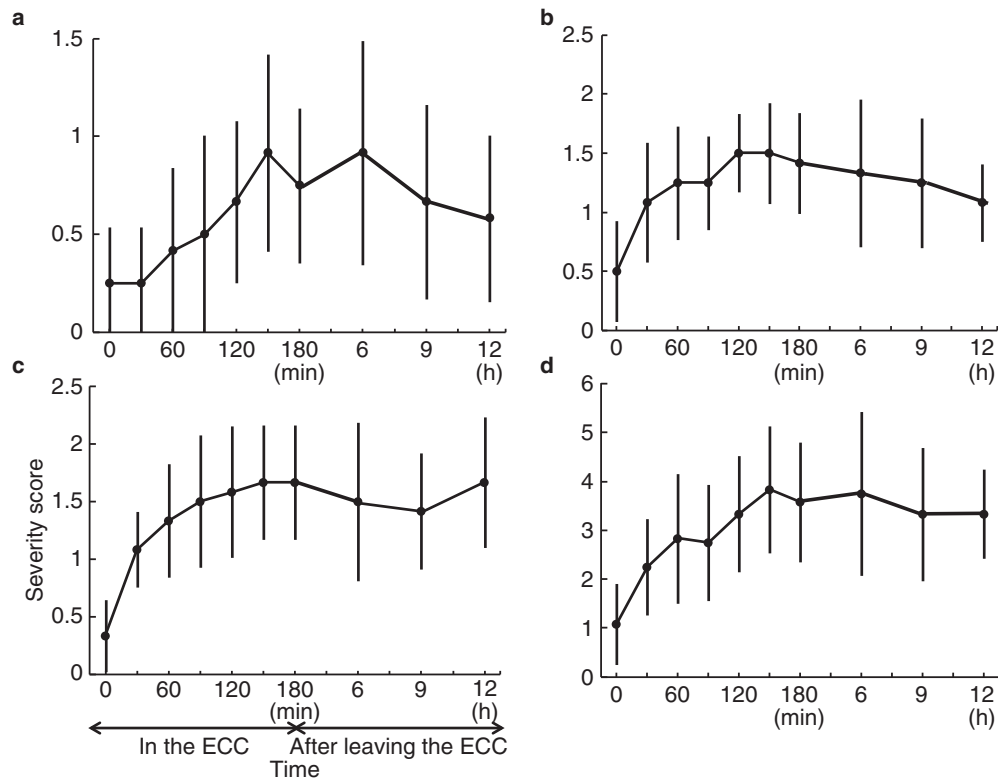


Fig. 5 Change of severity scores in 3-hour pollen exposure of 8000 grains/m³. Subjects recorded their symptom scores every 30 min in the chamber (0-180 min) and every 3 h after leaving the chamber (3-12 h). Symptoms reached a plateau in about 120 min after the start of exposure. (a) Sneezing. (b) Rhinorrhea. (c) Obstruction. (d) TNSS. Data are shown as the mean $\pm$ 95%CI.

