

Original article

Evaluation of food-pollen cross-reactivity by nose–mouth cross-challenge in pollinosis with oral allergy syndrome

Background: Oral allergy syndrome (OAS) is often associated with pollen-induced rhinitis, and there are preferential associations between causative substances. If OAS and rhinitis are both immunoglobulin (Ig)E-mediated and there are cross-reacting proteins, it is expected that similar reactions can be elicited in the nose and mouth. In order to test this hypothesis we performed a series of 'cross-challenges' with foods and pollens in both the nose and the mouth.

Methods: Nine patients with ascertained OAS due to vegetables and rhinitis due to pollens were studied. On the first day a nasal challenge with pollen extracts and an oral challenge with fresh food was carried out. After a week, washout nasal challenge with food and an oral challenge with pollens were performed. Immediate symptoms, mucosal tryptase and soluble eosinophil cationic protein (ECP) were assessed after each challenge.

Results: The administration of pollen into the nose and food into the mouth elicited symptoms as expected, but the cross-challenge had no clinical effect. In parallel, tryptase and ECP increased after nasal challenge with pollens, whereas foods did not elicit a measurable response.

Conclusion: The cross-reactivity between foods and pollens, when evaluated at the shock organ, was not clinically evident. This data can be explained with a low concentration of cross-reagent epitopes in pollen extracts and food homogenized because of degradation. The different behaviour upon challenge suggests that different immunological mechanisms may act in the nose and mouth.

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Oral allergy syndrome (OAS) has been recognized as a clinical entity for several decades. It is characterized by oral itching/swelling of the mouth and, sometimes, oedema of the tongue immediately after the contact of specific foods with oral mucosa (1). Foods associated with OAS are usually fresh vegetables and/or fresh fruit. Since its earliest descriptions, it has been observed that OAS is frequently associated with allergic rhinoconjunctivitis due to pollens and there was a preferential association between some vegetables causing OAS and some pollens causing rhinitis (2, 3). The most frequently described and studied associations are that of birch and apple (4) and of mugwort and peach (5), but many other combinations are well-known (e.g. grass-tomato, mugwort-celery-spice). This led to the hypothesis that a cross-reactivity exists between some proteins of foods and plants, and molecular biology techniques detecting cross-reacting allergens (sometimes called pan-allergens), such as profilins and tropomyosin, which are presently considered responsible for the cross-reactions (6–9).

OAS is considered an immunoglobulin (Ig)E-mediated reaction (10), based on its early onset and its similarity with other allergic disorders. Radioallergosorbent test (RAST)-inhibition experiments have confirmed that IgE can recognize cross-reacting epitopes of fruit and plants (11). Nevertheless, to our knowledge there is presently no direct experimental proof that OAS involves an IgE-triggered event. Again, most of the cross-reactivity described has been tested only by immunological assays, but not by the direct and simultaneous application of the allergens to the target organs. Our hypothesis is that if a cross-reactivity exists and the mechanism is common, the cross-reacting allergens should elicit the clinical symptoms when administered to either the nasal and oral mucosa. Moreover, if a degranulation of local mast cells occurs, an increase of tryptase should be observed. To test this hypothesis we studied nine subjects with allergic rhinitis and concomitant OAS and administered the culprit foods and pollens both orally and nasally. The results of these challenges were evaluated by grading the symptom's severity and by measuring mucosal tryptase and eosinophil cationic protein (ECP).

Methods

Study design

Nine patients with pollinosis and OAS were studied. Briefly, after assessing baseline parameters (nasal and oral tryptase and ECP), a nasal challenge with pollen and an oral challenge with foods were performed. Symptom scores were recorded and samples were collected for the measurement of mucosal tryptase and ECP. After a 7-day washout, patients underwent the cross-challenge: nasal challenge with the food and oral challenge with the aeroallergen, and tryptase and ECP were measured again. In the case of multiple food positivity, further challenges were performed after 7-day washout.

