

Development of pollen-induced allergic rhinitis with early and late phase nasal blockage in guinea pigs

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Abstract. *Objective and Design:* Development of nasal blockage and sneezing during repeated inhalation challenges with Japanese cedar pollens was evaluated in guinea pigs.

Subjects: Male Hartley guinea pigs.

Treatment: Guinea pigs were sensitized by intranasal instillation of cedar pollen extracts + Al(OH)₃ 2 times a day for 7 days. The animal was then forced to inhale the pollens for challenge, which was restrictively trapped in the upper airways, once a week.

Methods: Change of specific airway resistance (sRaw), sneezing frequency, and titers of anaphylactic antibodies in the serum were measured after each of the 30 challenges.

Results: At the first challenge, no obvious increase in sRaw was observed. However, the second and third challenges to the animals caused modest biphasic elevations of sRaw, with peaks at the first and the fourth to sixth hour. At the fourth to tenth challenges, marked elevations of sRaw were observed. However, with repetition of the inhalation challenge, the early and the late responses became almost indistinguishable because of partial overlapping as the responses expanded. All guinea pigs sneezed immediately after each pollen inhalation challenge. Apparent increases of both circulating γ 1 and IgE antibodies were seen after the seventh challenge.

Conclusions: These results indicate that the experimental allergic rhinitis established in the present study can be a valuable model for analyzing the pathogenesis of the disease and developing new therapeutic drugs.

Key words: Allergic rhinitis – Nasal blockage – Sneezing – Pollen – Airway resistance

Introduction

People suffering from allergic rhinitis have IgE antibodies against specific allergens including the pollens of trees, grasses or weeds in their sera, and characteristic symptoms

of the disease are sneezing, rhinorrhea and nasal congestion [1]. The antigen provocation test indicated that over 90% of patients showed these symptoms immediately and that approx. 50% of them also developed a late phase reaction with the predominant symptom of nasal blockage, which resulted mainly from sustained nasal congestion [2, 3].

It has been reported that sneezing and rhinorrhea in allergic rhinitis could be substantially inhibited by treating patients with anti-histaminic drugs, because these symptoms are triggered via the stimulation of the nasal terminals of the trigeminal nerve mainly by histamine [4, 5]. On the other hand allergic nasal blockage, another symptom, is relatively serious because only glucocorticoids [6, 7] and α -stimulants [8, 9] are effective against it. The cells and mediators involved and the detailed sequence of events leading to the onset of nasal congestion are not yet fully elucidated. To investigate allergic rhinitis including nasal blockage using experimental animals, models that closely resemble the human case are indispensable.

Recently we reported a guinea pig, allergic rhinitis model developed by repetitive inhalation challenges with Japanese cedar pollens as the antigen [10]. Distinguishing features of the model are: (1) Antigen challenge by repeated quantitative inhalation with cedar pollens, (2) ability to induce allergy restricted to the upper airway and (3) use of nasal lavage without sacrificing the animals. Thus, our model is very useful for long-term investigation of the pathogenesis of allergic rhinitis in the same guinea pig.

In the present study, we investigated the development of nasal blockage and sneezing induced by repetitive pollen inhalation challenges to guinea pigs that had been sensitized by repeated instillation of the pollen extract + Al(OH)₃ into their nostrils.

Materials and methods

Animals

Male 3-week-old Hartley guinea pigs weighing 250–300 g were purchased from Japan SLC (Hamamatsu, Japan). The animals were

housed in an air-conditioned room at a temperature of $23 \pm 1^\circ\text{C}$ and $60 \pm 10\%$ humidity with lights on from 8:00 a.m.–8:00 p.m.; they were fed a standard laboratory diet and given water ad libitum. The first sensitization was started 2 weeks after breeding.

This animal study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Materials

Japanese cedar pollens were donated from the laboratory of Torii Pharm. Co., Ltd., Chiba, Japan. Evans' blue was purchased from Merck (Darmstadt, Germany). Ovalbumin (OA) was from Wako Pure Chem. (Osaka, Japan). The other reagents used were the highest grade of commercial products available.

