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Original Article

Repeated antigen challenge in patients with perennial allergic rhinitis to house dust mites

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Abstract

Background: In allergic rhinitis, antigen–antibody reactions occur in the nasal mucosa through antigen exposure. A strong reaction occurs following exposure to a large amount of antigen, whereas no reaction occurs in the absence of antigens. In seasonal allergic rhinitis, nasal hypersensitivity increases during the pollen-dispersing season, which is known as the 'priming effect'. The purpose of the present study was to clarify whether repeated nasal challenges bring about increased nasal hypersensitivity in patients with perennial allergic rhinitis.

Methods: Fourteen patients with perennial allergic rhinitis to house dust mites were enrolled in the present study. Repeated challenge tests were performed once daily for 8 consecutive days with a fixed amount of antigen.

Results: Sneezing and nasal secretion were slightly enhanced by repeated challenges only on the 2nd and 3rd days, whereas nasal resistance remained unchanged. Increased sneezing and nasal secretion was marked in a group of subjects who were not sneezing at the first challenge, whereas changes in nasal reaction following repeated challenge were less obvious in subjects who were sneezing at the first challenge.

Conclusions: In contrast with pollinosis, nasal provocation reactions were not clearly enhanced by repeated provocation. To further understand nasal reactions induced by antigen challenge, studies should be performed under specified conditions (i.e. in an experimental room) with a prescribed quantity of antigens administered, as well as within a study environment.

Key words: nasal provocation test, perennial allergic rhinitis, priming effect, repeated antigen challenge.

Introduction

In type I allergic diseases, antigen–antibody reactions occur in the nasal mucosa, bronchus and/or skin through antigen exposure, leading to local allergic reactions and local sensitivity to antigens. The intensity of the allergic reaction depends on the quantity of antigen; a strong reaction occurs following exposure to a large amount of antigen, whereas no reaction occurs in the absence of antigens. In seasonal allergic rhinitis, Japanese cedar pollinosis, the most common pollinosis in Japan, antigen-specific nasal hypersensitivity increases during the pollen-dispersing season, which is known as the 'priming effect', but returns to a level comparable with that of non-allergic people during the off-season.[1]

Connell proposed the term 'priming effect' for the first time on the basis that repeated daily pollen challenges on 7 successive days decreased the nasal threshold for allergic rhinitis and that environmental exposure during the ragweed pollinating season primed ragweed-sensitive individuals.[1] Thereafter, Bacon *et al*.[2] and Borum *et al*.[3] confirmed this phenomenon. In contrast, Schumacher and Pain,[4] van Wijke *et al*.,[5] Grammer *et al*.,[6] Small *et al*.[7] and Doyle *et al*.[8] failed to demonstrate this phenomenon. In Japan, Konno[9] conducted repeated nasal challenges for 4 consecutive days in patients with Japanese cedar pollinosis using the paper disc method and revealed an increased manifestation of sneezing and discharge, but not nasal airway resistance, comparing provocation reactions on the 1st and

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4th days. Yoshida *et al*.[10,11] also performed repeated nasal challenges for 6 consecutive days in patients with Japanese cedar pollinosis using the paper disc method and demonstrated that the early phase reaction progresses following repeated challenge.

To our knowledge, studies of 'priming effects' have been performed by repeated challenge with pollen in patients with pollinosis but have not been documented in rhinitis patients with house dust mite allergy, the most common perennial allergy in Japan. This phenomenon, although it remains controversial, has practical importance in mite allergy compared with pollinosis, because allergen exposure is perennial and prolonged, leading to stronger priming of the nasal mucosa in mites allergy. For the first time, we have attempted to confirm whether this phenomenon occurs following repeated nasal challenge with discs containing dust mite allergen.

