

Comparison of Cedar Pollen-Induced Allergic Rhinitis in Passively and Actively Sensitized Guinea Pigs

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Received August 29, 2000 Accepted January 20, 2001

ABSTRACT—We have developed an allergic rhinitis model in guinea pigs using Japanese cedar pollen as antigen. In the present study, we examined whether provocation by pollen induces similar magnitudes of rhinitis symptoms in passively and actively sensitized guinea pigs. One group of animals was actively sensitized by intranasal application of pollen extract, and another was passively sensitized by intraperitoneal injection with anti-pollen serum. Actively and passively sensitized groups were then challenged by repeated and a single pollen inhalation, respectively. In both groups, sneeze was induced immediately after the challenge. The actively sensitized animals developed not only early but also late nasal blockage, whereas the passively sensitized animals showed only early nasal blockage. In both groups, an H₁ antagonist, mepyramine, inhibited the occurrence of sneezing but did not inhibit nasal blockage. Nasal hyperresponsiveness to intranasal instillation of leukotriene D₄ was obvious only in the actively sensitized animals. We thus conclude that although early nasal blockage is induced by a single antigen-antibody reaction, repetitive anaphylactic reaction is required for occurrence of late nasal blockage and hyperresponsiveness to stimuli. Furthermore, histamine plays a central role in induction of sneezing but not in nasal blockage, irrespective of whether animals are actively or passively sensitized.

Keywords: Allergic rhinitis, Cedar pollen, Histamine, IgE, Nasal blockage

Patients with allergic rhinitis have serum IgE antibody against specific allergens including pollens of trees, grasses or weeds, and characteristic symptoms of the condition are sneezing, rhinorrhea and nasal blockage (1). When specific allergens are applied to the nasal cavities of the patients, over 90% immediately respond by sneezing, developing rhinorrhea and nasal blockage (2). In addition, about 50% of these patients further develop a late phase reaction with the predominant symptom being nasal blockage, which results mainly from sustained nasal congestion (2, 3). Furthermore, the nasal responsiveness of allergic rhinitis patients to stimuli other than a specific allergen increases compared with those of healthy individuals (1, 4). The increase in nasal reactivity to stimuli that occurs after allergen provocation may resemble the reaction provoked by increased sensitivity to histamine (5).

We have established a Japanese cedar pollen-induced allergic rhinitis model of guinea pigs that develop symp-

toms similar to those described above. Following intranasal active sensitization by instillation with pollen extracts and aluminum hydroxide adjuvant, levels of antigen-specific anaphylactic antibodies in the serum increase, and frequent sneezes and biphasic nasal blockage develop in proportion to the number of pollen inhalation challenges (6, 7). Furthermore, the nasal blockage response of sensitized animals to intranasal instillation of not only histamine (8) but also leukotriene (LT) D₄ (9) appears to be enhanced in proportion to the number of challenges. However, the mechanisms of the occurrence of allergic rhinitis symptoms, especially those of nasal blockage and nasal hyperresponsiveness to stimuli, are unclear.

Many researchers have reported developing allergic asthmatic models, and the induction mechanisms of the lower airway response have been extensively analyzed (10–15). In contrast, there have been relatively few reports of basic research into the mechanisms of allergic rhinitis.

In the present study, we initially examined whether passively sensitized guinea pigs develop sneezing, biphasic nasal blockage and nasal hyperresponsiveness to LTD₄

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after the challenge with Japanese cedar pollen inhalation. The magnitudes of their allergic rhinitis symptoms were compared with those in actively sensitized animals. We then assessed the effects of a classical H_1 -receptor antagonist, mepyramine, on nasal symptoms in both sets of animals.

MATERIALS AND METHODS

Animals

Four- (300–350 g) and 9-week-old (550–600 g) male Hartley guinea pigs (Japan SLC, Hamamatsu) were actively and passively sensitized, respectively. Anti-cedar pollen serum was raised and passive cutaneous anaphylaxis (PCA) titers were measured in male Hartley guinea pigs (Japan SLC) weighing 250–300 and 500–1,000 g, respectively. 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 with a standard laboratory diet and provided with water ad libitum.