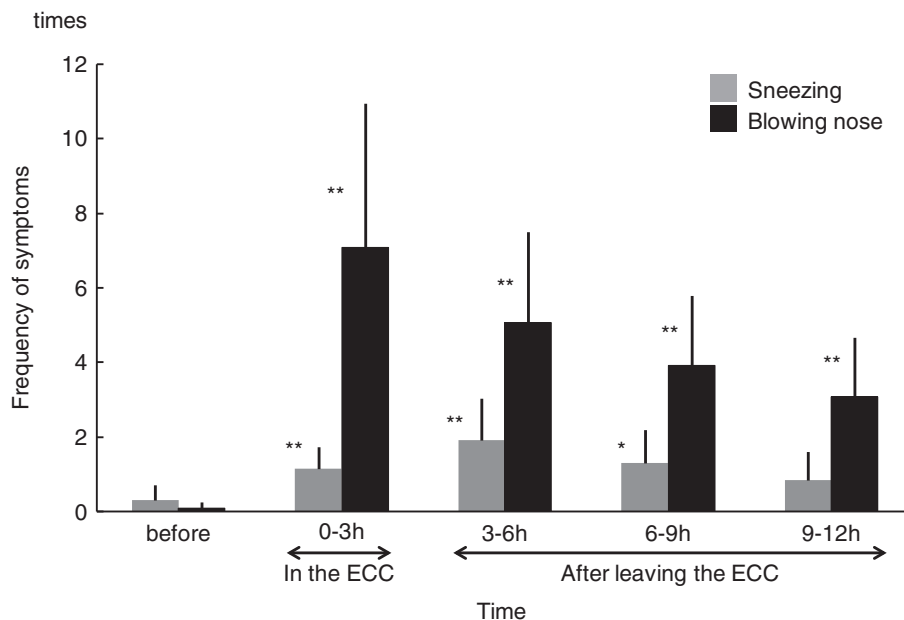


Fig. 6 Frequency of sneezing and nose blowing in 3-hour pollen exposure of 8000 grains/m³. Sneezing and nose blowing still occurred after the subjects left the chamber (3-12 h). Data are shown as the mean $\pm$ 95%CI. * <0.05 vs. before. ** <0.01 vs. before.

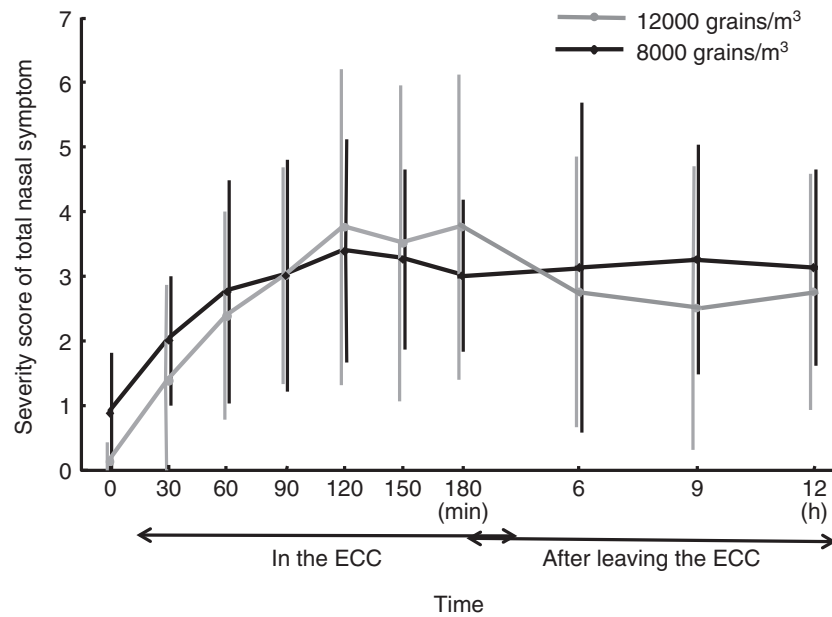


Fig. 7 Comparison of severity scores in pollen exposure at 8000 and 12000 grains/m³. There was no significant difference between exposure at 8000 and 12000 grains/m³. Data are shown as the mean ± 95%CI.

