



Preliminary study of a challenge test to the patients with Japanese cedar pollinosis using an environmental exposure unit

Kanako Ito^{a,*}, Tetsuya Terada^{a,*}, Ayumi Yuki^a, Takahiro Ichihara^a, Sawako Hyo^a,
Ryo Kawata^a, Hiroshi Takenaka^b, Kentaro Amano^b, Takeshi Ishiguro^b, Toshitami Ro^b

^a Department of Otorhinolaryngology, Osaka Medical College, 2-7 Daigaku-cho, Takatsuki City, Osaka 569-8686, Japan

^b Takenaka Corporation, 1-5-1 Otsuka Inzai City, Chiba 270-1395, Japan

Received 26 July 2009; accepted 1 April 2010

Abstract

Objective: Although clinical studies on pollinosis have been performed employing placebo-controlled double-blind comparative and field techniques, accurate evaluation is difficult because the scattering pollen count and climatic conditions vary between years and regions, leading to a bias in the results. Thus, we prepared a pollen challenge test unit (allergen challenge chamber: ACC) which facilitates quantitative pollen challenge at any time, and, so, the acquisition of objective data.

Methods: The control of constant conditions and maintenance of specified pollen concentrations in ACC were investigated. In addition, the pollen distribution in ACC was measured while maintaining the level at 10,000 counts/m³. The pollen levels were measured employing the aspiration and Durham methods, and the measured values were compared. Furthermore, whether symptoms are adequately induced in the chamber during the non-cedar pollen-scattering season was investigated in 14 volunteers with cedar pollinosis.

Results: When the pollen level in ACC was set at 6000 counts/m³ or higher, the rate of variation was $\pm 15\%$, within the adjustable range, and that of the pollen distribution in the chamber was within $\pm 20\%$. When the volunteers with cedar pollinosis were exposed to cedar pollen in ACC, pollinosis symptoms were induced, and challenge for 2 consecutive days significantly induced symptoms.

Conclusions: The temporal and spatial variations of the pollen level in ACC were small, facilitating stable pollen challenge, and pollinosis symptoms were induced in the volunteers with cedar pollinosis. The challenge chamber may be useful to judge the effects of therapy against pollinosis.

© 2010 Elsevier Ireland Ltd. All rights reserved.

Keywords: Allergen challenge chamber; Allergic rhinitis; Allergy symptom; Japanese cedar pollen

1. 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 the QOL of patients, and affects productivity [2–5], leading to economic and social problems.

The treatment of pollinosis is roughly divided into drug, surgical, and immunological therapies, but objectivity regarding the evaluation of their effects is insufficient. One reason is that many clinical studies evaluating the

effects employing placebo-controlled double-blind comparative study and field techniques involving cedar pollinosis patients [6,7], but the accuracy of evaluation may be hampered by variations in the scattering pollen count and climatic conditions between years and regions in these studies.

The maintenance of identical conditions during pollen challenge facilitates accurate induction tests and evaluation of the pharmacological effects of drugs not relying on subjective observation, such as impression, which can be achieved using a pollen challenge chamber. Such controlled pollen challenge chambers, allowing the acquisition of objective data, have been established mainly in Western countries and Japan [8–10]. We established the second

* Corresponding author. Tel.: +81 726 83 1221; fax: +81 726 84 6539.
E-mail address: tetsu@yc4.so-net.ne.jp (T. Terada).

Japanese pollen challenge test unit in Osaka. In this study, we verified the basic performance of our unit, and investigated the challenge conditions to adequately induce pollinosis symptoms.

2. Materials and methods

2.1. Materials

2.1.1. Outline of the pollen challenge test unit (challenge chamber)

The pollen challenge test unit consists of a challenge chamber in which patients are actually exposed to pollen, machine room to prepare pollen, air shower room to remove pollen on leaving the challenge chamber, consultation room in which a physician examines the patient regarding symptoms, test room for objective testing, clothing room for patients to change their clothes to a clean suit, and waiting room in which the test is explained to patients. The area and ceiling height of the challenge chamber are about 21.8 m² and 2.5 m, respectively, with a maximum capacity of 12 persons. Patients in the challenge chamber can be observed through a two-way mirror. The challenge chamber is controlled at a constant temperature (summer: 25 ± 3 °C, winter: 23 ± 3 °C) and relative humidity (summer: 50 ± 10%, winter: 45 ± 10%) using an outside air-processing air conditioner, and the air can be ventilated with clean air from which dust is removed using a high-performance filter (HEPA filter). Pollen in the challenge chamber can be partially removed and expelled outside through a filter box. The ceiling and walls are finished with antistatic cloth to avoid the adherence of sprayed pollen.

