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Co-recognition of lipid trasfer protein in pollen and foods in Northern Italy: clinician's view

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Key words

Lipid transfer protein, food allergy, cross-reactivity, pollen allergens.

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Summary

Background: *Lipid transfer proteins (LTP) are pan-allergens in plant derived foods that have been also detected in several pollens. The observed cross-reactivity between pollen and plant-food LTPs has led to hypothesize that primary sensitisation to this allergen may occur through the airways.* ***Objective:*** *The present study looked at the prevalence of hypersensitivity to different LTP-containing pollen sources among peach-allergic subjects sensitized to LTP.* ***Methods:*** *Sixty-six adults (M/F 25/41; mean age 33.7 yrs) with allergy to peach LTP living in the area of Milan underwent SPT with mugwort, plane and olive pollen extracts. IgE to the same allergen sources as well as to peach were measured in 16 cases.* ***Results:*** *Skin tests with Artemisia, Platanus, and Olea pollen extracts scored positive in 16 (24%), 10 (15%), and 10 (15%) patients, respectively. Peach-specific IgE were detected in 16/16 patients, whereas IgE to Artemisia, Platanus, and Olea pollen were found in 7 (44%), 10 (62%), and 8 (50%) cases. In all cases peach-specific IgE levels were higher than levels of IgE to the three pollens, and a strong correlation between peach-specific IgE levels and the levels of IgE specific for mugwort ($r = 0.84$; $p < 0.001$) and plane ($r = 0.96$; $p < 0.001$) pollen was recorded.* ***Conclusions:*** *In Northern Italy olive, plane, and mugwort pollen seem an unlikely source of LTP sensitisation and the most likely primary sensitizer to this protein remains the peach.*

Introduction

Lipid transfer protein (LTP), a heat- and pepsin-resistant allergen present in plant-derived foods is the major cause of both primary food allergy and food-induced anaphylaxis in Italy (1, 2). Although it is generally accepted that the peach represents the primary sensitizer to LTP (3), due to its highly conserved structure and amino acid sequence allergic subjects frequently cross-react to a number of botanically unrelated plant-derived foods. In view of its widespread distribution LTP is presently considered a typical example of plant pan-allergen. In recent years, LTPs have been detected also in several pollens (4) and this fact has led several groups to hypothesize and investigate the possible cross-reactivity between pollen and plant-food LTPs as well as the possibility that primary sensitisation to this allergen may occur through the airways.

With the exception of Par j 2, the major pellitory pollen allergen, a LTP that has no relationship with plant food LTPs, the mugwort pollen lipid transfer protein, Art v 3, has received the largest interest among pollen LTPs. The first studies by Diaz-Perales et al. found that Art v 3 and Pru p 3, the peach LTP, show about 50% identity and a certain degree of cross-reactivity and concluded that this forms the basis of the frequently observed co-sensitisation, although mugwort LTP shows substantially lower levels of specific IgE binding (5). Subsequently, based on clinical studies as well as on the results of cross-inhibition experiments, the same group concluded that Art v 3 might be the primary sensitising agent to the protein in some subjects (6, 7). This opinion was clearly

in contrast with that of Pastorello and co-workers who, based on their clinical and serological investigations, concluded that in peach-allergic patients Art v 3 reactivity is uniquely the result of primary sensitisation to Pru p 3 (8). More recently, another Spanish group suggested that cabbage lipid transfer protein (Bra o 3) may play a relevant role in food-pollen cross-reactivity and that Art v 3 hypersensitivity might identify Artemisia pollen-allergic subjects that are more likely to experience severe food allergy reactions (9). Finally, recent molecular biology studies confirmed the cross-reactivity between Pru p 3 and Art v 3 (10).

Interestingly, Ole e 7 (like Art v 3) displays a sequence identity with plant food LTPs of about 50% (4), and in 2002, Spanish researchers reported their observations in patients with olive pollen allergy a part of which also had a history of food-induced reactions, concluding that there was a strict association between severe food allergy and sensitisation to the olive pollen allergen Ole e 7, a lipid transfer protein (11). These conclusions have been recently challenged by Barber and co-workers who did not observe any correlation between sensitization to peach and olive sources across LTP sensitization (12).