$\text{Al}(\text{OH})_3$ gels were prepared with 0.5 N NaOH and 0.5 N $\text{Al}_2(\text{SO}_4)_3$ as previously described [11].

Anti-OA guinea pig serum was obtained by the method of Levine et al. [12]. In brief, guinea pigs were sensitized by i.p. injection of $10\text{ }\mu\text{g}$ OA/mg $\text{Al}(\text{OH})_3$ /animal, once every 2 weeks, for a total of 10 injections. Both the 4 h and 7 day homologous passive cutaneous anaphylaxis (PCA) titers of the serum were 1:500. This serum served as the standard for quantification of $\gamma 1$ and IgE antibody levels in sensitized guinea pig sera by PCA, and was stored at -80°C until use.

Preparation of Japanese cedar pollen extracts

The cedar pollen extracts used for the sensitization were prepared according to the previously described method [10]: The pollens were suspended in physiological saline at a concentration of 100 mg/ml and allowed to stand at 4°C for 18 h with mild stirring. After centrifugation at $1,700 \times g$ for 15 min at 4°C , the resultant supernatant was used as the sensitization antigen and stored at -80°C until use. The protein concentration in the solution was quantified according to the method of Bensadoun and Weinstein [13]; it was estimated to be $500\text{ }\mu\text{g}$ protein/ml.

Sensitization and challenge

As shown in Chart 1, guinea pigs were sensitized by intranasal (i.n.) instillation of cedar pollen extracts adsorbed onto $\text{Al}(\text{OH})_3$ gel at a concentration of $0.3\text{ }\mu\text{g}$ protein/ 0.3 mg $\text{Al}(\text{OH})_3$ / $3\text{ }\mu\text{l}$ /nostril 2 times a day for 7 days. Prior to each sensitization, the upper airway mucosal surface was anaesthetized by 2 min inhalation with 4% lidocaine hydrochloride solution (Fujisawa Pharm. Co., Osaka, Japan) mists, generated with an ultrasonic nebulizer (NE-U12, Omron, Osaka, Japan), to prevent the rapid elimination of antigen by ciliary movement. The sensitized animal was then intranasally challenged once every week by inhalation of cedar pollen using a hand-made inhalation apparatus [10], which allowed quantitative inhalation of pollen. The apparatus, into which 3 mg of the pollen was poured, was positioned in the left nostril of the conscious guinea pig for 1 min so that it could inhale the pollen by spontaneous breathing. Thereafter, the pollen was also inserted into the right nostril. During inhalation, the other nostril was plugged with a finger. When 3 mg of the pollen (1.07×10^5 pollens/mg) was applied to the respective nostrils, approx.

60% was inhaled with little variation. The rest of the pollen adhered to the inside wall of the apparatus. Almost all the inhaled pollens were trapped in the upper airways; less than 0.001% was found in the lower airways [10].

For the negative controls, groups were prepared that were non-sensitized – non-challenged or non-sensitized – challenged with the pollen.

Measurement of pulmonary function

As indicators of respiratory function, the specific airway resistance (sRaw) and the respiratory rate in 1 min (RR) were measured 1 h before and 10 min–10 h after the antigen challenge by inhalation in the conscious guinea pigs, using a two-chambered, double-flow plethysmograph system according to the method of Pennock et al. [14]. Briefly, the animal was placed with its neck extending through the partition of a two chambered box, and sRaw and RR measured with the Data analyzer Pulmos-I (MIPS, Osaka, Japan) and a PC 9801 FA computer (NEC, Tokyo, Japan) after detection of the airflow by sensors equipped to both the front and rear chambers.

Counting of sneezing frequency

Sneezing frequencies in the intervals 0–10 min, 10 min–1 h and 1–2 h after the pollen inhalation challenge were counted.

Passive cutaneous anaphylaxis (PCA)

To estimate specific $\gamma 1$ and IgE antibody titers of the serum in the sensitized guinea pig, which had been repeatedly challenged/boosted by pollen inhalation, 4-h and 7-day PCAs were performed according to the method of Ovary [15] and Levine et al. [12], respectively. The serum for the PCAs was obtained 1 day before the respective inhalations. The cedar pollen extracts described above were used as a challenging antigen after dialysis at a dose of $90\text{ }\mu\text{g}$ protein/ml/animal.