Patients and diagnosis

We studied nine patients (three male and six female, mean age 21.9 years, age range: 11–33). All patients suffered from OAS and rhinitis due to pollens. The OAS was diagnosed based on clinical history, prick-by-prick test positivity with the fresh food and *in vivo* oral open provocation (12). Also, a skin test with commercial food extract (Alk-Abellò, Lainate, Milan, Italy) and a RAST assay (UniCap IgE; Pharmacia, Uppsala, Sweden) were performed. The diagnosis of allergic rhinitis was based on typical clinical history (at least 2 years), skin test positivity and RAST positivity for pollen allergens. The characteristics of the patients are summarized in Table 1.

The prick tests were performed according to international guidelines (13) with commercial standardized extracts. Tests were carried out on the forearm, using positive (histamine 1%) and negative (saline) controls for comparison. The results were calculated as the mean of the major diameter of the wheal plus its orthogonal and expressed as class 0 to + + + +. Reactions > 3 mm were considered positive. All the patients (or parents) signed an informed consent and the study was approved by the Ethical Committee.

Nasal pollen challenge and oral food challenge

Patients were required to be symptom-free at the time of the study. They took no medication (topical or systemic antihistamines, topical or systemic corticosteroids, topical cromolyn) in the month preceding the study. On the first day, a nasal-specific challenge with

the responsible pollen (nasal pollen challenge, NPC) was carried out (14). The NPC was performed spraying into a nostril the allergenic extract at increasing concentrations, starting with the lower one. If no symptom appeared after 10 min, the subsequent concentration was administered. Concentrations were 2, 4 and 8 BU/ml (ALK-Abellò), corresponding to about 0.016, 0.032 and 0.064 µg of major allergen per puff. The challenge was preceded by the administration of the vehicle alone as a negative control. The severity of each symptom (itching, sneezing, rhinorrhoea and obstruction) was graded as follows: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. At the same visit, an oral challenge with the fresh food (oral food challenge, OFC) was performed. A small amount of the fresh food had to be kept in the mouth and chewed until symptoms appeared (usually < 1 min). The severity of symptoms (itching and sensation of foreign body) was graded from 0 (absent) to 3 (severe). Oral and nasal mucosal tryptase was measured after 30 min, whereas mucosal ECP was measured 24 h after challenge.

Oral pollen challenge and nasal food challenge

After at least 1 week, the same patients underwent a second series of challenges. In this occasion, 1 ml of the pollen extract (the same used for nasal challenge) was given sublingually at increasing concentrations at 10-min intervals. If the maximum concentration of 8 BU/ml did not elicit a response, the 100 BU/ml concentration (the same used for skin prick test, about 0.8 mcg of major allergen) was applied. The patients were instructed to keep the extract in their mouth for 5 min before spitting it out. This time was considered appropriate, as in the case of oral food challenge the symptoms invariably appeared within the first minute. Symptom severity was graded accordingly to the scale described above.

For the nasal food challenge (NFC), the whole fresh food was smashed and homogenized, then diluted with saline at 1 : 1, 1 : 10, 1 : 100, 1 : 1000 (w/v). Each dilution was then sprayed into one nostril (about 100 µl per puff) at 10-min intervals. Nasal symptoms were recorded and graded as described above. Again, nasal and oral mucosal tryptase and ECP were measured.

Measurement of mucosal tryptase and ECP

Tryptase and ECP levels were determined by means of enzyme-linked immunosorbent (ELISA) assays (UniCAP Tryptase System