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

Antigen and adjuvant

Japanese cedar (*Cryptomeria japonica*) pollen was harvested in Gifu and Shiga prefectures. The cedar pollen extracts used for the active sensitization were prepared as previously described (6). Pollen was suspended in physiological saline at 100 mg/ml and left at 4°C for 18 h with mild stirring. After centrifugation, the supernatant was stored at -80°C until its use as an active sensitization antigen.

Aluminum hydroxide [$\text{Al}(\text{OH})_3$] gel was prepared with 0.5 N NaOH and 0.5 N $\text{Al}_2(\text{SO}_4)_3$ as described (16).

Reagents

Reagents and their sources were as follows: lidocaine hydrochloride (Fujisawa Pharm. Co., Osaka), mepyramine maleate (Sigma Chem., St. Louis, MO, USA), leukotriene (LT) D_4 (Wako Pure Chem., Osaka) and Evans blue (Merck, Darmstadt, Germany). All other reagents were of the highest commercial grade available.

Passive sensitization and challenge with cedar pollen

Anti-cedar pollen serum was obtained according to the method of Levine et al. (17). In brief, 70 guinea pigs were sensitized by i.p. injections of cedar pollen extracts adsorbed on $\text{Al}(\text{OH})_3$ gel at $20 \mu\text{g protein} \cdot 20 \text{ mg Al}(\text{OH})_3^{-1} \cdot \text{ml}^{-1} \cdot \text{animal}^{-1}$ once every 2 weeks for 14 weeks. On the 7th day after the last sensitization, blood was withdrawn from the carotid artery of each animal and 25 sera possessing relatively high levels of IgE titer were pooled. The $\gamma 1$ and IgE titers of the serum were both 1:4,000 when estimated

by 4-h and 7-day PCA (17, 18), respectively.

Guinea pigs were passively sensitized by i.p. injection with 2 ml anti-cedar pollen serum/animal twice (both injections on the same day). Two days later, each animal was challenged by inhalation of a measured dose of cedar pollen using a hand-made inhalation apparatus (1.8 mg/nostril) (6).

The negative control was a non-sensitized, challenged animal of the same age.

Active sensitization and challenge with cedar pollen

As previously reported (7, 8), guinea pigs were actively sensitized by intranasal instillation of cedar pollen extracts adsorbed on $\text{Al}(\text{OH})_3$ gel at a dose of $0.3 \mu\text{g protein} \cdot 0.3 \text{ mg Al}(\text{OH})_3^{-1} \cdot 3 \mu\text{l}^{-1} \cdot \text{nostril}^{-1}$ twice each day for 7 days. Prior to each sensitization, the upper airway mucosal surface was topically anesthetized by 2-min inhalation of a 4% lidocaine mist generated using an ultrasonic nebulizer (NE-U12; Omron, Osaka) to prevent the rapid elimination of antigen by ciliary movement. The sensitized animal was intranasally challenged once each week for 15 weeks by inhalation of cedar pollen, using the apparatus and dose described above for passively sensitized animals.

Time-course changes in specific airway resistance (sRaw) and sneezing frequency were measured at the 7th pollen inhalation challenge. Nasal responsiveness to LTD₄ was assessed 2 days after the 15th challenge.

For the negative control, a group of non-sensitized, challenged animals was prepared.

Counting of sneezing frequency

In preliminary experiments, we confirmed differences between the respiratory patterns of sneezing and coughing using a two-chambered, double-flow plethysmograph system (Pulmos-1; MIPS, Osaka) in guinea pigs. A cough induced by a forced inhalation of stimuli [for example, fine mists of capsaicin solution ($50 \mu\text{M}$)] is characterized by a sudden expiration irrespective of inspiration or expiration immediately beforehand. In contrast, sneezing, which was only observed with the cedar pollen inhalation in sensitized animals, is characterized by an explosive expiration just after a deep inspiration. On the basis of this information, we determined sneezing frequency at 0–10 min and 10 min–1 h after the pollen inhalation challenge in unrestrained guinea pigs.

Measurement of sRaw

As an indicator of respiratory function (nasal blockage), the sRaw before and 10 min–10 h after antigen challenge in conscious guinea pigs was measured using a two-chambered, double-flow plethysmograph system (Pulmos-I) according to the method of Pennock et al. (19). Briefly, an animal was placed with its neck extending through the

partition of a two chambered box. The sRaw was measured using the Data analyzer Pulmos-I and a PC 9801 FA computer (NEC, Tokyo) after detection of the airflow by sensors equipped to both the front and rear chambers.