2.1.2. Dispersion of pollen

Cedar pollen (particle size: 20–30 µm, specific gravity: 1.4 g/m³) was used. Employing a cylinder feeder-type powdering device (Aerosol generator RBG-1000R, Palas Co.), cedar pollen was dispersed into the compressed air supply while controlling the level, and homogeneously sprayed in the challenge chamber via 12 Anemostat-type diffusers installed in the ceiling. 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 min, and the maintenance level was adjusted corresponding to the real-time measured level (every 4 min). Regarding the cedar pollen used, the allergen level was measured by ELISA, and its antigenicity was confirmed to be active beforehand.

2.2. Methods

2.2.1. Pollen level in the challenge chamber

The conditions in the challenge chamber were set at 23 ± 3 °C, 45 ± 10% relative humidity, and 10 times/h

ventilation using the outside air-processing air conditioner, and whether the pollen level in the challenge chamber could be maintained at specified levels (3000, 6000, and 10,000 counts/m³) was investigated. After reaching the specified level (20 min), the maintenance level was adjusted corresponding to the real-time measured level. Then, the pollen level was measured every 4 min for 60 min.

The pollen distribution was measured at a 1.2-m height from the floor at which subjects breathe (sitting on a chair during the test) under the conditions described above at a pollen level maintained at 10,000 counts/m³. The pollen level was measured at 35 points in the challenge chamber and 25 points within 1.2-m² areas with the patient at the center.

2.2.2. Measurement of pollen level

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.), and the Durham gravimetric method, using a Durham pollen sampler. Under conditions of 23 ± 3 °C, 45 ± 10% relative humidity, and 10 times/h ventilation controlled using the outside air-processing air conditioner in the challenge chamber, the level of dispersed pollen was measured using the aspiration and Durham methods, and the measured values were compared. Since the count of 20–50 µm particles was 0 on measurement by the aspiration method without pollen dispersion, the total count of 20–30 µm particles was regarded as the pollen level. On measurement using the Durham method, samples were collected at a 1.2-m height in the center of the chamber after pollen had been dispersed for 30 min, and the measured values were converted to 24-h values.

2.2.3. Symptom induction test in the non-cedar pollen-scattering season

Whether pollen challenge induces symptoms in the non-cedar pollen-scattering season was investigated in subjects with cedar pollinosis.

The subjects were volunteers who developed moderate or severer symptoms of cedar pollinosis in the latest cedar pollen-scattering season with a cedar-specific IgE antibody titer of 2 or higher on RAST Scoring. There were 14 subjects (8 males and 6 females) aged 19–24 years.

In September 2005, under conditions of 23 ± 3 °C, 45 ± 10% relative humidity, and 10 times/h ventilation in the challenge chamber, the cedar pollen level was maintained at 10,000 counts/m³, and the subjects were exposed to pollen for 120 min (single challenge test). About one month after this single challenge, the subjects were exposed to pollen for 120 min per day for 2 consecutive days under the same conditions (2-consecutive day challenge test). In both tests, 4 items (frequencies of sneezing and nose blowing, nasal obstruction, and itch) were investigated. The subjects recorded the frequencies of sneezing and nose blowing. For the evaluation of nasal obstruction and itch, the visual analog scale (VAS) was employed. Designating the

conditions in which the subject felt the worst and no symptoms as 10 and 0, respectively, the subject marked a site on a 10-cm line corresponding to the severity of the symptom. These were recorded before challenge and every 15 min after challenge initiation. The study was conducted in accordance with GCP guidelines and the declaration of Helsinki. The study protocol was reviewed and approved by the Research Committee at Osaka Medical College.

2.3. Statistical analysis

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

3. Results

3.1. Pollen level in the challenge chamber

When the pollen levels in the challenge chamber were set at 3000, 6000, and 9000 counts/m³, the rates of pollen level variation were ± 20 , ± 15 , and $\pm 15\%$, respectively, showing that the variation can be limited to within $\pm 15\%$ when the pollen level is set at 6000 counts/m³ or higher (Fig. 1).

