

Nasal challenge with pollen grains, skin-prick tests and specific IgE in patients with grass pollen allergy

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Summary

Nasal challenges with pollen grains are as close as possible to natural pollen exposure, but they are not well documented in grass pollen allergy. Forty-four grass pollen allergic patients and ten non-allergic volunteers were tested by means of nasal challenge, quantitative skin-prick tests with a standardized orchard grass pollen extract and serum-specific IgE. Nasal challenges were performed with lactose and increasing concentrations of orchard grass pollen grains (15–3645 grains, three-fold increase). The test was considered to be positive when a symptom score over 5 was obtained, since this score had been previously correlated with the release of PGD₂ in nasal secretions. All control subjects and 3/44 patients had a negative challenge. The number of orchard pollen grains required to elicit a positive challenge was 332 ± 440 (range: 15–1215 grains) and the distribution was Gaussian. This number is higher than expected according to pollen calendars performed during the season, but owing to the priming effect of the nasal mucosa by allergens it is compatible to natural exposure. The correlation between nasal provocation tests and skin-prick test end-points was significant ($P < 0.005$, Spearman rank test). Conversely there was no correlation between nasal challenge or skin-prick test end-point and serum-specific IgE.

Introduction

Nasal challenges are performed either with allergen extracts or pollen grains [1–7]. Intra-nasal application of allergen extracts is far from being natural and the insufflation of pollen grains into the nostrils was shown to be highly effective since it is closer to natural pollen exposure than allergen extracts in solutions or placed on discs [8,9]. Moreover, it was found that symptoms elicited by pollen grains were correlated with the occurrence of allergic mediators in nasal secretions [5,10]. The methods for assessing the positivity of nasal challenges with pollen grains often use the titration of

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mediators in nasal secretions [5,10,11] but cannot use the evaluation of nasal airway resistances that are increased after lactose insufflation [5,12]. In a previous study we have constructed a symptom score and shown that it was significantly correlated with the release of PGD₂ in nasal secretions [10].

Nasal challenges with ragweed pollens have been widely documented both before and after specific immunotherapy [3–5,13]. It has been observed that the threshold number of grains inducing symptoms, although far greater than the number of pollens present in the atmosphere during the pollen season, was compatible with the priming effect described by Connell [14]. However, although grass pollens represent the major pollen allergens in many parts of the world, data concerning nasal challenge with grass pollen grains are not yet totally evaluated [6,7].

Skin-prick tests represent the easiest and one of the major tools in the diagnosis of immediate type allergy [15] but their relation with nasal provocation tests performed with pollen grains is still poorly determined. The titration of serum-specific IgE is widely used but the relationship between the amount of serum IgE and the threshold dose eliciting a positive skin or nasal provocation test is still a matter of discussion [16–19]. However, the poor correlation between various investigations may be related to the lack of standardization of the tests or, alternatively, to the fact that nasal challenges were performed with a method being far from natural pollen exposure.

A study was undertaken using nasal challenges with orchard grass pollen grains in pollen-allergic individuals to determine the number of grass pollen grains required to induce nasal symptoms and to compare nasal challenges with both serum-specific IgE and the end-point skin-prick test titre.

Subjects and methods

Patients

Forty-four patients (twenty men, age: 13–64 years, 33.1 ± 11.5 years) volunteered to enter in the study after informed consent. They were selected on the following criteria: (a) all had symptoms of rhinitis during the grass pollen season and more than half also had symptoms of asthma and/or conjunctivitis (the duration of symptoms ranged from 2 to 23 years);

(b) all patients had a positive skin-prick test to a 1/100 (w/v) standardized orchard grass pollen extract (Stallergènes Laboratories, Fresnes, France) and the presence of orchard grass pollen-specific IgE;

(c) none of the patients had received any form of specific immunotherapy to pollen extracts.

Ten non-allergic individuals ranging in age from 18 to 45 years (28 ± 12 years) were taken as a control group.

Investigations

Skin-prick tests. Skin-prick tests (SPT) were performed according to the modified method of Pepys [20], using a hypodermic needle on the volar surface of the forearm. None of the patients had any factor that could modify the performance of skin tests [21]. All allergic patients had quantitative SPT with a standardized orchard grass pollen extract (Stallergènes Laboratories, Fresnes, France) prepared according to a method previously described in detail [22] and controlled by *in-vivo* and *in-vitro* methods (RAST inhibition, iso-electric focusing and cross-immuno-electrophoresis).