Statistical analyses

Statistical analyses were performed by one-way analysis of variance (ANOVA). If a significant difference was detected, the individual group difference was determined by Bonferroni's multiple test. A probability value (p) of less than 0.05 was considered to be statistically significant.

Results

Time-course changes of sRaw

Figure 1 shows the time-course changes in sRaw of the sensitized guinea pigs at challenges 1–3 (A); 4, 7 and 10 (B); and 13, 20 and 30 (C), induced by pollen inhalation.

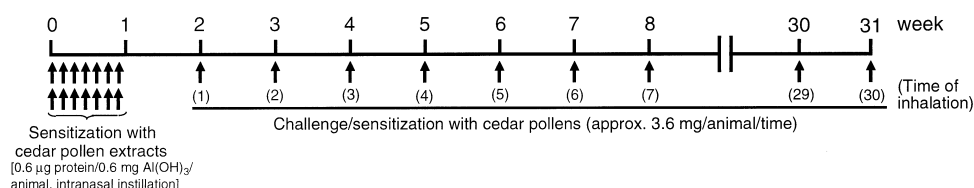


Chart 1. Schedule for sensitization with the cedar pollen extracts and subsequent nasal challenge/sensitization by inhalation of the cedar pollens in the guinea pig.

At the first challenge, no obvious changes were observed in the sensitized guinea pigs. The second and third provocations caused modest but significant biphasic elevations of sRaw, with peaks at 1 h and 4 to 6 h after the provocation (Fig. 1A). At the fourth challenge, these early and late sRaw elevations were greater than at the earlier challenges. A further increase in peak value of the early phase at the seventh challenge masked the biphasic response (Fig. 1B). Although almost the same degree of biphasic response to that of the fourth challenge was observed at the tenth provocation (Fig. 1B), at the thirteenth to thirtieth challenges both the responses gradually diminished, and the early and late responses became indistinguishable (Fig. 1C).

In the non-sensitized – non-challenged group, no

obvious time course changes in sRaw were observed at any time. In addition, when non-sensitized guinea pigs were exposed to the pollen at the time corresponding to the first, fourth and twentieth challenges, no alterations of sRaw were seen throughout the measurement time (Figs. 1A, B and C).

Time-course changes in RR

Figure 2 shows time-course changes in RR at the fourth challenge in the sensitized guinea pigs compared with those of the non-sensitized – non-challenged and the non-sensitized – challenged groups.