The pollen distribution in the challenge chamber was 80–110%, showing that the variation in the chamber can be limited to within $\pm 20\%$ (Fig. 2a). Regarding the pollen level at the sites of subjects (center) as 100%, the level within a 1.2-m regular square area with the center being the subject's seat was 87–109%, showing that the variation was within $\pm 15\%$ (Fig. 2b).

3.2. Measurement of pollen level

The pollen levels measured by the aspiration and Durham methods were well correlated ($R^2 = 0.9775$)

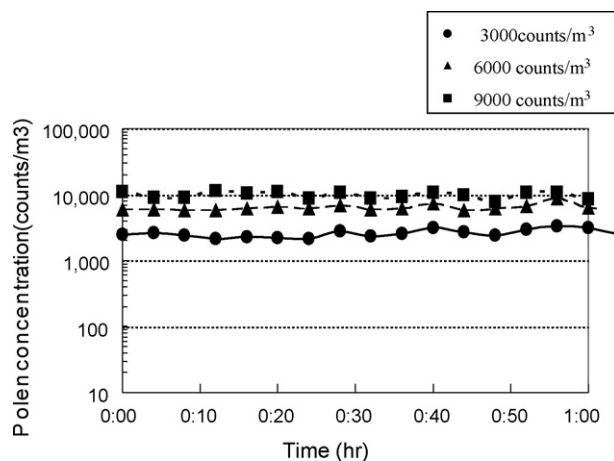


Fig. 1. When the pollen level in the challenge chamber was set at 6000 counts/m³ or higher, the rate of variation could be limited to within 15%.

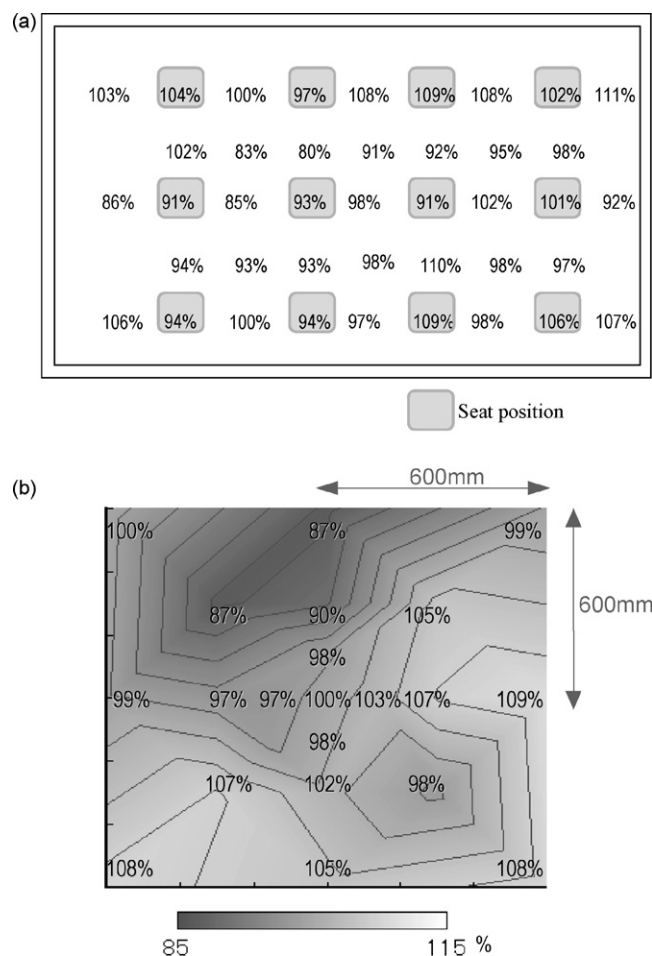


Fig. 2. (a) When the pollen level was set at 10,000 counts/m³, the rate of variation could be limited to within $\pm 20\%$. (b) When the pollen level was set at 10,000 counts/m³, the rate of variation within a 1.2-m regular square with the subject's seat at the center was within $\pm 15\%$.

(Fig. 3). Based on the regression line, challenges at 10,000 and 50,000 counts/m³ corresponded to 2876 and 14,380 counts/cm²·24 h on measurement using the Durham method, respectively.

