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Allergen-induced nasal hyperreactivity appears unrelated to the size of the nasal and dermal immediate allergic reaction

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It has been previously demonstrated in nasal challenge studies that there is an increased sensitivity to allergen following an initial allergen challenge. A similar feature has been demonstrated following natural allergen exposure in patients with seasonal allergic rhinitis. To further explore the characteristics of this "priming" phenomenon and its relationship to other expressions of their allergic airway disease, 28 hay fever patients with strictly seasonal disease were studied. Skin tests with the relevant pollen allergen and histamine were performed and the size of the immediate and late phase allergic reaction was determined. An initial nasal allergen challenge was followed by a rechallenge of the nose with allergen 24 h later using a lavage technique. Determinations of TAME-esterase activity, as a biochemical marker of the allergic reaction, were made in the returned lavage fluid. The number of sneezes was counted and nasal symptoms were also assessed using a scoring technique. 19 of 28 patients (67%), displayed an increased responsiveness at rechallenge with similar findings in terms of symptom scores and TAME-esterase measurements. The increase was statistically significant for the symptoms of nasal blockage, which increased from 0.7 ± 0.1 (mean ± SEM) to 1.1 ± 0.2 ($P < 0.05$), and nasal secretion which rose from 1.1 ± 0.2 to 1.7 ± 0.2 ($P < 0.01$). A composite nasal symptom score which also took account of the number of sneezes, increased from 2.9 ± 0.4 to 4.0 ± 0.3 ($P < 0.01$). The TAME-esterase activity in the recovered lavage fluid following challenge with the same allergen dose increased from 11.7 ± 2.4 to 18.7 ± 2.8 CPM/10^3 ($P < 0.01$). No correlation was found between the increased responsiveness and the size of initial immediate reaction either in the nose or in the skin, or the presence or size of any late phase dermal reaction. There was, however, a correlation between the size of initial allergen response in the nose and skin ($r = 0.43$). As an increased responsiveness to allergen appears to be of clinical significance at natural allergen exposure, further studies of the pathophysiology of this phenomenon are called for.

Key words: allergic rhinitis; hyperreactivity; nasal allergy; nose; skin tests

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It has been previously demonstrated in nasal challenge studies that there is an increased sensitivity to allergen following an initial allergen challenge (2, 8, 20). This phenomenon has been called "priming" (8). In a recent study it was shown that this increased responsiveness was not only an increased sensitivity to mediators at the receptor level but an increased mediator generation as well (20). A finding similar to the priming phenomenon described in laboratory challenge experiments has been reported at natural allergen exposure. Thus, patients with hay fever display a seasonal increase in nasal responsiveness to allergen (7, 17). In the lower airways there seems to be a relationship between an increased responsiveness to unspecific

and specific stimuli and the presence of a late phase response at bronchial allergen challenge (13). To our knowledge it is not known whether a similar relationship exists in the upper airways and whether this observation extends to allergic late phases in other organs such as the skin.

The aim of the present study was to to study the incidence of the "priming" phenomenon in an unselected hay fever patient population and to establish whether there was a relationship between the size of the immediate reaction in the nose and skin or dermal late phase and the increased nasal responsiveness to repeated nasal allergen challenge.

PATIENTS AND METHODS

Patients

A total of 28 patients were recruited (20 males and 8 females) with ages ranging from 18 to 36 years (mean age 25 years). They all had a positive history of allergic nasal disease. They all had a positive skin prick test for timothy (*Phleum pratense*) and/or birch (*Betula verrucosa*) pollen allergens. They had been free from any medication for at least 3 weeks prior to entry into the study. The study was performed in pollen-free autumn and winter months. The study was approved by the Ethics Committee at the University of Lund.