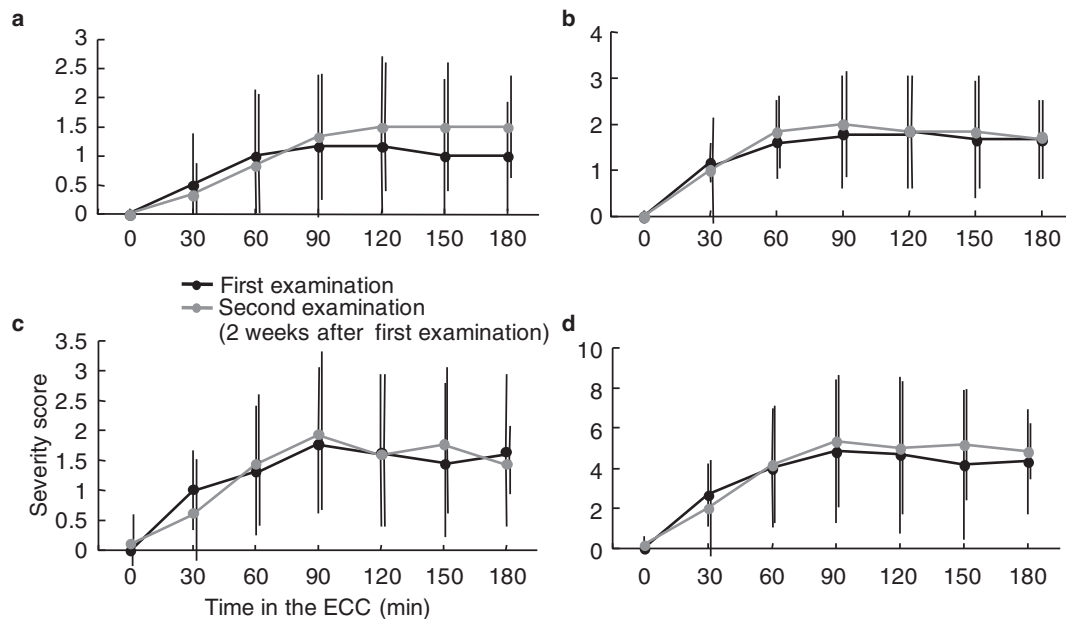


Fig. 8 Comparison of severity scores between exposures at a two-week interval. No aggravation of symptoms was found in the second study. Data are expressed as the mean ± 95%CI. (a) Sneezing. (b) Rhinorrhea. (c) Obstruction. (d) TNSS. Data are shown as the mean ± 95%CI.

DISCUSSION

ECCs were developed to induce SAR symptoms artificially under standard conditions. The Vienna Challenge chamber was the first ECC worldwide and was first described in 1987.¹⁴ An ECC needs to fulfill the

following conditions¹⁵: studies are not limited to the period of natural pollination, controlled and uniform allergen exposure, no impact of weather conditions, no impact of personal context, ensured compliance (medication administration, timeliness and completion of symptom assessment), and instantaneous and

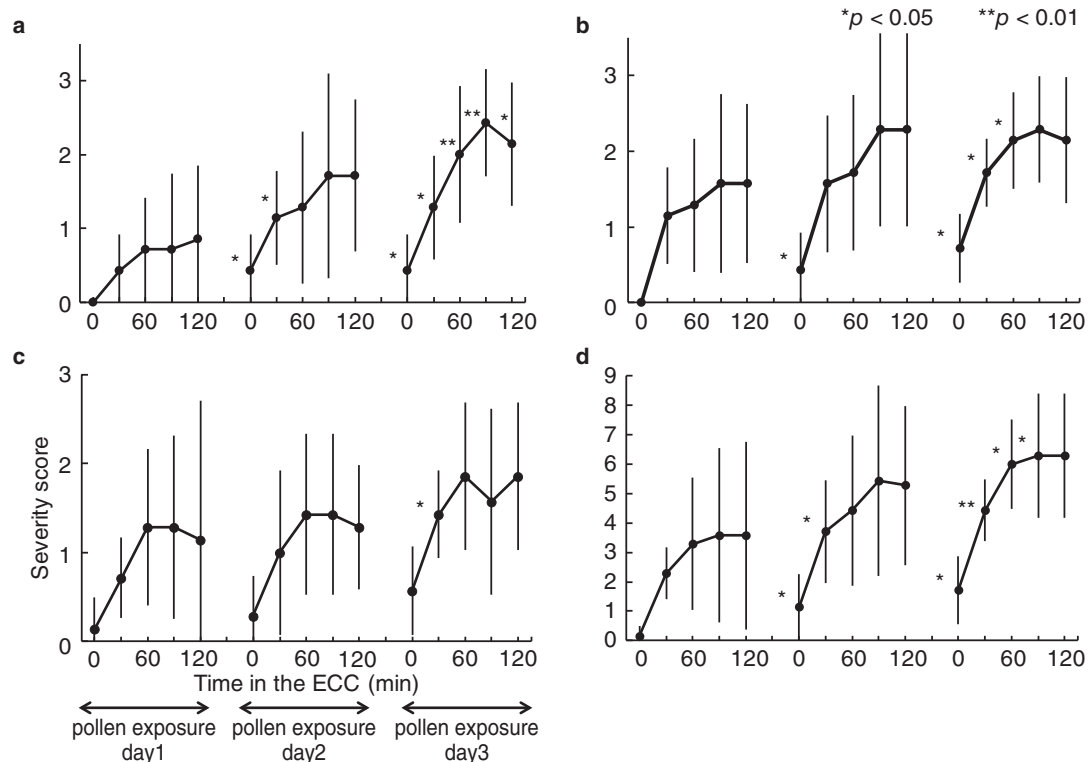


Fig. 9 Change in severity scores in the EEC in a challenge test for 3 consecutive days. Symptoms were increased by 3-day exposure and progression of symptoms became faster and more marked. (a) Sneezing. (b) Rhinorrhea. (c) Obstruction. (d) TNSS. Data are shown as the mean $\pm$ 95%CI. * $p < 0.05$ vs. day 1 at the same time point. ** $p < 0.01$ vs. day 1 at the same time point.