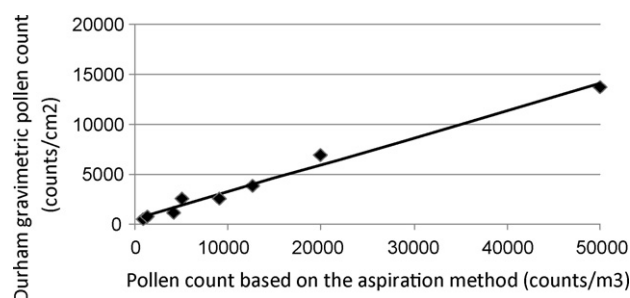


Fig. 3. When the pollen level in the challenge chamber was measured by the aspiration and Durham methods, the values were well correlated ($R^2 = 0.9775$).

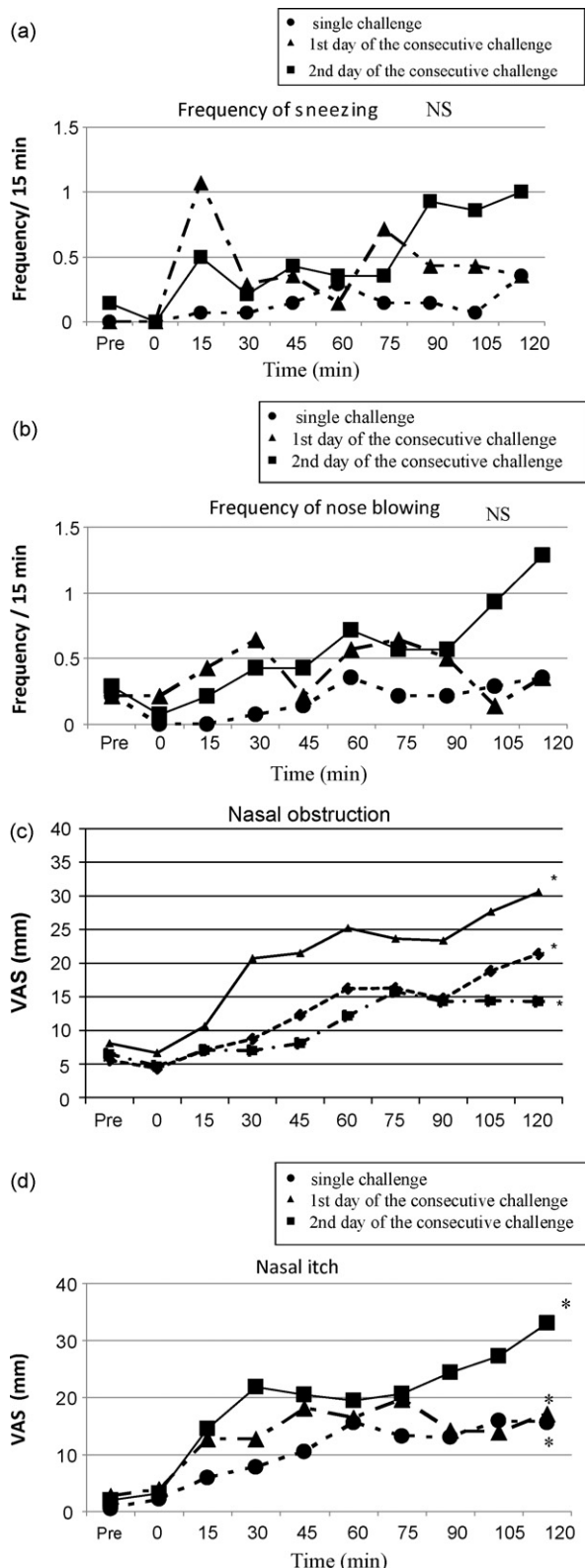


Fig. 4. Changes in the symptoms after challenge initiation were investigated in the single and 2-consecutive day challenge tests. Nasal obstruction and itch were significantly exacerbated after challenge on all days of both tests, particularly on the 2nd day of the consecutive challenge test (*Wilcoxon singed-ranks test: $P < 0.05$).

3.3. Symptom induction test in the non-cedar pollen-scattering season

Changes in symptoms were investigated before and after single and 2-consecutive day challenges. Changes in the 4 items are shown in Fig. 4.

The 4 items were compared between those before challenge and at 120 min after challenge initiation. Nasal obstruction and itch were significantly exacerbated after challenge on all days of single and 2-consecutive day challenge tests, particularly on the 2nd day of the consecutive challenge test. The frequencies of sneezing and nose blowing also rose on the 2nd day, although these increases were not significant.