Skin prick test procedure

At entry, a skin prick test was performed on the volar aspect of one forearm. A recently introduced standardized prick test technique using preloaded prick test needles (Phazet , Pharmacia, Uppsala, Sweden) was used (10, 16). Each individual was tested with the two pollens, birch and timothy, as well as a histamine and a negative control. Fifteen minutes after the prick tests were performed the outline of the induced weal and flare was marked with a pen and transferred onto an adhesive film. The area of the skin and flare reaction was measured with the aid of microcomputer connected to a digitizer (Hewlett Packard, USA) as previously described (1). Any late phase skin reaction was subsequently marked and measured 6 h after the initial challenge in the same way. If both pollens showed a positive reaction we chose the pollen allergy yielding the largest immediate weal for the subsequent nasal challenge and further evaluation.

Nasal challenge procedure

The technique used for the nasal challenge which involves repeated nasal lavages has previously been described in detail (18, 20, 21). Briefly, the protocol involved prechallenge lavages with saline solution to reduce the levels of cell-free mediators that are present initially in nasal secretions to a stable baseline. Then 100 μl of oxymethazoline hydrochloride (0.5 mg/ml, Nezeril , Draco, Sweden) was then sprayed into each nasal cavity to maintain nasal patency during the allergic reaction and thereby permit the collection of nasal secretions. It has been shown previously that this dose of oxymethazoline does not affect mediator release (18). Subsequently, two challenges with diluent (the same as for the pollen extracts) were employed to control for the effect of the delivery system. Thereafter, serial challenges with three doses of antigen each increasing 10-fold. The doses used were $0.1*10^3$, $1*10^3$ and $10*10^3$ BU of birch (*B. verrucosa*) or timothy (*P. pratense*) allergen solution (Pharmalgen , Pharmacia, Uppsala, Sweden). Lavages with saline solution were collected 10 min after all challenges. In addition, a follow-up lavage was performed 20 min after the last antigen dose. 24 h later the same challenge procedure was repeated, but using only a single allergen challenge, $0.1*10^3$ BU, the lowest one from the previous day. The levels of TAME-esterase in the nasal lavages were measured. The number of sneezes after each challenge, (diluent and active) was counted. Prior to each lavage the symptom blockage and secretion was rated on a scale of 0–3 where 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms and 3 = severe symptoms. Furthermore, a composite nasal symptom score was calculated which included the sum of the block-