The RR of the sensitized guinea pig before the fourth

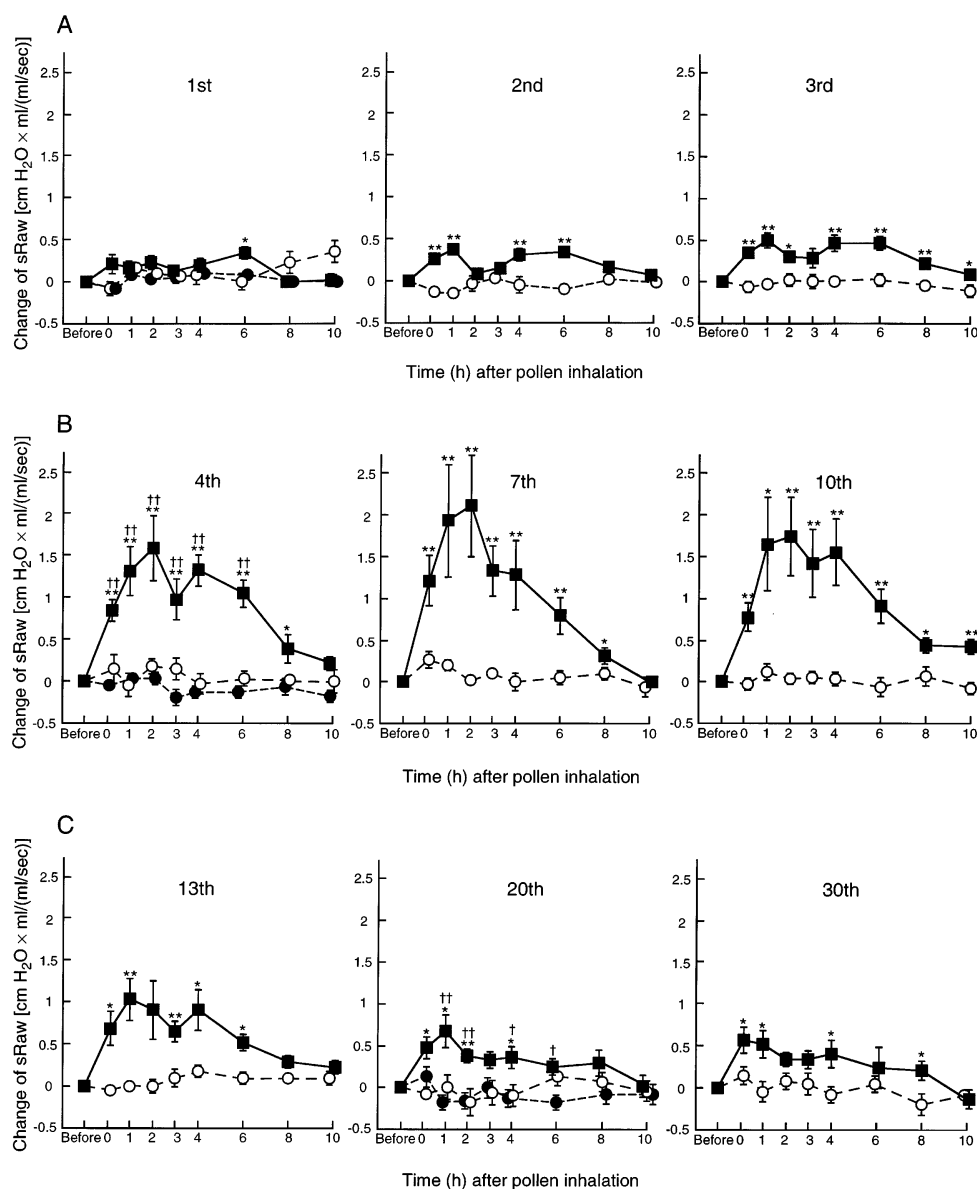


Fig. 1. Time-course changes in specific airway resistance (sRaw) by the 1st, 2nd and 3rd (A), 4th, 7th and 10th (B), and 13th, 20th and 30th (C) inhalations of Japanese cedar pollen in sensitized guinea pigs. Each point represents the mean $\pm$ SEM of 11 or 12 animals. (○) non-sensitized – non-challenged, (●) non-sensitized – challenged, (■) sensitized – challenged. Significantly different from the non-sensitized – non-challenged group (* p < 0.05, ** p < 0.01) or from the non-sensitized – challenged group († p < 0.05, †† p < 0.01).

pollen inhalation was approx. 100 times in 1 min, which was almost the same as those of the negative control groups. The pollen inhalation challenge produced an obvious and prolonged decrease in the indicator of pulmonary function 10 min–6 h after the inhalation, which was almost recovered at the eighth and the tenth. In the non-sensitized groups, no significant changes were observed at any time during the experiment.

Time-course changes in sneezing frequency

Figure 3 shows time-course changes in sneezing frequency during the first to thirtieth cedar pollen inhalations in the sensitized guinea pigs.

At the first to thirteenth challenges, the animal exhibited the highest sneezing frequency within 10 min of each challenge, followed by a decreasing frequency or absence of sneezing at 10 min to 2 h after the challenge. After the 2nd h, little or no sneezing was observed at any challenge time (data not shown). At the first to seventh challenges, the total number of sneezes increased with increasing challenge number. On the other hand, the peaks of the frequency at the twentieth and thirtieth challenges shifted to the period between 10 min and 1 h after the challenge.

When the non-sensitized guinea pigs were forced to inhale the pollen at the time corresponding to the first, fourth and twentieth challenges, the total number of sneezes was approximately one quarter (first and fourth) or less (twentieth) that of the sensitized ones.