They were standardized in biologic units (BU) according to Aas & Belin [23]. Skin-prick tests were done using three-fold dilutions from 2 to 39 000 BU/ml. The end-point titre was calculated as the threshold concentration giving a weal similar to that of the 9% codeine phosphate solution.

Serum-specific IgE. Serum-specific IgE was titrated by Phadebas® RAST (Pharmacia Diagnostics AB, Uppsala, Sweden) using the same orchard grass pollen extract as that used for SPT. RAST discs were prepared according to the method of Ceska *et al.* [24]. In all allergic patients, orchard grass-IgE was titrated with serum undiluted, and diluted 1/5 and 1/10, in duplicate, during the same experiment series, and results were averaged. They were expressed in arbitrary units according to their level and the dilution of the serum.

Nasal provocation tests. All patients were tested between 08.00 h and 12.00 h to avoid possible circadian variations, by the same investigator. They were tested within 3 months (December–February) at a time when no grass pollen was found in the atmosphere. None of the patients was under treatment that might affect the performance of nasal challenges. Anti-H₁ histamines, nasal beclomethasone and oral corticosteroids were eventually withdrawn 1 week before challenge. Nasal cromoglycate was withdrawn 2 days before testing. Ketotifen and imipramines were eventually stopped 1 month prior to testing. None of the patients had been suffering from any form of allergic or infectious rhinitis for the past 2 weeks before the challenge.

Capsules containing lactose or increasing amounts of orchard grass pollens from 15 to 3645 (15, 45, 135, 405, 1215, 3645) grains were prepared by the Laboratoires des Stallergènes (Fresnes, France). They were insufflated into one of the nostrils according to a technique modified from Rosenberg *et al.* [25] using a nasal sp inhaler® (Fisons Laboratories, Loughborough, U.K.), the patient refraining from breathing during the insufflation. For each concentration, the sp inhaler was actuated five times. Each capsule was opened after insufflation to assess that it was completely empty.

Lactose was initially insufflated and eventual symptoms were recorded for 15 min. Then, increasing numbers of grains were deposited on the nasal mucosa every 15 min until a symptom score of 5 was obtained (Table 1). This scoring system was defined according to the observations of Østerballe [26], Wihl [27] and Mygind & Borum [2]. We subsequently confirmed the validity of symptom scores in a previous study [10]. It

Table 1. Symptom score

Sneezing: 3–4	1
Over 5	3
Rhinorrhoea: Anterior and moderate	1
Posterior rhinorrhoea	1
Both symptoms	2
Important anterior and posterior	3
Blockade: The patient can breathe freely	0
The patient can only breathe with difficulty (changing frequency and depth)	1
One nostril blocked	2
Both nostrils blocked	3
Pruritus: Of the nose	1
Of the palate or ear	1
Conjunctivitis	1

was observed that the release of PGD_2 in nasal secretions was significantly ($P < 0.005$) associated with a symptom score over 5.

Design of the study

Before any challenge, subjects were selected on the basis of symptoms and positive SPT and RAST with orchard grass pollen extracts. All allergic and non-allergic individuals had a nasal challenge. Only allergic subjects had a quantitative SPT and a RAST with diluted serum.

Statistical analysis

Statistical analysis was performed using non-parametric tests. The Spearman rank test was used for regression analysis, the Wilcoxon W test for paired data and the Mann-Whitney U test for unpaired comparisons.

Results

Nasal challenge

None of the subjects tested had significant symptoms with lactose. Although selected on the basis of a suggestive history and both positive SPT and RAST, three patients had a negative nasal provocation test with 3645 grains. It was not possible to find any obvious difference in the skin-test titre or the level in serum orchard grass-IgE in responders and non-responders to nasal challenge. In the remaining forty-one patients, the number of orchard grass pollen grains required to elicit typical symptoms of rhinitis ranged from 15 to 1215 with a Gaussian distribution (Fig. 1). Conversely, no control subject had a symptom score over 3 for up to 3645 grains. Three subjects, however, had some incomplete nasal blockade that subsided within 5 min and another had minor rhinorrhoea (score of 1 or 2).