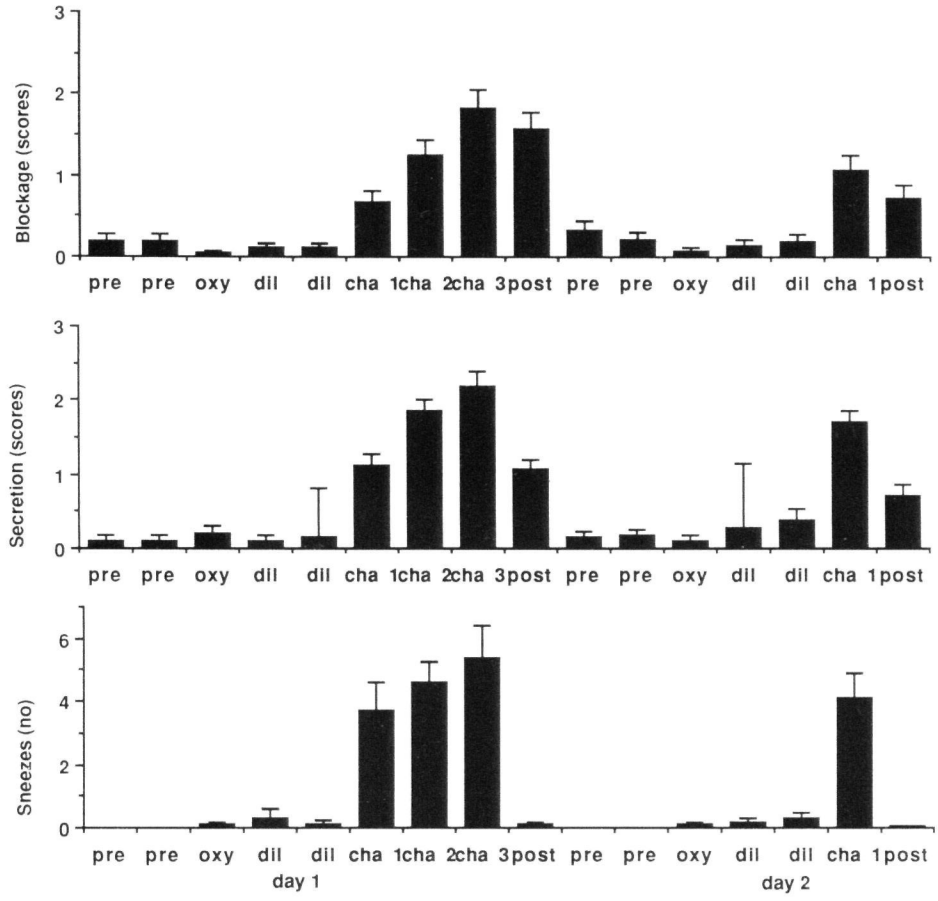

Fig. 1. Protocol of the lavage and challenge procedure. After three quick initial lavages a lavage was performed every 10 min. Immediately after the lavage a challenge was given as shown on the x-axis. The order of the challenges was as follows. Oxymethazoline, diluent, diluent, $0.1*10^3$ BU, $1*10^3$ BU, and $10*10^3$BU on day 1. The same protocol was used on the second day up to the first allergen challenge. Symptoms scores for the nasal symptoms of blockage and secretion (mean ± SEM) at the initial challenge day and at rechallenge day 2 are shown.

age and secretion score and a sneezing score (0 sneezes = 0,1–4 = 1,5–9 = 2, ≥10 = 3) according to (23).

TAME-assay

TAME-esterase in the nasal supernatant was measured using a radiochemical method essentially the one described by Imanari et al. (15) adapted for the nasal lavage procedure (18) which is based on the liberation of H^3-labeled methanol from the synthetic substrate H^3 TAME. In short: 40 µl of the lavage fluid was added to a propylene microtube containing 10 µl of a 0.2 M Tris buffer (pH, 0.8) and the reaction was initiated by the addition of 10 µl of H^3 TAME. The microtube was then immediately suspended in 10 µl of econofluor and 50 µl of stop solution (1 vol glacial acetic acid to 10 vol 0.02 TAME) in a closed counting vial. The microtube was incubated for 1 h at room temperature in the closed vial before the reaction was terminated by shaking. The vial was then counted in a liquid scintillation spectrometer for 1 min. Enzymes which are active in this assay are designated as TAME-esterases. The TAME esterase levels following diluent challenge were substracted from the levels following allergen challenge in order to evaluate the increase after the specific challenge alone.

Statistical analyses

The statistical evaluation was performed on a microcomputer (Macintosh , Apple Computer, Cupertino, USA) with the aid of statistical software package (Statview 512 + by Brainpower Inc. , Calabasas, CA, USA). Student's t-test for paired data was used to compare the values obtained day 1 with day 2. A regression analysis was used in order to evaluate whether there was any correlation between the induced hyper-

reactivity on the second day, expressed as the increase in TAME-esterase activity on day 2 as compared to day 1, and the size of the immediate allergic reaction on day 1 in the nose or the skin or the size of any dermal late phase. A chi-square test was used in order to see whether the mere presence of a dermal late phase, irrespective of size, was indicative of hyperresponsiveness. T-values < 0.05 were considered significant (two-tailed test).

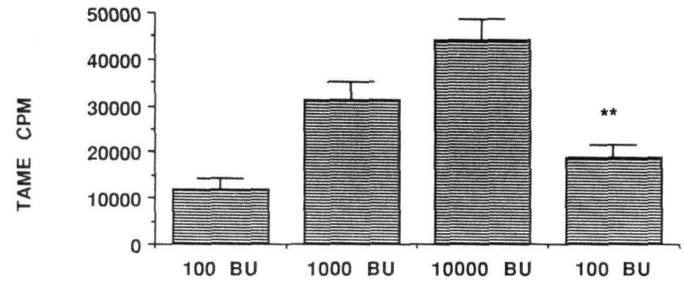

Fig. 2. TAME-esterase levels in lavage fluids following the allergen challenges on day 1 (10^2, 10^3, 10 BU[4]) and day 2 (10^2 BU) (mean ± SEM). The increase seen on day 2 for the same challenge dose is statistically significant ($P < 0.01$).