precisely timed symptom assessments. Currently, there are 10 facilities in the world. These chambers should permit objective evaluation of the severity of symptoms, but there are some differences in the standards among the chambers.

We conducted a validation study of the newly built ECC at Chiba University, which is currently the second largest ECC worldwide. Our results showed that the pollen distribution and concentration were constant based on detailed pollen counts throughout the chamber using 56 automatic real time pollen counters. Movement of subjects in the chamber only caused a slight and temporary change in the concentration. Circulation of pollen in the chamber maintained a stable distribution and avoided accumulation of pollen on the floor, which may cause unstable conditions. We also examined the pollen by microscope on glass slides placed in the chamber and found that less than 1% of pollens were damaged (data not shown). These results show that the pollen supply system in the α -chamber can be used for stable pollen exposure.

In the pollen exposure studies in patients with Japanese cedar pollinosis, significant and reproducible symptoms were induced in the α -chamber. The duration of exposure and the concentration for induc-

tion of symptoms were based on the results of validation studies by Krung *et al.*¹⁶ and Hashiguchi *et al.*,¹⁷ in which exposure for at least 2 h and a concentration of 8,000 grains/ m^3 were found to be necessary to observe symptoms. Exposure to cedar pollen at 8,000 grains/ m^3 induced symptoms in all of the subjects in our studies and these symptoms reached a peak after 2 h during exposure in the chamber. The severity and pattern of symptoms did not differ from those induced by exposure at 12,000 grains/ m^3 . We did not examine the effects at a lower concentration; however, exposure at 8,000 grains/ m^3 has been used in previous chamber studies with Japanese cedar pollen¹⁷ and we conclude that this is an appropriate pollen concentration.

The slight increase of TNSS at baseline may have occurred because patients with SAR are frequently aware of their nasal symptoms when they are not in the chamber. There was no carryover effect after an interval of two weeks, which indicates that a cross-over study with this interval will give comparable data. In contrast, consecutive daily exposure exaggerated the symptoms and carryover effects were clearly detected. Pollen exposure in the chamber results in enhanced nasal allergic inflammation and the priming of allergic inflammation in the nasal mucosa may