4. Discussion

Regarding the basic performance of our ACC established in Osaka, the dispersed pollen level did not change with time (temporal stability), the antigen level distribution in the challenge chamber was homogenous (spatial homogeneity), and symptoms were adequately induced in subjects with cedar pollinosis.

In an ACC, allergic rhinitis symptoms, therapeutic effects, and a comparison of therapeutic drugs can be studied in subjects under strictly controlled conditions. ACCs were initially established in the 1980s [11], and the first clinical report was published in 1988 [12]. The number of effective ACCs is limited in the world because a system capable of strictly controlling antigen particles is necessary. One condition required of challenge chambers is the capability of maintaining a specific pollen level for a certain time. It is also necessary to induce moderate to severe nasal allergic symptoms in allergic subjects while inducing no symptoms in non-allergic subjects [13].

The technological concept necessary for an ACC is to realize accurate evaluation in the presence of a sufficiently controlled antigen level and homogeneous antigen distribution in a room, overcoming the difficulty caused by heterogeneous conditions, which cannot be avoided in clinical studies performed under natural conditions. In previous clinical studies on pollinosis, placebo-controlled double-blind comparative and field techniques were mainly employed in the pollen-scattering season [6,7], but cedar pollen-scattering varied among regions and years in the former, and the latter was affected by the climatic conditions on the test day, preventing some studies from being completed, although subjects could be subjected to identical conditions.

The real-time pollen level in the challenge chamber was measured (every 4 min) by the aspiration method to monitor and maintain it at a specific level. The Durham method is generally employed for the measurement of outdoor pollen levels. As shown in Fig. 3, the pollen counts measured by the two methods were well correlated,

and 10,000 counts/m³ using the aspiration method corresponded to 2876 counts/cm²·24 h using the Durham method. Since the highest ever recorded pollen level in Kinki region is 1500 counts/cm²·24 h using the Durham method, the pollen level in the chamber, 10,000 counts/m³, corresponded to about 1.9 times the maximum count in the pollen-scattering season. The challenge test corresponded to staying outside for 2 h during peak pollen-scattering, but the induced allergic symptoms were not severe. The season in which the challenge test was performed was significant in that it revealed the necessity of producing a higher pollen count than that in the natural environment to induce symptoms in the challenge chamber. The test was performed about 4 months after the end of the cedar pollen-scattering season, i.e., symptoms were induced in the non-scattering season. A priming effect of repeated antigen challenge increases sensitivity to the antigen in the latter half of the pollen-scattering season [8,14], whereas single challenge in the off-season may require a high dose to sufficiently induce symptoms. On the 1st day of the consecutive challenge, an allergic reactivity to the pollens more increased than on the single challenge reaction. In the 2-day challenge test, symptoms were significantly more exacerbated on the 2nd than on the 1st day. These may have demonstrated a priming effect, but further investigation of the challenge method, including the duration of antigen challenge is necessary.

Consideration of the site of pollen measurement and the calculation method comprises another reason for the necessity of a higher pollen count to induce symptoms in the challenge chamber than in the natural environment. Since the outdoor scattering pollen count is presented as a mean value, the daytime count may be underestimated because the count at night is lower. Moreover, the Durham-type pollen sampler is generally placed on the roof of buildings, but pollen counts taken at a height at which humans breathe, about 1.6 m from the ground, may be higher than such roof-top recordings [15].

There may be several problems in clinical studies employing a challenge chamber, other than the priming effect, establishment of the pollen challenge level, and its interpretation described above. The conditions in the challenge chamber and behavioral patterns of subjects during the test do not precisely reproduce those in actual life in the pollen-scattering season [16]. It is controversial whether the evaluation of symptoms in the presence of a behavioral pattern in a challenge chamber, different from that during normal life activities, is reliable, even though the data are collected under strictly controlled conditions. Moreover, it may be difficult to investigate the developmental mechanism of allergic rhinitis symptoms in cases positive for multiple antigens based on a single antigen challenge test in a challenge chamber.

The antigen challenge chamber established by us showed a satisfactory basic performance and was capable of

inducing nasal allergic symptoms. It was suggested that the challenge chamber is useful to judge the effects of therapy against pollinosis. However, it could not be concluded whether nasal allergic symptoms induced in the challenge chamber are identical to pollinosis symptoms that develop during the actual pollen-scattering season. Further investigation of the challenge method, including the appropriate dose and duration of antigen challenge, and careful evaluation are necessary.