RESULTS

Of the 28 patients nine were challenged with birch pollen extracts and 19 with timothy pollen extracts. The individual symptom scores are presented in Fig. 1. As shown, the symptoms increased in a dose-dependent manner following challenge with the three allergen doses on the first day. In the comparison between the two challenges using the same allergen dose ($0.1*10^3$ BU) on days 1 and 2, the symptom score for blockage increased from 0.7±0.1 (mean ± SEM) to 1.1 ± 0.2 on the second day ($P < 0.05$), despite the administration of the decongestant, and for secretion from 1.1 ± 0.2 to 1.7 ± 0.2 ($P < 0.01$). The equivalent mean number of sneezes also increased from 3.7 ± 0.9 to 4.1 ± 0.8 (NS). The composite nasal symptom scores increased from 2.9 ± 0.4 to 4.0 ± 0.3 ($P < 0.01$), as shown in Fig. 2, together with the levels of TAME-esterase activity following the allergen challenges. As shown, the TAME-esterase activity increased from 11.7 ± 2.4 to 18.7 ± 2.8 CPM*10^3 ($P < 0.01$) following challenge with the same allergen dose on the 2 days. Individually, 19 of the 28 patients had an increase TAME-esterase activity in the lavage fluid on the second day compared to the first, while the remainder had an unchanged or slightly reduced generation, as shown in Fig. 3A. The corresponding number for the combined nasal symptom score was 18 as shown in Fig. 3B, with 15 individuals being the same. There were no differences in terms of age and sex between those patients who displayed hyperreactivity and those who lacked this expression of their allergic disease. The areas of the induced weal and flare reaction in the skin

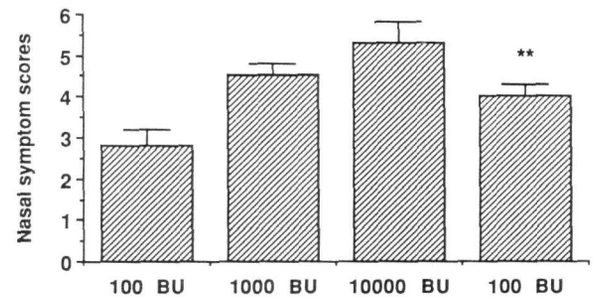

Fig. 3. Composite nasal symptom score following the allergen challenges on day 1 (10^2, 10^3, 10 BU[4]) and day 2 (10^2 BU) (mean ± SEM). The increase seen on day 2 for the same challenge dose is statistically significant ($P < 0.01$).

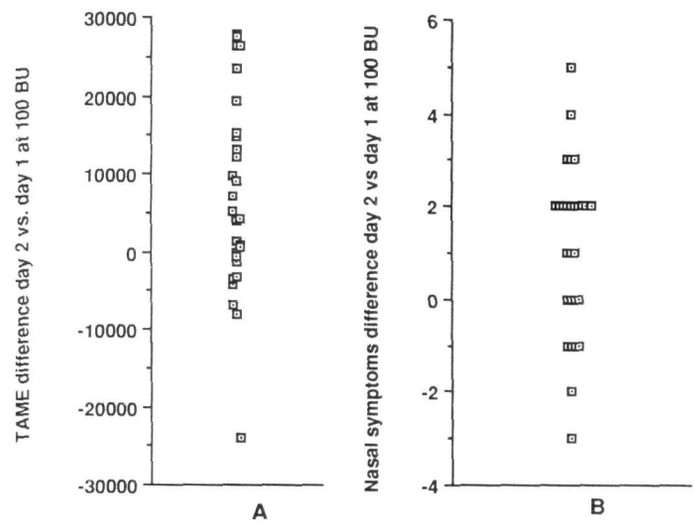

Fig. 4. Individual increase at rechallenge for the combined nasal symptom score and TAME-esterase activity.