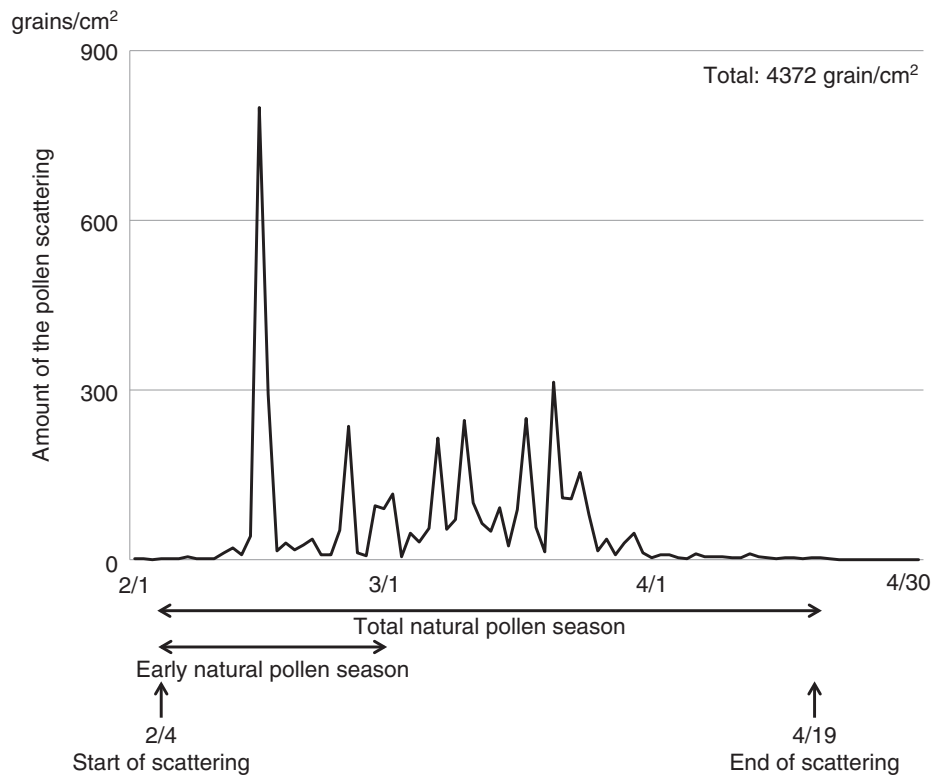


Fig. 10 Scattering pattern of Japanese cedar pollen in 2009. Pollen was scattered from February 5 to April 19. The total amount of cedar pollen in 2009 was 4372 grain/cm²/day. The early natural pollen season was defined as the period from February 5 to 28.

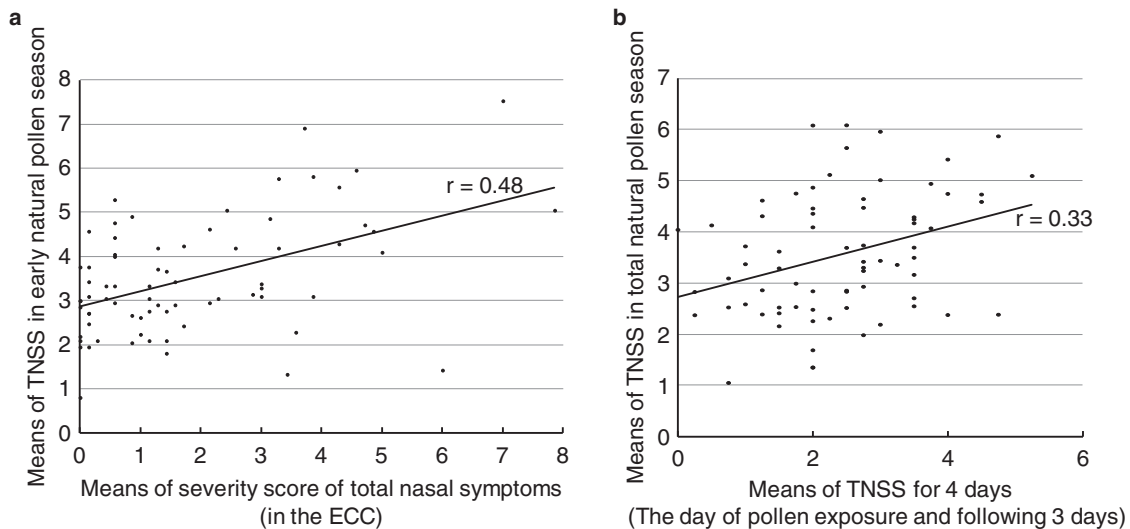


Fig. 11 Correlation between symptoms induced in the ECC and in the natural cedar pollen season. **(a)** Correlation between the mean severity score in the ECC and the mean TNSS in the early natural pollen season. **(b)** Correlation between the mean TNSS for 4 days (day of pollen exposure and the following 3 days) and the mean TNSS over the whole natural pollen season.

cause severe symptoms.¹⁸

Interestingly, persistent symptoms were detected in all subjects after leaving the chamber. Sneezing,

rhinorrhea and nasal congestion occurred for 3 days after a single pollen exposure for only 3 h. The severity of nasal symptoms in the natural pollen season did

not correlate with those in the chamber, but showed a mild relationship with the mean TNSS for 4 days, including the late phase. A previous comparison of results from an ECC and the natural season showed that TNSS for 24 h after 4-h ECC exposure correlated with TNSS during natural exposure.¹⁹ Symptoms are likely to be variable among patients in the natural pollen season because of differences in nasal mucosal sensitivity and in the amount of pollen exposure due to the region and lifestyle of each patient. However, there seem to be a correlation between the symptoms in the ECC on non-pollen season and the symptoms in the natural pollen season, which indicates the value of performing studies using the ECC. Thus, our results show that the α -chamber can be used to perform clinical studies and evaluate symptoms in SAR, including the late phase reaction. The ECC is likely to accelerate the development of treatment and understanding of the detailed mechanisms of allergic inflammation.