Quantitative skin tests

Quantitative skin tests performed with serial dilutions of orchard grass pollen extracts

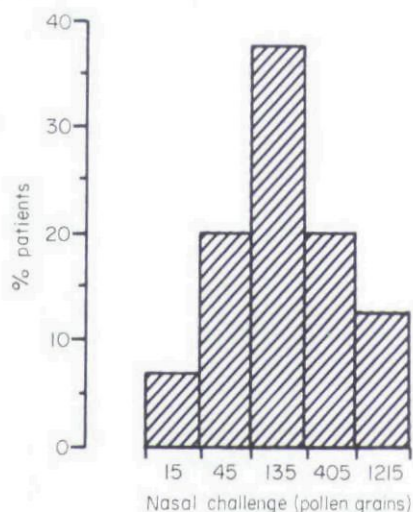


Figure 1. Distribution of positive nasal challenges with *Dactylis glomerata* pollens in allergic patients.

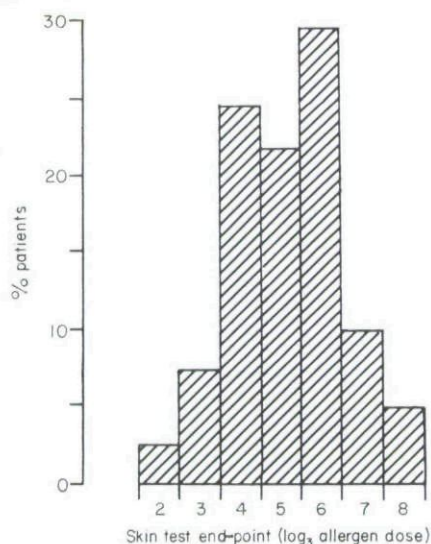


Fig. 2. Distribution of skin-prick tests with a standardized extract of *Dactylis glomerata* pollen in allergic patients.

were only performed in allergic individuals and results are shown in Fig. 2. The end-point titre ranged from 2 to 8.

Orchard grass-specific IgE

With undiluted serum all patients had serum orchard grass-specific IgE in RAST class 3 or 4 in the Pharmacia scoring system. Sixty-five per cent of patients had a RAST class of 4. With diluted serum, orchard grass-IgE ranged from 2 to 300 arbitrary units.

Correlations between nasal challenges, skin tests and specific IgE

Correlations between these parameters are shown in Figs 3, 4 and 5. It can be seen that there is a significant correlation between the threshold dose eliciting positive nasal

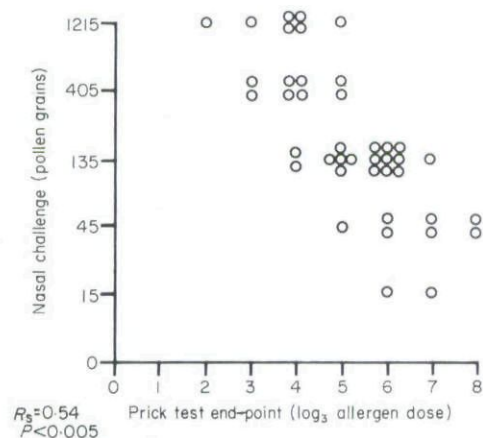


Fig. 3. Relationship between nasal challenges with pollen grains and skin-prick test end-points in patients allergic to *Dactylis glomerata* pollens. Statistical analysis by Spearman rank correlation test.

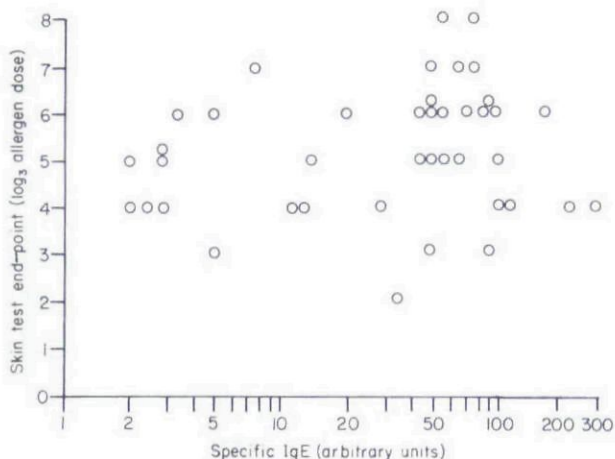


Fig. 4. Relationship between skin-prick test end-points and the titre of serum-specific IgE (RAST) in patients allergic to *Dactylis glomerata* pollens.