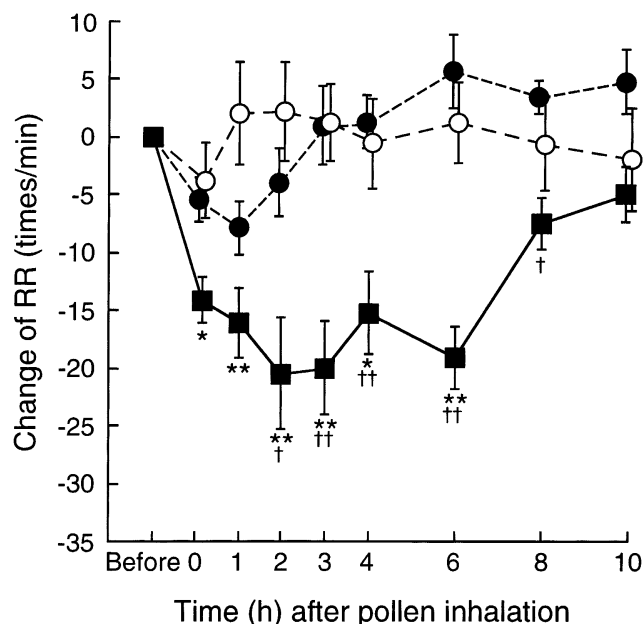


Fig. 2. Time-course of changes in respiratory rate in 1 min (RR) by the 4th inhalation of Japanese cedar pollen in sensitized guinea pigs. Each point represents the mean $\pm$ SEM of 12 animals. (○) Non-sensitized – non-challenged, (●) non-sensitized – challenged, (■) sensitized – challenged. Significantly different from the non-sensitized – non-challenged group (* p < 0.05, ** p < 0.01) or from the non-sensitized – challenged group († p < 0.05, †† p < 0.01).

$\gamma 1$ and IgE antibody levels in the serum during sensitization/challenge

The levels of $\gamma 1$ and IgE antibody in the serum obtained one day before the repetitive inhalation challenges with the cedar pollen in the sensitized guinea pigs were quantitated by measuring the titers PCA at 4 h and 7 day, respectively. These results are shown in Figure 4.

There were no PCA-positive animals at the first challenging inhalation. Only 1 and 3 out of 12 animals were positive for 4 h and 7 day PCA, respectively, even at the fourth challenge. However, increased numbers of both PCA-positives and elevated levels of both antibodies as a whole were observed at the seventh and the thirteenth challenges. At the later challenges (13–30), all animals were positive for both PCA. The levels of $\gamma 1$ and IgE antibodies appear to remain unchanged, at least until the thirtieth provocation.