Nasal responsiveness to LTD₄

Two days after the 15th and single challenge of the actively and passively sensitized animals, respectively, doses of 10 μ l/nasal cavity of 10^{-8} and 10^{-6} M of LTD₄ were consecutively instilled into the bilateral nasal cavities at intervals of 20 min. The sRaw value was measured before and 10 min after each set of two LTD₄ instillations.

Statistical analyses

Statistical analyses were performed using the 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*) below 0.05 was considered statistically significant.

RESULTS

Occurrences of sneezing and nasal blockage in the passive and active sensitization animal models

Figure 1A shows sneezing frequency induced at 0–10 min and 10 min–1 h following inhalation challenge with cedar pollen in the passive sensitization model. The pollen exposure induced an average of 3 sneezes at 0–10 min in the non-sensitized guinea pigs. In passively sensitized animals, sneezing frequency was 15 sneezes at 0–10 min and 5 sneezes at 10 min–1 h, but after the 1st hour

few sneezes were observed (data not shown). Sneezing frequency of actively sensitized animals at 0–10 min and 10 min–1 h increased significantly after the 7th pollen inhalation challenge (Fig. 1B), a finding consistent with the results for the passively sensitized group and the results of our previous study (7).

Time-courses of sRaw in the passively and actively sensitized animals after pollen challenge are shown in Fig. 2, A and B, respectively. As reported in our previous study (7), the elevation of sRaw in the actively sensitized animals was biphasic after provocation, and peaked at the 2nd hour (early blockage) and 4th hour (late blockage) (Fig. 2B). In contrast, pollen provocation of the passively sensitized guinea pigs induced a swift increase in sRaw that peaked at the 10th min, but sRaw elevation then declined (rapidly at first and then gradually) until the 6th hour. Very little elevation of sRaw was observed after the 6th hour (Fig. 2A). In contrast, no increase in sRaw was observed in the non-sensitized animals after inhalation (Fig. 2: A and B).

Effect of mepyramine on occurrences of sneezing and nasal blockage in the passive and active sensitization models

Table 1 shows effects of 10 mg/kg mepyramine [a dose which strongly suppressed the early asthmatic response of guinea pigs (15)] on the occurrences of sneezing and nasal blockage in the passively and actively sensitized guinea pigs. When mepyramine was orally administered 1 h before pollen provocation, sneezing frequency of both the actively and passively sensitized guinea pigs within 10 min after antigen challenge was reduced by approximately 50%

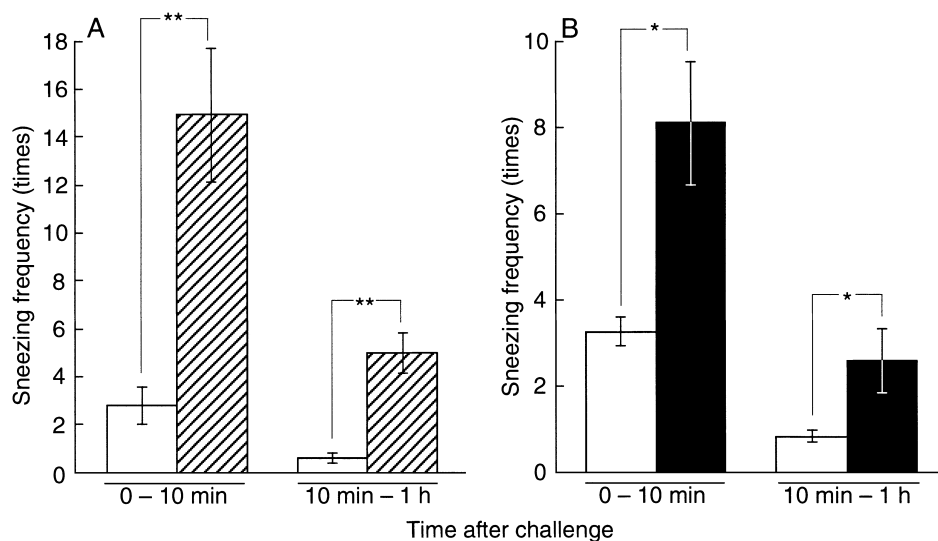


Fig. 1. Cedar pollen-induced sneeze in passively (A) and actively (B) sensitized guinea pigs. Open squares, non-sensitized; hatched squares, passively sensitized; closed squares, actively sensitized. Each column represents the mean $\pm$ S.E.M. of 15 (passively sensitized) and 12 (actively sensitized) animals. **P*<0.05, ***P*<0.01.