Table 1. Patients' characteristics

Patients	Age	Sex	Pollinosis		Oral allergy syndrome (oral challenge positive with the reported allergens)		
			Skin test	RAST (kU/l)	Skin test	Prick-by-prick	RAST (kU/l)
MG	11	M	Grass +++	75.6	Tomato +	Tomato ++++	Tomato <0.35
PC	32	F	Birch +++	21.3	Apple ++	Apple +++	Apple 6.95
BM	28	M	Grass +++	15.3	Celery +	Celery +	Celery 0.87
					Chestnut ++	Chestnut ++	Chestnut 1.5
VP	25	F	Birch +++	5.29	Apple +++	Apple +++	Apple 1.12
					Peach +++	Peach ++	Peach <0.35
AA	11	M	Mugwort +++	2.65	Tomato ++	Tomato ++++	Tomato 2.5
			Grass +++		Apple ++	Apple ++	Apple 6.98
MR	33	F	Cypress +++	16.2	Pine nuts +	Pine nuts +++	ND
PM	25	M	Grass +++	1.2	Tomato +++	Tomato ++++	Tomato 1.27
MV	18	F	Birch ++++	6.56	Apple +++	Apple ++++	Apple 23.2
					Celery +++	Celery +++	Celery 3.8
DM	16	F	Birch +++	3.9	Tomato +++	Tomato ++	Tomato 1.12
				3.2	Apple ++	Apple +++	Apple 3.2

Table 2. Symptom scores in the nose and mouth after challenge with pollens and foods

Patients	Nasal challenge*				Oral challenge**			
	Pollen	Score	Food	Score	Food	Score	Pollen	Score
MG	Grass	5	Tomato	0	Tomato	4	Grass	0
PC	Birch	6	Apple	1	Apple	3	Birch	0
BM	Grass	8	Celery	1	Celery	4	Grass	0
			Chestnut	0	Chestnut	3		
VP	Birch	7	Apple	1	Apple	3	Birch	0
			Peach	2	Peach	2		
AA	Mugwort	4	Tomato	1	Tomato	3	Mugwort	0
	Grass	4	Apple		Apple	4		
MR	Cypress	6	Pine nuts	1	Pine nuts	4	Cypress	0
PM	Grass	6	Tomato	0	Tomato	3	Grass	0
MV	Birch	6	Apple	0	Apple	4	Birch	0
			Celery	0	Celery	3		
DM	Grass	5	Tomato	0	Tomato	4	Grass	0
	Birch	5	Apple	0	Apple	4	Birch	0
Mean		5.6		0.5		3.4		0

* Student's $P = 0.02$; ** Student's $P = 0.01$

FEIA and UniCAP ECP System FEIA; Pharmacia), adapted for mucosae (15, 16). Samples for tryptase assays were collected 30 min after the challenge, whereas the measurement of ECP was made after 24 h. Briefly, the sponges of the Cap system, coated with antitryptase or anti-ECP antibodies were washed with physiological solution, then mounted on an appropriate plastic stick. The plastic stick carrying the sponges were then inserted into a nostril or in the sublingual region and maintained in site for 5 min with a tape fixed on the skin of the nose or lips. The sponges were then removed and frozen in buffered ($\text{NaCl} + \text{NaN}_3$, 0.1%) vials. All the assays were performed at the same time. The concentration of tryptase and ECP were expressed in $\mu\text{g/l}$, according to the calibration curve provided by the manufacturer. Mucosal ECP and tryptase were also measured in 12 healthy subjects to obtain a reference baseline value.

Statistical analysis

The evaluated parameters were analysed by Student's t -test for paired samples (intragroup comparisons), and unpaired samples (intergroup comparisons). Statistical significance was set at 0.05.

Results

All patients were challenged with one pollen and in some cases (four of nine) with more than one food, depending on their clinical history and allergy tests, as shown in Table 1. The nasal challenge with pollen extracts and the oral administration of foods immediately provoked the clinical symptoms, as expected (mean symptom score: 5.6 ± 1.2 in the nose and 3.4 ± 0.8 in the mouth). On the contrary, the nasal food challenge and the oral pollen challenge were invariably unable to elicit measurable symptoms, even using the maximum concentrations (mean symptom score: 0.5 ± 0.4 in the nose and 0 in the mouth). The difference between the two challenges was highly significant (0.02 in the nose and 0.01 in the

mouth). The clinical results of the challenges are summarized in Table 2.