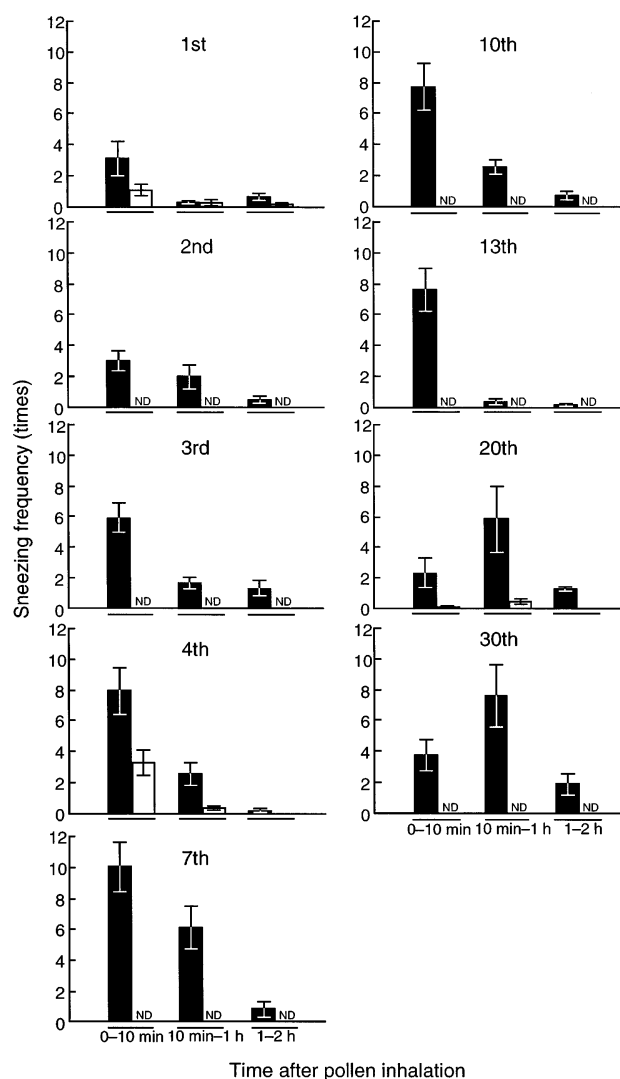


Fig. 3. Time-course changes in sneezing frequency by the 1st, 2nd, 3rd, 4th, 7th, 10th, 13th, 20th and 30th inhalations of Japanese cedar pollen in sensitized guinea pigs. Each column represents the mean $\pm$ SEM of 11 or 12 animals. (□) Non-sensitized – challenged, (■) sensitized – challenged. ND = not done.

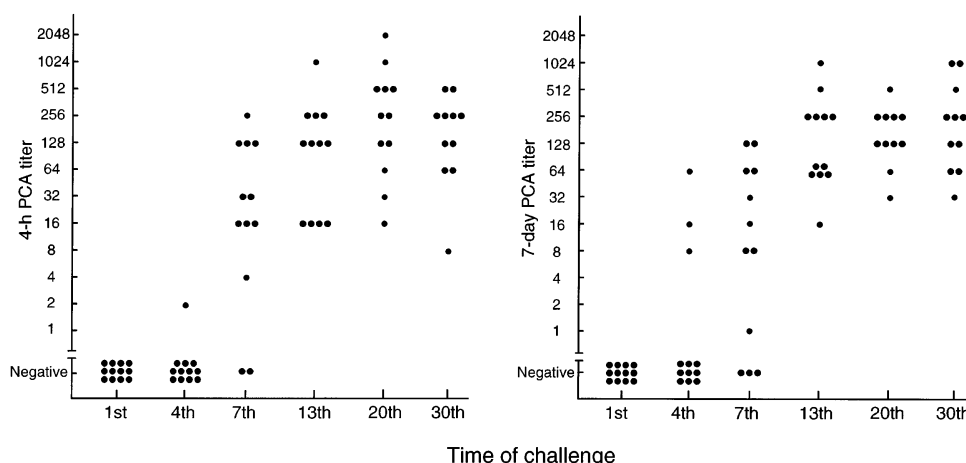


Fig. 4. Four-h and 7-day homologous passive cutaneous anaphylaxis (PCA) titers of the sera from sensitized guinea pigs. Sera were drawn on the day before the respective challenges.

Discussion

We previously reported that when guinea pigs were sensitized by i.p. injection the cedar pollen extracts + $\text{Al}(\text{OH})_3$ gels and then challenged with the pollen repetitively, elevations of $\gamma 1$ and IgE antibody levels were seen and examination of the nasal cavity lavages indicated that there was immediate leakage of albumin and sustained eosinophil influx into the fluid [10]. In the present study, nasal blockage and sneezing, the main symptoms of patients with allergic rhinitis, were evaluated when intranasally sensitized guinea pigs were forced to inhale the pollen on several occasions over a long term period. As a result, $\gamma 1$ and IgE levels in sera from the sensitized guinea pigs increased in a relatively gradual manner, unlike the i.p. sensitized guinea pigs of the previous experiment [10], followed by maintenance of high levels of these antibodies at the thirteenth to thirtieth challenges. Sneezes were observed immediately after each challenge to the sensitized guinea pigs. On the other hand, increases in sRaw, which is taken as an index of the nasal blockage, was barely noticeable at the first challenge. Weak but distinguishable biphasic increases of sRaw were observed at the second and third challenges. At the fourth to tenth challenges, peak values of the early and late phases of sRaw were elevated. At the thirteenth to thirtieth challenges, the level of response diminished as the pollen inhalation challenge was repeated. As shown in the fourth challenge experiment, the time-course changes of sRaw correlated well with those of RR.