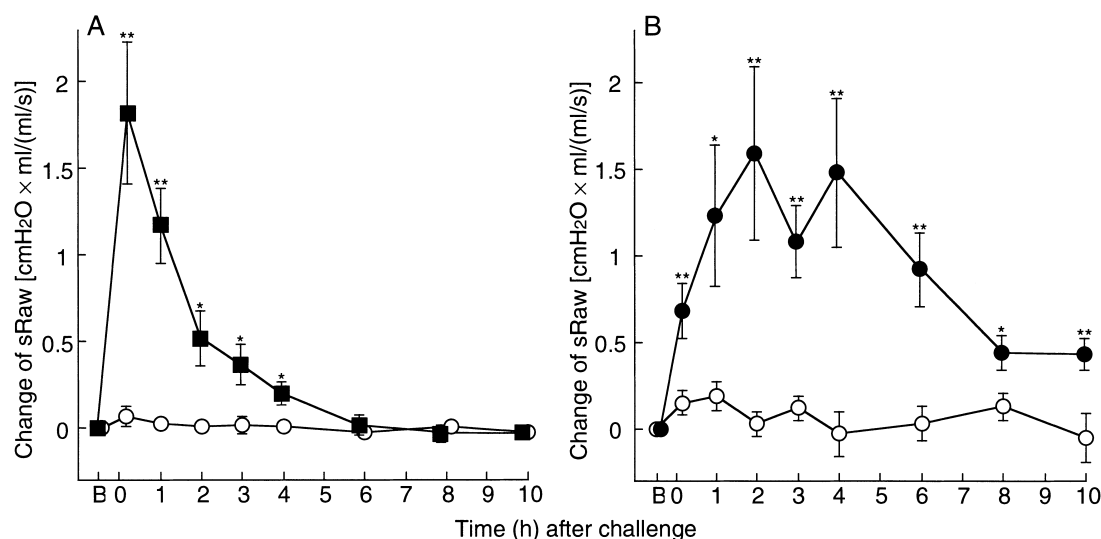


Fig. 2. Cedar pollen-induced increase of specific airway resistance (sRaw) in passively (A) and actively (B) sensitized guinea pigs. Open circles, non-sensitized; closed squares, passively sensitized; closed circles, actively sensitized. Each point represents the mean $\pm$ S.E.M. of 15 (passively sensitized) and 12 (actively sensitized) animals. * P <0.05, ** P <0.01 vs non-sensitized. B, Before.

Table 1. Effect of mepyramine on sneeze and increased specific airway resistance (sRaw) induced by inhalation challenge with Japanese cedar pollen in passively and actively sensitized guinea pigs

Group	Sneezing frequency (times/10 min)	Change of sRaw (AUC)	
		Early phase (0 – 3 h)	Late phase (3 – 10 h)
Passively sensitized			
Control	14.9 ± 2.8	2.68 ± 0.48	0.48 ± 0.23
Mepyramine	7.0 ± 1.1*	1.95 ± 0.46	0.53 ± 0.18
Actively sensitized			
Control	8.1 ± 1.4	3.71 ± 0.70	5.55 ± 0.75
Mepyramine	4.3 ± 1.0*	2.95 ± 0.67	5.40 ± 1.40

Mepyramine (10 mg/kg) was administered orally 1 h before challenge. Each value represents the mean $\pm$ S.E.M. of 15 (passively sensitized) and 12 (actively sensitized) animals. * P <0.05 vs control.

(P <0.05) (Table 1). Since an average of 3 sneezes at 0–10 min was induced in non-sensitized guinea pigs following pollen inhalation (as described above), the inhibitory ratios of mepyramine on antigen-induced sneezing in the passively and actively sensitized animals can be calculated as 80% and 65%, respectively.

In contrast, the area under the nasal blockage response curve at 0–3 and 3–10 h after antigen challenge, in both passively and actively sensitized guinea pigs, was not significantly reduced by the mepyramine treatment (Table 1).