METHODS

Subjects

Fourteen patients with perennial allergic rhinitis to house dust mites, a major causative antigen in Japan, were enrolled in the present study. According to the *Japanese Practice Guideline for Allergic Rhinitis* (revised 3rd edn),[12] patients were diagnosed as having allergic rhinitis if they had allergy like symptoms together with positive results in two or more of the following three tests: (i) skin test or determination of serum antigen-specific IgE; (ii) nasal provocation test; and (iii) test for nasal eosinophilia.[12] Fourteen male patients with allergic rhinitis, having a class 3 or higher for serum antigen-specific IgE antibodies (CAP-RAST), and who were positive in the nasal provocation test (degree 2 or more severe) were enrolled in the present study (Table 1). These 14 subjects were also confirmed to have a negative response following nasal challenge with a control disc without allergen. Subjects ranged in age from 21 to 29 years, with a mean age of 24.2 years. None suffered from seasonal rhinitis and rhinoscopy did not reveal any gross anatomical abnormalities. There was a 1 month washout period before the present study and none of the subjects received astemizol in the 6 weeks prior to the study. None had received immunotherapy. All patients gave informed consent. The study was conducted under controlled conditions with hospitalization.

Table 1 Subjects' background

	n	Total
Type of disease		
Sneeze/secretion	1	14
Sneeze/secretion/obstruction	13	
Severity		
Mild	3	14
Moderate	6	
Severe	5	
Duration of disease		
5–< 10 years	1	14
10–< 20 years	12	
> 20 years	1	

Methodology

The nasal challenge test was performed using the paper disc method[13] for 8 consecutive days at 24 h intervals using an equal quantity of antigens at 19.00 h. Changes in nasal symptoms induced during the 5 min period after provocation were evaluated as follows: (i) sneezing or an itchy sensation of the nose; (ii) watery nasal secretion; and (iii) nasal mucosal swelling. Nasal secretions were collected by blowing the nose with a tissue after antigen provocation for 5 min and the tissue was then weighed. The difference in weight between tissues before and after nose blowing gave the quantity of secretion induced. The weight of the tissue just before provocation was also analyzed, because this indicates the natural secretion of nose. Nasal resistance (P = 100 Pa) was measured with a rhinoanemometer (anterior induction method) 20 min after antigen provocation (Fig. 1). Nasal resistance was hypothesized as 100 Pa/cm^3 per s when scaled out.

In the nasal challenge test, two discs (Allergen Disc 'Torii' House Dust; Torii Pharmaceutical, Tokyo, Japan) were placed on the anterior edge of the inferior turbinate mucosa on each side; each disc is a piece of small, round filter paper, 3 mm in diameter, that contained 250 μg crude allergen extract from house dust mites (33.3 ng Der f 1, a major allergic component). The concentrations of the major house dust mites allergens, Der p 1 and Der p 2, were measured in dust collected by vacuuming the hospital room. The allergen levels for both Der p 1 and Der p 2 were < 0.10 μg/g fine dust.

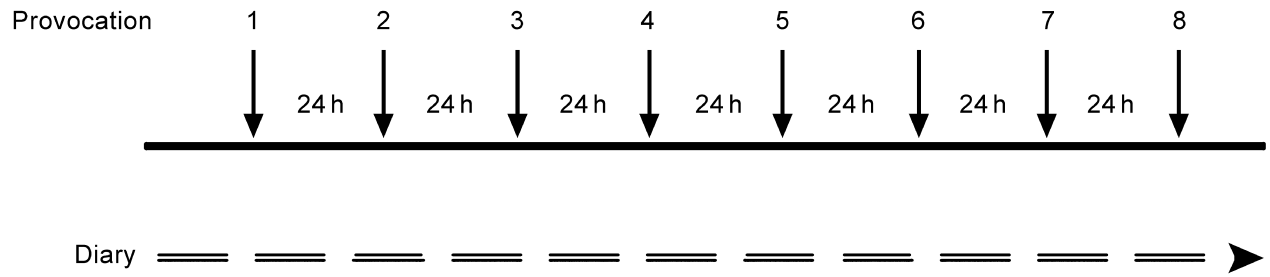

Fig. 1 Time-course of nasal antigen challenge. The arrows indicate nasal challenge with the house dust mites disc.