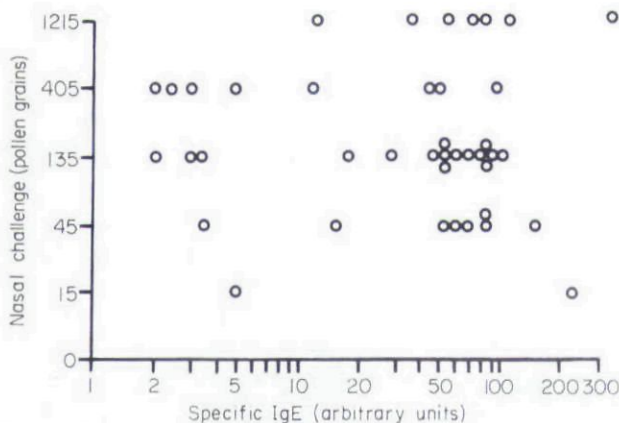


Fig. 5. Relationship between nasal challenges with pollen grains and the titre of serum-specific IgE (RAST) in patients allergic to *Dactylis glomerata* pollens.

challenges and skin tests ($R_s = 0.54$, $P < 0.005$). The majority of patients (41/44) had positive prick tests, nasal challenges and RAST demonstrating an overall correlation of 93.2%, but when the threshold dose eliciting a positive nasal challenge or prick test was compared to the titre of orchard grass-IgE there was no significant correlation.

Discussion

Nasal challenges performed with pollen grains are easy to do and symptoms elicited were shown to correlate with the release of mediators in nasal secretions [5,10]. Mediators were not titrated in the present study but, owing to our previous experience [10], symptoms of allergic origin could be assessed accurately. The number of pollen grains insufflated was chosen according to pollen calendars suggesting that during the season 5–100 grains/³ of grass pollens induce symptoms in most patients [28–29]. The results of nasal challenges are not, therefore, always compatible with palynological observations. These differences may be due either to the lower allergenic concentration of stored pollen grains or alternatively to the priming effect described by Connell [14]. The latter hypothesis was confirmed by nasal challenges with ragweed pollen grains

(R. N. Naclerio and P. S. Norman, personal communication) and it is likely that the greater number of pollen grains usually required to elicit nasal symptoms a few months after the pollen season may correspond to a lower reactivity of the nose. Davies [6] used a different method to measure the positivity of the challenge but numbers of grass pollen grains eliciting a positive nasal challenge were similar. A few patients (3/44) did not present a positive nasal challenge although they had positive SPT and RAST. This observation is noticed in most studies [19] and may be related to the heterogeneity of mast cells and basophilic cells between the skin and the nose [29, 30] or, alternatively, to an insufficient number of pollen grains insufflated owing to the priming effect.

Quantitative SPT have been widely used for standardization purposes [23] or to assess the efficacy of anti-allergic treatments [22]. Skin-prick tests performed with the method described by Pepys are reproducible and the coefficient of variation of this method is under 20% [31]. In the present study we observed a significant correlation between the skin and the nose when challenged by the specific allergen. These results observed with natural nasal challenge accord with previous data. [17, 19]. In another study we observed that the evolution of the nasal sensitivity to allergen after specific immunotherapy was significantly ($P < 0.001$) correlated with that of the skin sensitivity [32]. It should be pointed out that the correlation is only moderately significant ($R_s = 0.54$) since mast cells of the skin and those of the superficial layers of the nose do not appear to be identical [29].

Although the overall correlation between the percentage of patients with positive RAST, SPT and nasal challenges is high, we did not find a significant correlation between the degree of nasal or skin sensitivity to the allergen and the titre of specific IgE. Although in many studies a positive correlation has been found [16, 17, 32], these results accord with Nickelsen *et al.* [34] who failed to demonstrate any correlation between titres of serum allergen-specific IgE and symptoms in untreated patients with seasonal allergic rhinitis, and with Conroy *et al.* [35] who noticed that there was a positive correlation between the number of IgE bound to the basophil membrane but the releasability of basophils appeared to be independent of the number of IgE bound to the membrane. Taken altogether, these findings suggest that the severity of rhinitis is largely independent of the RAST titre, and that the releasability [35] of basophils and mast cells may be an important parameter in determining mediator release and symptom severity.

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