In parallel, after the NPC, a significant increase of nasal mucosal tryptase at 30 min ($P < 0.01$ vs baseline) and ECP at 24 h ($P < 0.05$ vs baseline) was found, whereas the challenge with fresh food modified neither nasal tryptase nor nasal ECP. These results are shown in Fig. 1.

As far as oral mediators are concerned, oral tryptase was invariably below the detection limit of the method ($1 \mu\text{g/l}$) at baseline and after challenge with both pollens and foods in all patients. The same was seen in the 12 healthy controls. The level of oral ECP was low and highly dispersed, although detectable in almost all patients. No significant change could be seen after oral pollen challenge (OPC) ($6.23 \pm 5.3 \mu\text{g/l}$ vs $6.9 \pm 6.1 \mu\text{g/l}$; $P = \text{ns}$) and OFC ($4.7 \pm 3.6 \mu\text{g/l}$ vs $5.1 \pm 3.8 \mu\text{g/l}$; $P = \text{ns}$). There was no difference from the baseline values assessed in 12 healthy subjects ($4.32 \pm 4.1 \mu\text{g/l}$).

Discussion

It is accepted that OAS is sustained by an IgE-mediated reaction (1, 10, 17) and that some cross-reacting proteins contained in pollens and foods can explain the frequent clinical association between OAS and pollinosis. Indeed, several cross-reacting antigens (e.g. profilins and lipid transfer proteins) have been identified on immunochemical basis, especially by means of the RAST-inhibition technique and the Western blot separation of protein bands. It is therefore expected that if a cross-reactivity exists and both nasal and oral reactions are IgE-mediated, the administration of the pollen extract (that elicits a response in the nose) to the mouth should provoke OAS