for the same allergen as was used in the nasal challenge procedure and the histamine control are presented in Fig. 4. The skin prick test technique which was used produced an allergen-induced but not a histamine-induced late phase with a mean area of 1011 ± 273 mm^2

(Mean ± SEM) in 12 patients (43 %). To evaluate whether the induced hyperreactivity, expressed as an increased TAME-esterase generation on the second day, could be predicted by any of the *in vivo* allergy tests used, a regression analysis was performed in which the increased TAME-esterase generation was compared with the size of the immediate dermal weal and flare reaction, and the size of the dermal late phase reaction and immediate nasal allergic reaction (combined nasal symptom score and TAME-esterase activity). As shown in Table 1, there seems to be no obvious correlation between these parameters. The presence of a dermal late phase, regardless of size, was not indicative of hyperresponsiveness as shown in a chi-square test (Table 2). There was, however, a correlation between the size of the weal for the allergen in the skin prick test and the TAME-esterase activity for the first allergen dose (r = 0.43, $P < 0.03$) in the nasal challenge, and this supports the validity of these test procedures as shown in Fig. 5.

Table 1

Correlation between the increased TAME-esterase generation on the second day as compared with the same allergen challenge on the first day and other *in vivo* allergy tests

Allergy test parameter	Correlation coefficient	*P*-value
TAME generation provocation 1 day 1	0.29	0.12
Size of dermal late phase	0.19	0.33
Nasal symp. score provocation 1 day 1	0.13	0.52
Size of dermal weal	0.11	0.58
Size of dermal flare	0.10	0.61
Total allergy-induced TAME generation	0.06	0.75

Table 2

Chi-square test for the comparison between the frequency of dermal late phase reactions and increased response at nasal rechallenge.

	Dermal late phase		Totals
	present	absent	
Increased response at rechallenge	9	9	18
Unchanged or lower response	3	7	10
Totals	12	16	28

P = 0.31 (NS)

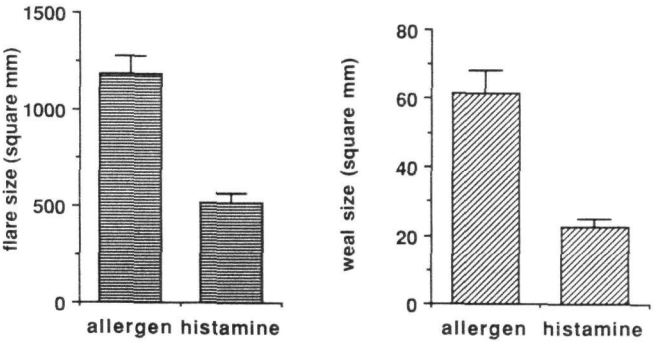

Fig. 5. Area of the allergen and histamine induced weal and flare reaction.

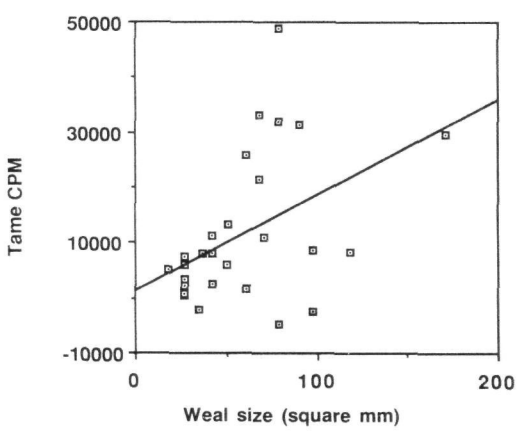

Fig. 6. Correlation between the area of the allergen-induced weal and TAME-esterase activity in the lavage fluid following nasal challenge with $0.1*10^3$ BU. The obtained correlation coefficient of r = 0.43 yields a statistically significant correlation ($P < 0.03$).