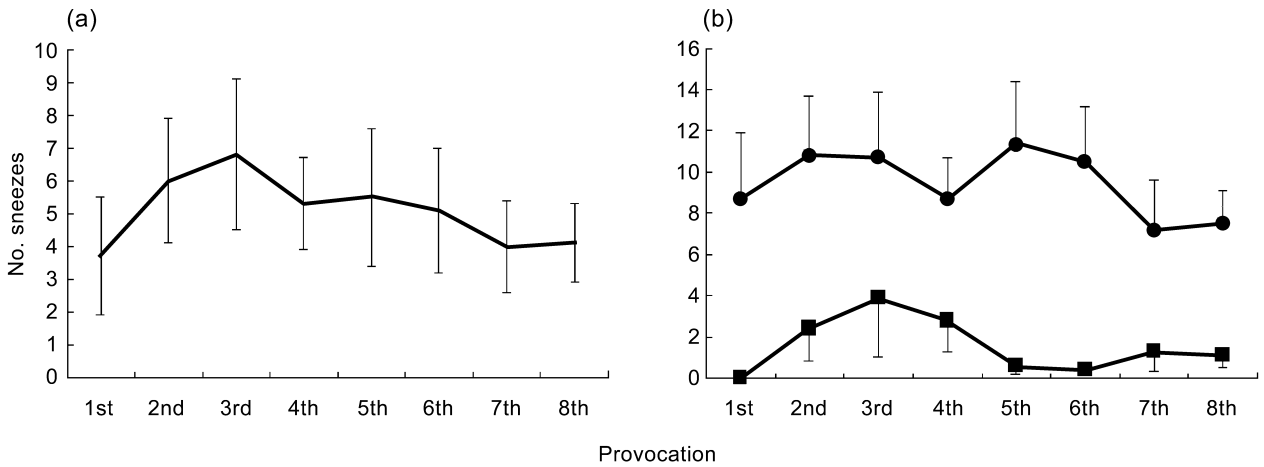

Fig. 2 Changes in the number of sneezes of (a) all subjects and (b) the low- (■; $n = 7–8$) and high-reaction (●; $n = 6$) groups. Data are the mean ± SEM.

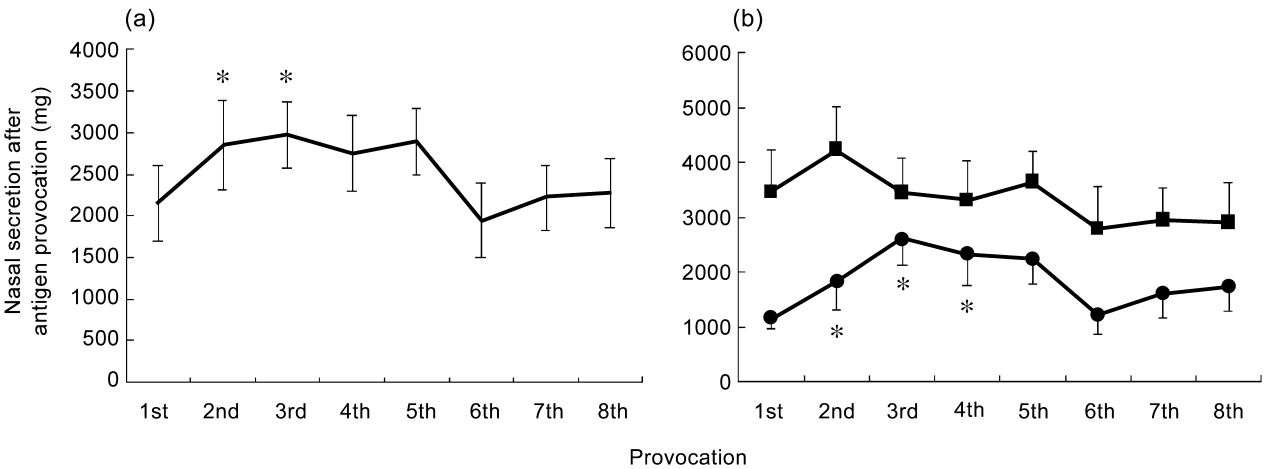

Fig. 3 Changes in the quantity of nasal secretion of (a) all subjects and (b) the low- (■; $n = 7–8$) and high-reaction (●; $n = 6$) groups. Data are the mean ± SEM. *$P < 0.05$ compared with the initial provocation.

Statistics

Nasal symptoms at each measurement point were compared with those observed at the first challenge with antigen. The Wilcoxon signed-rank test was used for statistical analysis and $P < 0.05$ was considered statistically significant.

RESULTS

Of 14 subjects enrolled, 13 completed the scheduled tests; one subject dropped out after the fourth provocation.