In 2007, Lauer et al. identified a plane pollen lipid transfer protein (Pla a 3) that behaved as a major allergen in those patients living in the Mediterranean basin with both plane pollen allergy and peach allergy. Pla a 3 showed a variable allergenic potency in comparison with Pru p 3, which led no general conclusion about the primary sensitizer. (13).

Thus, despite the efforts of several research groups the relationships between peach (or other plant food) LTP and pollen LTPs remain poorly defined. The present study looked at the relationship between peach LTP and homologous allergens in different pollens from a clinical point of view in a cohort of Italian LTP-allergic subjects; for the first time, the 4 allergenic sources were investigated contemporarily.

Patients and methods

Patients and admission criteria

Adult patients living in the area of Milan with a history of peach allergy (either oral allergy syndrome or urticaria/angioedema) and shown to be allergic to lipid transfer protein by means of a positive SPT with a commercial extract (ALK-Abello, Madrid, Spain) containing LTP but lacking both the PR-10 allergen, Pru p 1, and profilin, Pru p 4, (14) seen at the allergy department of the Clinica San Carlo during the last 5 years represented the starting population. In order to avoid the interference of cross-reacting allergens present both in pollen and in plant-derived foods such as PR-10 or profilin, patients scoring positive on SPT with birch extract were excluded from the study. The area of Milan is virtually olive trees-free, although very rare cases of primary sensitizations to Oleaceae (most probably primarily induced by Privet pollen) may be found. Mugwort pollen monosensitization is very unusual and most positive skin tests to Artemisia pollen are observed in subjects primarily sensitized to short ragweed pollen which has led to hypothesize a possible cross-reacting pollen allergen shared by the two species (15). Plane pollen monosensitization has never been detected over thousands of patients during the last 5 years, although positive SPT are rather commonly observed in subjects sensitized to other tree pollens, particularly birch.

Skin tests and specific IgE measurements

Patients underwent SPT with a large panel of commercial (Allergopharma, Reinbeck, Germany) seasonal airborne allergen extracts including grass, mugwort, ragweed, pellitory, plantain, birch, plane, olive, and cypress. Skin tests were carried out and read at 15 min following established methods (16). Mugwort, plane, and olive pollen extracts contained 50000, 50000, and 5000 BU/ml, respectively.

IgE specific for peach, mugwort, plane, and olive pollen were measured by ImmunoCAP (Phadia, Uppsala, Sweden). Levels were expressed in kU/L; values < 0.35 kU/L were considered negative.

Statistics

Correlation coefficient after Pearson was calculated for specific IgE levels. Probability (p) values < 5% were considered statistically significant.

Results

Skin tests

Sixty-six patients (M/F 25/41; mean age 33.7 yrs, range12-79 years) fulfilled the admission criteria and were included in the study. Skin tests with Artemisia, Platanus, and Olea pollen extracts scored positive in 16 (24%), 10 (15%), and 10 (15%) cases, respectively. Only 3 patients reacted to all 3 pollen extracts. Eight patients reacted to 2 pollens (mugwort and plane in 4 cases, plane and olive in 3, and mugwort and olive in 1), whereas single skin reactivity to mugwort and

olive was found in 8 and 3 cases, respectively. Plane sensitivity was always associated with skin reactivity to one of the two other pollens. Skin reactions to the 3 pollen extracts were weaker than the one induced by the positive control (histamine 10 mg/ml) in most instances.
Seven of the 16 (44%) mugwort reactors were strongly sensitized to ragweed as well; 4/8 (50%) single mugwort reactors were co-sensitized with ragweed.