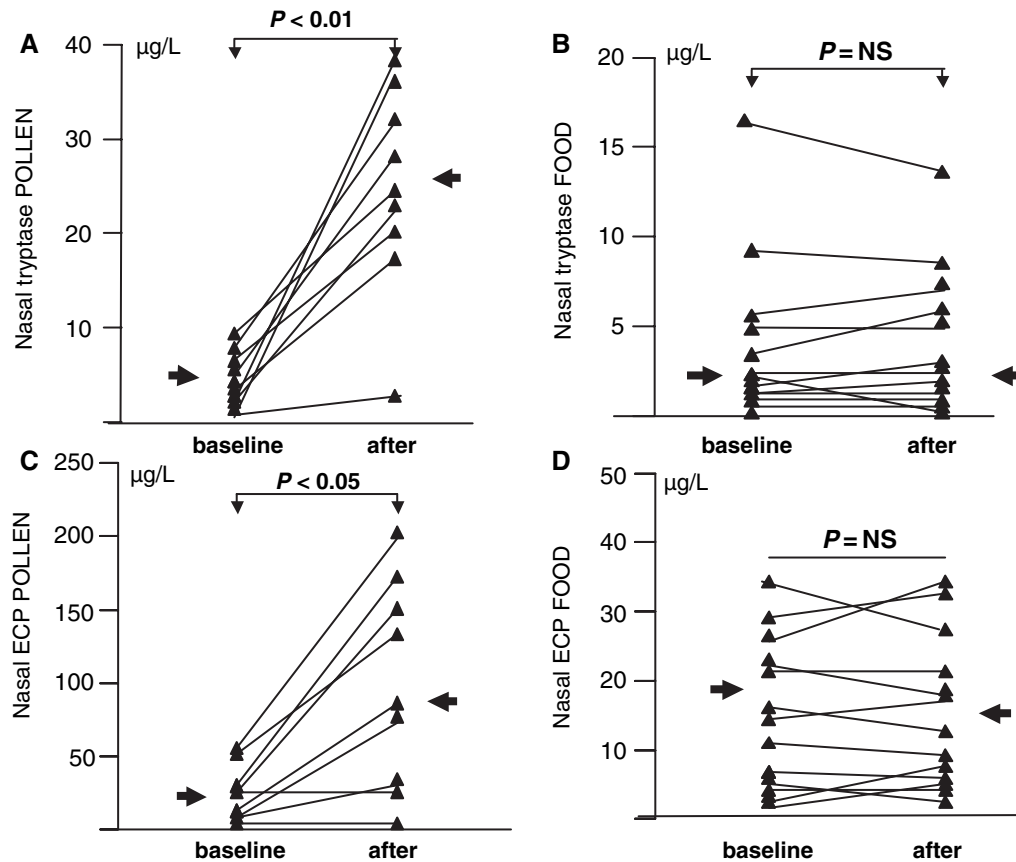


Figure 1. Nasal tryptase measured at baseline and 30' after the challenge with pollens (A) and food (B). Nasal eosinophil cationic protein (ECP) measured at baseline and 24 h after the challenge with pollens (C) and food (D). The results are expressed in mcg/l. The mean values are shown as arrows.

symptoms. Conversely, the nasal administration of the food causing OAS, should elicit rhinitis symptoms. To our knowledge, no experimental evidence of this fact has been provided so far in humans by administering the responsible allergens to both target organs (nose and mouth). Therefore, we set-up a 'cross-challenge' procedure in order to evaluate the effects of the different allergen-containing substances in different organs.

Our study, performed with crossover nasal and oral challenges in patients with concomitant OAS and rhinitis, could not confirm the aforementioned hypothesis. All patients promptly reacted to the nasal administration of the inhalant allergen and to oral administration of the food, but the opposite never happened. This discrepancy cannot be simply explained by the different preparation of the allergens. It is true that some food allergens could have been degraded, but it seems to be unlikely that all proteins were altered, as no chemical or thermal stimulus were applied when preparing the smashed food. It can be argued that the dose of the allergen is crucial, but, for instance, the maximum concentration of the pollen extract (100 BU/ml = 0.8 mcg/ml major allergen), the same used for the skin test, is ineffective in the mouth and the maximum dose of fresh food is ineffective in the nose.

A possible cause of the negativity of oral challenge with pollens may be the anatomical structure of the oral mucosa, which has a keratinized surface, so that pollen antigens cannot reach effector cells. Nevertheless, foods are capable of immediately eliciting the reaction in the mouth, probably because the concentration of allergens is much higher than in pollen extracts, whereas they are indifferent in the nose where mucosa is naked. As expected, the nasal challenge with pollens elicited the mast cell degranulation (testified by tryptase release) and the activation of eosinophils (increased ECP) but, on the contrary, this did not occur with foods. Also in this case, degradation of allergens during homogenization may occur (18) and this may explain why prick-by-pricks with fresh food are much more sensible than prick tests with standard extracts. Noteworthy, no mediator release could be detected, although foods clearly induced the clinical symptoms. This is in agreement with previously reported experiments, showing the lack of correlation between oral symptoms and tryptase/ECP levels in serum and tryptase in saliva (18). This fact was also observed in patients showing oral symptoms after the administration of sublingual immunotherapy (19). In addition, it has been shown that oral symptoms are infrequent in patients with

OAS (20) receiving sublingual immunotherapy with possible cross-reacting allergens. Concerning immunotherapy, although its beneficial effect on OAS has been demonstrated, the effects are variable and patients usually relapse their food sensitization after immunotherapy (IT) discontinuation (21). Finally, it is well-known that in OAS, the diagnostic value of skin prick tests (classically employed to detect IgE-sensitization) is weak (22) and

OAS without allergic respiratory symptoms has also been found due to the cross-reactive pollens (5).

The clinical and immunological results of our cross-challenge study suggest that the immunological mechanism intervening in OAS and rhinitis are, at least in part, different (as observed also in food allergy conditions) (23), and that oral and nasal mucosa behave in a different manner when specifically challenged.

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