Number of sneezes

Sneezing tended to increase as the nasal challenges were repeated but gradually decreased after the forth challenge. However, the increase in sneezing was not statistically significant when compared with the initial challenge baseline.

Subjects were divided into groups depending on whether they were positive or negative for sneezing. The high-reaction group ($n = 6$) were positive for sneezing, whereas the low-reaction group ($n = 7$–8) did not sneeze at the initial challenge. There were no marked changes in the sneezing reaction in the high-reaction group during the course of the study, whereas sneezing tended to increase in the low-reaction group until the third provocation (Table 2; Fig. 2a,b).

Quantity of nasal secretion

The overall quantity of nasal secretion increased at the second and third antigen provocation compared with the initial challenge ($P < 0.05$). This change was more marked in the low-reaction group, like changes in the number of sneezes. Nasal secretion tended to decrease in the high-reaction group, but increased at the second–fourth provocations in the low-reaction group ($P < 0.05$; Table 2; Fig. 3a,b).

The quantity of nasal secretion just before each antigen challenge was followed up during the present study, but remained unchanged, even after the challenge had been repeated.

Nasal resistance

Changes in nasal resistance showed no clear tendency in response to repeated antigen challenge. Because

Table 2 Changes in induced nasal reactions

	Provocation							
	1st ($n = 14$)	2nd ($n = 14$)	3rd ($n = 14$)	4th ($n = 14$)	5th ($n = 13$)	6th ($n = 13$)	7th ($n = 13$)	8th ($n = 13$)
No. sneezes	3.7 ± 1.8	6.0 ± 1.9	6.8 ± 2.3	5.3 ± 1.4	5.5 ± 2.1	5.1 ± 1.9	4.0 ± 1.4	4.1 ± 1.2
Nasal secretion								
Before provocation (mg)	353.4 ± 97.1	307.6 ± 71.4	378.7 ± 127.6	233.6 ± 57.0	279.6 ± 93.1	157.4 ± 23.3	255.5 ± 70.7	194.8 ± 24.4
After provocation (mg)	2149.6 ± 456.6	2855.0 ± 537.9*	2976.0 ± 395.5*	2751.0 ± 452.7	2892.8 ± 404.4	1946.5 ± 450.7	2225.2 ± 390.1	2274.5 ± 422.5
Nasal resistance (Pa/cm^3 per s)	28.81 ± 12.49	21.72 ± 11.34	0.27 ± 0.04	7.51 ± 7.12	7.95 ± 7.67	15.62 ± 10.39	7.99 ± 7.67	8.09 ± 7.66
Positivity score	2.1 ± 0.1	2.2 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	1.9 ± 0.2

Data are the mean ± SEM. *$P < 0.05$ compared with the initial provocation.
Thirteen subjects completed the schedule and only one subject dropped out after the fourth provocation.

nasal resistance scaled out in subjects with severe nasal obstruction, it was difficult to evaluate nasal resistance in all cases using the anterior induction method (Table 2; Fig. 4).

Overall positivity of nasal provocation reaction

Positivity of the nasal challenge reaction remained unchanged in the overall evaluation, even after antigen challenge had been repeated (Table 2; Fig. 5).

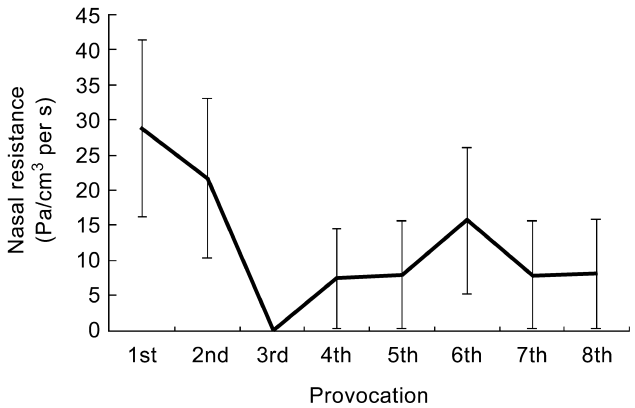

Fig. 4 Changes in nasal resistance. Data are the mean ± SEM. When scaled out, nasal resistance was hypothesized as 100 Pa/cm³ per s.