Nasal responsiveness to LTD₄ in the passive and active sensitization models

When LTD₄ was instilled into the nasal cavities of non-sensitized guinea pigs 2 days after the pollen inhalation,

sRaw did not increase even at a concentration of 10^{-6} M. In contrast, the administration of 10^{-8} and 10^{-6} M LTD₄ to actively sensitized, challenged animals 2 days after the 15th pollen challenge increased sRaw in a concentration-dependent fashion (P <0.001) (Fig. 3B). On the other hand, in the passively sensitized, challenged animal, the degree of hyperresponsiveness was obviously weaker than that seen in the actively sensitized model (Fig. 3A).

DISCUSSION

Terashi et al. (11) compared the time-course of asthmatic response in actively sensitized guinea pigs with that in passively sensitized animals. Guinea pigs that had been actively sensitized by repeated inhalation of antigen (ovalbumin, OA) mists developed not only early- but also

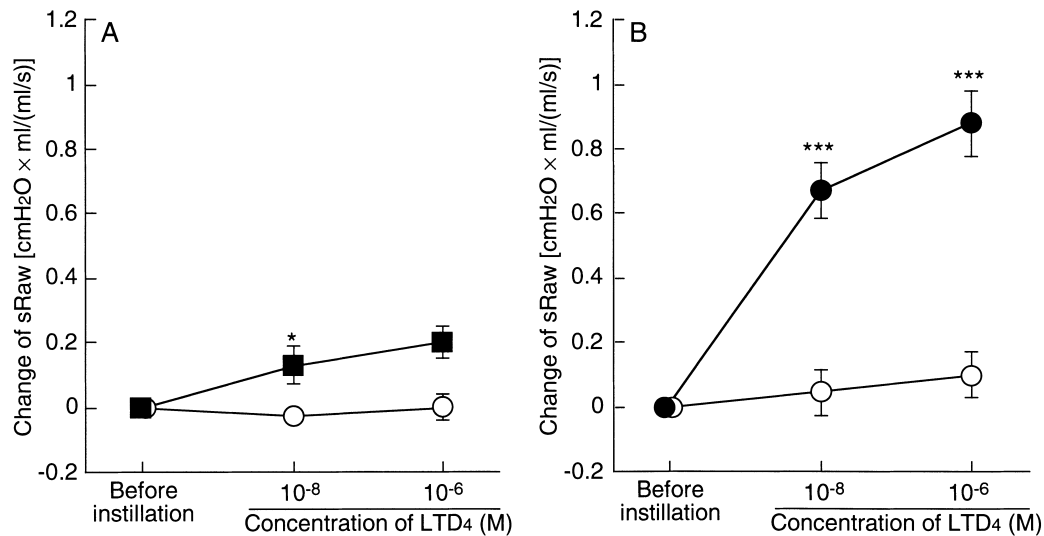


Fig. 3. Nasal responsiveness to leukotriene (LT) D₄ in passively (A) and actively (B) sensitized guinea pigs. LTD₄ was instilled into the nasal cavities of sensitized animals 2 days after Japanese cedar pollen inhalation challenge. Actively sensitized guinea pigs were challenged with LTD₄ 2 days after the 15th pollen inhalation. Open circles, non-sensitized; closed squares, passively sensitized; closed circles, actively sensitized. Each point represents the mean $\pm$ S.E.M. of 15 (passively sensitized) and 12 (actively sensitized) animals. * $P < 0.05$, *** $P < 0.001$ vs non-sensitized.

late-phase asthmatic responses following OA inhalation challenge. However, only the early bronchoconstrictive response was induced by OA inhalation in guinea pigs sensitized passively with anti-OA antiserum. Results of other studies (10, 12, 13), including our previous report (14), have also indicated that repeated antigen inhalation causes a biphasic asthmatic response. In contrast to the situation with lower airway obstructive responses, research on experimental models of allergic rhinitis has not been very extensive. We recently developed an animal model of allergic rhinitis that exhibits not only sneezing but also biphasic nasal blockage and marked nasal hyperresponsiveness to histamine and LTD₄ (7–9). In the present study, intensities of these nasal symptoms in passively and actively sensitized guinea pigs were compared.