Acknowledgments

We thank Mr. K. Nishibata and M. Koizumi for their technical support.

References

- [1] Kaneko Y, Motohashi Y, Nakamura H, Endo T, Eboshida A. Increasing prevalence of Japanese cedar pollinosis: a meta-regression analysis. *Int Arch Allergy Immunol* 2005;136(April (4)):365–71 [Epub 2005 March 2].
- [2] Stuck BA, Czajkowski J, Hagner AE, Klimek L, Verse T, Hörmann K, et al. Changes in daytime sleepiness, quality of life, and objective sleep patterns in seasonal allergic rhinitis: a controlled clinical trial. *J Allergy Clin Immunol* 2004;113(April (4)):663–8.
- [3] Settiple RA. Complications of allergic rhinitis. *Allergy Asthma Proc* 1999;20(July–August (4)):209–13. Review.
- [4] Thompson AK, Juniper E, Meltzer EO. Quality of life in patients with allergic rhinitis. *Ann Allergy Asthma Immunol* 2000;85(November (5)):338–47. quiz 347–8.
- [5] Schoenwetter WF, Duplay Jr L, Appajosyula S, Botteman MF, Pashos CL. Economic impact and quality-of-life burden of allergic rhinitis. *Curr Med Res Opin* 2004;20(March (3)):305–17.
- [6] Hyo S, Fujieda S, Kawada R, Kitazawa S, Takenaka H. *Ann Allergy Asthma Immunol* 2005;94(April (4)):457–64. The efficacy of short-term administration of 3 antihistamines vs placebo under natural exposure to Japanese cedar pollen.
- [7] Bronsky EA, Aaronson DW, Berkowitz RB, Chervinsky P, Graft D, Kaiser HB, et al. Dose ranging study of mometasone furoate (Nasonex) in seasonal allergic rhinitis. *Ann Allergy Asthma Immunol* 1997;79(July (1)):51–6.
- [8] Horak F, Stübner P, Ziegelmayer R, Kavina A, De Vos C, Burtin B, et al. Controlled comparison of the efficacy and safety of cetirizine 10 mg o.d. and fexofenadine 120 mg o.d. in reducing symptoms of seasonal allergic rhinitis. *Int Arch Allergy Immunol* 2001;125(May (1)):73–9.
- [9] Day JH, Briscoe MP, Welsh A, Smith JN, Clark A, Ellis AK, et al. Onset of action, efficacy, and safety of a single dose of fexofenadine hydrochloride for ragweed allergy using an environmental exposure unit. *Ann Allergy Asthma Immunol* 1997;79(December (6)):533–40.
- [10] Krug N, Hohlfield JM, Geldmacher H, Larbig M, Heermann R, Lavalley N, et al. Effect of loteprednol etabonate nasal spray suspension on seasonal allergic rhinitis assessed by allergen challenge in an environmental exposure unit. *Allergy* 2005;60(March (3)):354–9.
- [11] Horak F, Jäger S. The Vienna challenge chamber (VCC)—a new method for allergen exposition test. *Wien Klin Wochenschr* 1987;99:509–10.
- [12] Horak F, Jäger S. VCC—Vienna challenge chamber—the protective effects of Astemizole. *Allergy* 1988;43(Suppl. 7):110.

- [13] Day JH, Briscoe MP. Environmental exposure unit: a system to test anti-allergic treatment. *Ann Allergy Asthma Immunol* 1999;83(August (2)):83–9. quiz 89–93 [Review].
- [14] Connell JT. Quantitative intranasal pollen challenges. 3. The priming effect in allergic rhinitis. *J Allergy* 1969;43(January (1)):33–44 [no abstract available].
- [15] Nielsen L, Johnsen CR, Bindslev-Jensen C, Poulsen LK. Efficacy of acrivastine in the treatment of allergic rhinitis during natural pollen exposure: onset of action. *Allergy* 1994;49(September (8)):630–6.
- [16] Day JH, Ellis AK, Rafeiro E, Ratz JD, Briscoe MP. Experimental models for the evaluation of treatment of allergic rhinitis. *Ann Allergy Asthma Immunol* 2006;96(February (2)):263–77. quiz 277–8, 315. Review.