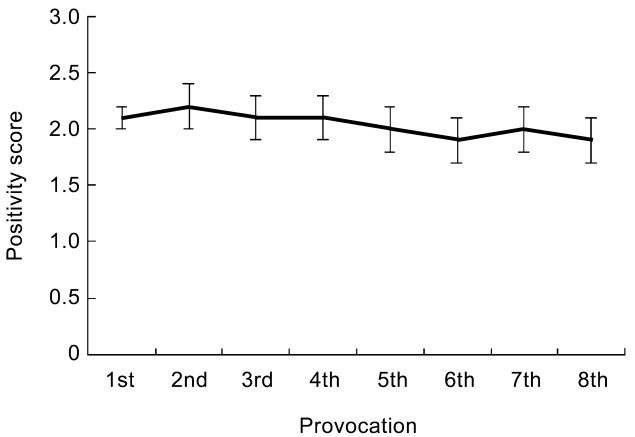

Fig. 5 Changes in positivity score. Data are the mean ± SEM.

Discussion

It has been reported that nasal hypersensitivity increases in patients with Japanese cedar pollinosis when antigen provocation is performed repeatedly.[9–11] In these studies, the quantity of antigens could be prescribed without inhalation of unexpected antigens from the natural environment because challenge tests were performed out of the pollen-dispersing season. In contrast, if patients with perennial allergic rhinitis are examined in a similar manner, it is impossible to strictly control the quantity of antigens, other than those used intentionally for provocation. Thus, the present study was conducted under controlled conditions with hospitalization, where the prevalence of both Der p 1 and Der f 1 was < 0.10 μg/g fine dust, to examine all subjects using the same quantity of antigens.

In the present study, the number and intensity of sneeze attacks tended to increase, peaking 3 days after the start of antigen challenge. The amount of nasal secretion after each challenge increased significantly on days 2–3 after the initial challenge. However, nasal resistance and overall intensity after the challenge test remained unchanged. Although nerve reflex-mediated changes, including changes in the number of sneezes and nasal secretion, were intensified by repeated challenge, no remarkable changes occurred in nasal resistance, which is mainly associated with vascular system reactions. Under the study schedule used in the present study, antigen provocation-induced nasal reactions, which involve the nervous system, increased, whereas no changes were observed in reactions such as nasal swelling or obstruction, in which the vascular system and migrating cell infiltration are thought to be deeply involved. It is suggested that individual symptoms may appear at different times according to the mechanisms of onset.

We failed to demonstrate clearly the priming effect in patients with house dust mite allergic rhinitis, unlike in ragweed pollinosis patients.[1] This controversial result may be dependent upon the types of allergen and the methods of provocation. Connell[1] challenged patients with pollen grains during both the pollen-dispersing and off seasons, whereas we did challenged patients with allergen discs in a mite-free environment and made baseline priming with mites the nasal challenge on the first day. Connell[1] and we repeated nasal allergen challenge on a daily basis for 7 consecuative days. Connell evaluated the priming effect by a decreased threshold of

allergen amount, whereas our evaluation examined changes in intensity of the overall response. Our method of evaluations is similar to that of Konno *et al*., who examined patients with Japanese cedar pollinosis.[9] They showed a statistically significant difference in the degree of symptoms induced between the 2nd and 4th days. However, the changes reported by Konno *et al*.[9] were very small (i.e. 4.0 ± 0.74 *vs* 5.3 ± 3.5 sneezes; 1.95 ± 0.24 *vs* 3.05 ± 0.31 g nasal secretion; 80.7 ± 29.8 *vs* 90.7 ± 21.2% nasal airway resistance). These results, taken together with those of the present study and reports in the literature as mentioned in the Introduction, indicate that intensive prolonged exposure to pollen may induce the priming effect in patients with pollinosis, whereas acute short-time exposure to dust mites does not clearly cause the priming effect. This is a practical convenience if perennial exposure to dust mite allergen does not cause this phenomenon (i.e. increase in hypersensitivity, nasal provocation test can be repeated at short intervals for diagnosis and the duration of drug effects can be followed up by repeated provocation).

If it is true that the priming effect occurs in pollinosis but not in perennial dust mite rhinitis, what is the reason for this? The final conclusion needs further study regarding factors such as the quantity of allergen used, provocation in a hospital room, time intervals of antigen challenge and provocation methods.

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