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REFERENCES

1. Horak F, Stuebner UP, Ziegelmayer R, Kavina A, Lemell P. Levocetirizine has a longer duration of action than fexofenadine after single administration. *Br J Clin Pharmacol* 2005;**60**:24-31.
2. Horak F, Ziegelmayer UP, Ziegelmayer R *et al.* Azelastine nasal spray and desloratadine tablets in pollen-induced seasonal allergic rhinitis. A pharmacodynamic study of onset of action and efficacy. *Curr Med Res Opin* 2006;**22**:151-7.
3. Patel P, Roland PS, Marple BF *et al.* An assessment of the onset and duration of action of olopatadine nasal spray. *Otolaryngol Head Neck Surg* 2007;**137**:918-24.
4. Guidance for Industry. Allergic Rhinitis: Clinical Development Programs for Drug Products. Draft Guidance. CDER-US Food and Drug Administration, 2000.
5. U.S. Department of Health and Human Services. Food and Drug Administration, Center for Drug Evaluation and Research. Allergic Rhinitis: Clinical Development Programs for Drug Products. Draft Guidance. April 2000.
6. Committee for Medicinal Products for Human Use (CHMP) at the European Medicines Agency (EMA). Guideline on the Clinical Development of Products for Specific Immunotherapy for the Treatment of Allergic Diseases. London, UK, November 2008. Draft CHMP/EWP/18504/2006.
7. Horak F, Ziegelmayer P, Ziegelmayer R *et al.* Early onset of action of a 5-grass-pollen 300-IR sublingual immunotherapy tablet evaluated in an allergen challenge chamber. *J Allergy Clin Immunol* 2009;**124**:471-7.
8. Okamoto Y, Horiguchi S, Yamamoto H, Yonekura S, Hanazawa T. Present situation of cedar pollinosis in Japan and its immune responses. *Allergol Int* 2009;**58**:155-62.
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. Patel P, Patel D. Efficacy comparison of levocetirizine vs montelukast in ragweed sensitized patients. *Ann Allergy Asthma Immunol* 2008;**101**:287-94.
11. Patel P, D'Andrea C, Sacks HJ. Onset of action of azelastine nasal spray compared with mometasone nasal spray and placebo in subjects with seasonal allergic rhinitis evaluated in an environmental exposure chamber. *Am J Rhinol* 2007;**21**:499-503.
12. Muradil M, Okamoto Y, Yonekura S *et al.* Reevaluation of pollen quantitation by an automatic pollen counter. *Allergy Asthma Proc* 2010;**31**:422-7.
13. Yonekura S, Okamoto Y *et al.* Randomized Double-Blind Study of Prophylactic Treatment with an Antihistamine for Seasonal Allergic Rhinitis. *Int Arch Allergy Immunol* 2013;**162**:71-8.
14. Horak F, Jäger S. Die Wiener Provokations-Kammer (Vienna Challenge Chamber). Eine neue Methode des Allergenexpositionstests. *Wien Klin Wochenschr* 1987;**99**:509-10 (in German).
15. Day JH, Horak F, Briscoe MP *et al.* The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper. *Clin Exp Allergy Rev* 2006;**6**:31-59.
16. Krug N, Hohlfeld JM, Larbig M *et al.* Validation of an environmental exposure unit for controlled human inhalation studies with grass pollen in patients with seasonal allergic rhinitis. *Clin Exp Allergy* 2003;**33**:1667-74.
17. Hashiguchi K, Tang H, Fujita T *et al.* Validation study of the OHIO Chamber in Patients with Japanese Cedar Pollinosis. *Int Arch Allergy Immunol* 2009;**149**:141-9.
18. Roquat A, Ihre E, van Hage-Hamsten M, Halldén G, Zetterström O. Allergen-induced inflammation in the nose: a comparison of acute and repeated low-dose allergen exposure. *Allergy* 1996;**51**:42-8.
19. Hohlfeld JM, Holland-Letz T, Larbig M *et al.* Diagnostic value of outcome measures following allergen exposure in an environmental challenge chamber compared with natural conditions. *Clin Exp Allergy* 2010;**40**:998-1006.