Elevations of sRaw after pollen inhalation in the sensitized guinea pigs appeared to reflect some responses taking place in the upper airway, but not in the lower airway, since we have previously demonstrated that our inhalation procedure deposits 99.999% of the pollen in the upper airway [10]. The present elevations of sRaw (nasal blockage) may be mainly due to the complication in mucosal congestion and increases in mucosal vascular permeability followed by mucosal edema, which are elicited by (chemical) mediators released or produced from various cells such as mast cells and other leukocytes [1].

The two phases of sRaw increase at the fourth–tenth

challenges were less distinguishable than those at the second and third challenges, because their peaks were closer to each other due to the peak of the early response being delayed. The time at which the late phase nasal blockage occurred is similar to that observed in patients with allergic rhinitis following nasal allergen application [2]. In recent experiments in our laboratory, an antagonist against cysteinyl leukotrienes (CysLTs), pranlukast, effectively suppressed the late phase response while the early response at the fourth challenge was barely influenced by the drug, strongly suggesting that the early and the late responses are induced by different mechanisms (unpublished data). In addition, an H_1 -receptor antagonist, mepyramine, did not inhibit either the early nor the late phase of the nasal blockage in the present model (unpublished data). The ineffectiveness of the H_1 -receptor antagonist on the nasal blockage closely parallels the clinical results [1, 4, 5].

To date, investigations of nasal blockage have focused on the immediate response occurring within 30 min of a single antigen challenge using guinea pigs sensitized passively or by two exposures to antigen mists [16–18]. These reports do not describe whether the late phase is also induced in their models. In the present study, not only the early but also the late phase nasal blockage could be detected at the fourth and subsequent challenges. However, one day before the fourth challenge, most of the sensitized guinea pigs had no detectable levels of the anaphylactic antibodies in their sera. These results strongly suggest that the local immune system is largely responsible for the occurrence of the dual response. Furthermore, although the sensitized guinea pigs maintained high levels of anaphylactic antibodies in their sera at the thirteenth to thirtieth challenges, both early and late responses gradually diminished with repeated challenges indicating that the decrease of these responses is not directly related to antibody levels in the sera.

The good correlation between elevations of sRaw and decreases of RR was almost always observed not only at the fourth challenge but at other times as well. Increase in respiratory volume at one time (tidal volume) was also seen when RR was reduced, indicating that guinea pigs breathe deeply and slowly while the nasal allergic reaction occurs.

As generally recognized, in the asthmatic model of guinea pigs shallow and fast respiration emerged when the early asthmatic response was observed, and no obvious changes from the normal levels of RR were seen at the time of the late asthmatic responses (unpublished data). This further emphasizes that the mechanisms of ventilatory obstruction following nasal allergy are quite different from those of allergic asthma.

On the other hand, the immediate appearance of sneezes after the pollen inhalation challenge in our model was consistent with results from allergic rhinitis patients [19, 20]. As has been suggested by clinical observations [4], sneezes occurring after the pollen inhalation challenge in the sensitized group were induced in response to histamine, which is probably released from nasal mucosal mast cells after the antigen-antibody reaction; this released histamine then stimulates sensory nerve endings followed by the sneeze reflex via the sneeze center. The sneezes observed were considerably inhibited by mepyramine (unpublished data) and increased amounts of histamine in nasal cavity lavage fluid were seen immediately after the pollen inhalation challenge in the present model [10]. Therefore, unlike nasal blockage but as with clinical observations, this model strongly suggests that sneezes are at least partly caused by histamine released. However, it appears that stimulatory activities of the pollen itself participate in the reflex because pollen provocation of non-sensitized guinea pigs also elicited sneezes.

In conclusion, we have established an experimental allergic rhinitis model using cedar pollen as an antigen, demonstrating the development of biphasic nasal blockages by repeated pollen inhalation challenges, immediate sneezing at the challenge and an increase in specific anaphylactic antibodies in the sera. These events are similar to clinical situations, indicating that the model is suitable for analyzing the pathogenesis of allergic rhinitis and the development of new types of therapeutic drugs.

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