In this study, although the cedar pollen inhalation challenge induced biphasic nasal blockage in the actively sensitized model, passively sensitized guinea pigs showed only the early phase response, a finding similar to results of studies on the asthmatic response (10–14). This finding suggests that the early phase nasal blockage is induced by a single antigen-antibody reaction and that multiple anaphylactic reactions are required for the induction of the late phase response. On the other hand, mepyramine did not affect early nasal blockage in either of our models, suggesting that histamine is not substantially involved in the nasal blockage, a finding that contrasts with the data obtained in guinea pig asthmatic models (10–15), but which is consistent with clinical reports (20–22). When histamine solution was administered into the nasal cavities of non-

sensitized guinea pigs, sRaw increased by approximately $0.3 \text{ cmH}_2\text{O} \times \text{ml}/(\text{ml}/\text{s})$ only, even at a dose of 10^{-2} M (8). However, in the actively sensitized model, a similar level of sRaw increase was achieved using 10^{-6} M histamine at 10 h and 2 days after the 20th–24th pollen challenges (8). Interestingly, we recently found that the hyperresponsiveness to histamine was already evident at 4 h after challenge (unpublished data), meaning that the upper airways of the actively sensitized animal were capable of responding to histamine during the late phase. Nevertheless, mepyramine did not affect the late phase nasal blockage. Furthermore, terfenadine, a second-generation H₁-receptor antagonist whose duration of action is considerably longer than that of mepyramine (23), also produced no inhibition of the late phase response (M. Yamasaki et al., submitted manuscript). Therefore, it is suggested that histamine is not released in an amount sufficient to induce nasal blockage during the late phase. In fact, we have evidence that only a small amount of histamine is present in the nasal cavity lavage fluid of the actively sensitized guinea pig at 5 h after challenge in comparison with an amount at the 20th min (M. Yamasaki et al., submitted manuscript).

We found that respiratory rate was reduced when nasal blockage was observed in the actively (7) and passively (data not shown) sensitized animals. However, it has been reported that rapid and shallow breathing is induced during the early asthmatic response (10). These findings, and the fact that inhaled pollen is restrictively trapped in the upper airways (6), indicate that the increase in sRaw in pollen-induced allergic rhinitis models almost entirely reflects

nasal obstruction but not lower airway obstruction. In addition, the ineffectiveness of mepyramine on nasal blockage suggests that the mechanisms of occurrence of airway obstructive responses in upper and lower airways are significantly dissimilar.

The magnitude of nasal hyperresponsiveness to LTD₄ after antigen challenge was markedly stronger in the actively sensitized guinea pigs than those in the passively sensitized animals. When non-sensitized guinea pigs were forced to inhale the cedar pollen repeatedly, their nasal responsiveness to LTD₄ was slightly increased (9). In addition, nasal responsiveness to LTD₄ in guinea pigs that had been treated only with the present active sensitization procedure (intranasal instillation with pollen extract + Al(OH)₃ twice a day for 7 days) without repeated pollen inhalation was also only slightly potentiated (9). However, these increases in responsiveness to LTD₄ were considerably less marked than the hyperresponsiveness observed in the actively sensitized, repeatedly challenged guinea pigs. These results indicate that acquisition of the marked hyperresponsiveness to LTD₄, as well as the antigen-induced late phase nasal blockage, may be due to repeated antigen-antibody reaction at the local site. Nevertheless, details of the mechanisms leading to the induction of the hyperresponsiveness are unclear. The question of whether the occurrences of the hyperresponsiveness and the late phase nasal blockage are related to each other is also under investigation.

In the present study, immediate sneezing was strongly suppressed by mepyramine in both models, a finding consistent with results of clinical experiments (20–22). This further suggests that histamine (probably released from nasal mucosal mast cells following antigen-antibody reaction) plays an important role in the induction of nasal symptoms. In addition, occurrence of sneezing may not be substantially linked to that of nasal blockage.

We thus conclude that, although early phase nasal blockage is probably induced by a single antigen-antibody reaction in the nasal mucosa, multiple anaphylactic reactions are necessary for the occurrence of late phase nasal blockage and hyperresponsiveness to stimuli. Furthermore, it is likely that histamine plays a central role in the induction of sneezing but not nasal blockage.

Acknowledgment

A part of this work was supported by the Promotion and Mutual Aid Corporation for Private Schools of Japan.

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