DISCUSSION

The present study was performed in order to find the incidence of allergen-induced nasal hyperresponsiveness among an unselected hay fever population, and to find out whether it was possible to predict the presence and strength of any induced nasal hyperreactivity using the standard clinical *in vivo* allergen test. Two of the reasons for the attempt to obtain this information were indications from studies of the lower airways that hyperreactivity is an important

feature of asthma disease (13) and that hay fever patients appear to display a hyperresponsiveness to allergen as part of their disease during seasonal allergen exposure (7, 17). Using the present technique we found that we could induce a nasal hyperresponsiveness in two-thirds of the patients studied. Thus, it appears to be a common expression of the hay fever disease. In the present study we chose a challenge model which involves repeated nasal lavages of the nasal cavity and the measurements of allergen-induced inflammatory mediators. In the present study we chose only to measure TAME-esterase activity in the returned lavage fluids, since it has been previously demonstrated that this is the parameter that best reflects the allergic inflammatory response in challenge models (18). This may be due to the fact that the TAME-esterase activity found in nasal lavages following allergen challenge represents three major components involved in the allergic reaction, mast cells, glands, and plasma leakage (5). This is also demonstrated by the fact that there was a correlation between the skin test and the TAME-esterase generation at nasal allergen challenge.

Although one might expect the strength of the hyperresponsiveness to be attributable to the size of the immediate allergic reaction on the preceding day, we found no such relationship. In the lower airways, hyperreactivity following allergen challenge seems to be closely related to the presence of an allergic late phase (13). It is not possible to determine whether there was a correlation between the induced hyperresponsiveness and a nasal late phase from the presnt study since no active measurements were made during the time period in between. The expression of an allergic late phase that was measured in the skin did not correlate with the hyperreactivity found in the nose.

In the present study we chose to rechallenge after 24 h. This appeared to be a reasonable choice, not only from a practical point of view but also in relation to previous findings. In allergen challenge studies a nasal-induced hyperreactivity was not demonstrated 4 h after the initial allergen challenge, but was present 11 h and 48 h after the initial allergen provocation (6). Not surprisingly, we could also demonstrate the presence of hyperreactivity 24 h after the initial allergen challenge. On the other hand, intranasal challenge with histamine or metacholine does not appear to induce any nasal hyperreactivity (14). It therefore appears more likely that the hyperreactivity is the combined result of the action of multiple mediators.

The nature of the increased responsiveness is not clear. It has been previously demonstrated that the hyperreactivity found in the nasal challenge models is not only an increased sensitivity to released mediators but actually an increased mediator release (20). It has been suggested that this feature is due to an increased number of mediator cells on the nasal surface giving the antigen better access to the mediator cells (4). This feature appears to be relevant not only in challenge models but also during natural allergen exposure (11, 12). Another cell type that also increases on the surface of the nasal epithelium following allergen challenge, as well as during natural allergen exposure, is the eosinophil (3, 19). The eosiniphil which can also be considered a candidate for the increased sensitivity through the activation and release of proteins such as eosinophilic cationic protein and major basic protein with marked cytotoxic potential for human epithelium as well (9).

Attempts have been made to influence the induced hyperreactivity pharmacologically. Glucocorticosteroids, effective agents in the treatment of allergic airway disease, have been shown to ameliorate the induced hyperreactivity (20). As glucocorticosteroids were previously thought to have no effect on the immediate allergic reaction it has been suggested that this feature of glucocorticosteroids is one important mode of action of this drug (22). One expression of the glucocorticosteroid effect is a reduction in the flow of cells from the circulation to the surface of the nasal epithelium (3, 4). It is not known whether these effects are dependent on the continuous presence of glucocorticosteroid.

In conclusion, induced nasal hyperreactivity is a common feature of hay fever. However, it cannot be predicted by any of the common *in vivo* allergen tests. As it appears to be clinically

important, further studies of this phenomenon are needed.

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