Specific IgE measurement

Specific IgE were measured in 16 patients (table 1). As expected, peach-specific IgE were detected in sera from all 16 patients. IgE specific for Artemisia, Platanus, and Olea pollen were detected in 7 (44%), 10 (62%), and 8 (50%) cases, respectively, although in the case of plane and olive pollen levels were often low (Table 1).
The concordance between skin tests results and specific IgE levels in these 16 patients was rather good although some discrepancies were observed (patients B, C, O, P, table 1). In general, ImmunoCAP scored more frequently positive for olive pollen than SPT. In all cases peach-specific IgE levels were much higher than levels of IgE to the three pollens, and a statistically highly significant relationship between peach-specific IgE levels and the levels of IgE specific for mugwort ($r= 0.84$; $p < 0.001$) and plane ($r= 0.96$; $p < 0.001$) pollen, but not with olive pollen ($r= 0.31$, $p=$ NS), was recorded. Nonetheless, even in the case of peach/olive relationship olive-positive patients were in most cases those showing the highest peach-specific IgE levels.

Discussion

Previous studies demonstrated that some pollen lipid transfer proteins show a variable degree of cross-reactivity with plant food LTP, and some researchers suggested the possibility that primary sensitization to LTP occurs via the respiratory tract (5-8). An analysis of the cross-reactivity between peach and pollen LTPs by cross-inhibition experiments was out of scope of the present study, which was performed on a population of peach-allergic subjects sensitized to LTP but (most probably) not to other plant-food allergens, such as the Bet v 1-homologous protein or profilin. This was the first clinical study investigating hypersensitivity to mugwort, plane, and olive pollen at the same time in a population of peach LTP hypersensitive patients. Although a study based on the use of purified or recombinant LTP rather than on the use of allergenic extracts would have been preferable, the results seem to suggest rather clearly that the primary sensitizer to lipid

Table 1 - Clinical and serological findings in 16 LTP-allergic patients patients

				SPT			ImmunoCAP			
No.	Sex	Age	Symptoms	Mugwort	Plane	Olive	Peach	Mugwort	Plane	Olive
A	M	33	U + CU	+++	++	+	7,04	1,81	2,04	0,98
B	M	42	OAS	+	++	Neg	4,58	0,24	0,49	0,09
C	M	39	OAS + CU	++ (R)	Neg	Neg	0,41	0,15	0,21	0,54
D	F	26	U	+++	++	++	11,6	1,0	2,32	1,61
E	F	20	U	Neg	Neg	Neg	1,75	0,01	0,03	0,01
F	M	13	OAS	Neg	Neg	Neg	0,96	0,06	0,16	0,01
G	M	36	U	+++ (R)	++	Neg	3,81	1,05	1,22	0,39
H	M	21	U	Neg	Neg	Neg	12,1	1,03	1,42	0,05
I	F	23	U	Neg	Neg	Neg	1,08	0	0,14	0,01
J	F	34	U	Neg	Neg	Neg	3,12	0,02	0,04	0,44
K	F	79	U	Neg	Neg	Neg	1,11	0,13	0,38	0,02
L	F	50	U	+++	++	Neg	58,1	3,21	20,8	0,79
M	F	26	OAS	Neg	Neg	Neg	1,44	0,25	0,31	0,14
N	F	27	OAS	+++	++	++	11,4	1,77	2,2	1,48
O	F	25	OAS	Neg	Neg	Neg	16,6	0,61	0,44	0,16
P	F	44	U	++	+++	Neg	2,75	0,32	0,74	0,51

Symptoms: peach-induced symptoms (U= urticaria/angioedema; OAS: oral allergy syndrome; CU: contact urticaria).
Skin test reactivity is expressed by comparison with the wheal induced by a SPT with histamine 10 mg/ml. (+++ = equal; ++ 50-100%; + < 50%). The symbol (R) means co-sensitization with ragweed.
Specific IgE levels are expressed in kU/L. Positive results are highlighted in red (strong reactivity) or yellow (weak reactivity).

transfer protein is the peach and not pollen. This conclusion is supported by several facts. First, sensitization to the studied pollens was found in a limited number of peach allergic subjects both in-vivo and in-vitro; in other words, in the majority of peach-allergic subjects no cross-sensitivity to any of the 3 pollens could be observed. Second, in all cases peach-specific IgE levels largely exceeded the levels of IgE specific for the study pollens, a fact that, in the presence of putative cross-reacting allergen, suggests primary sensitisation to the peach. Finally, the level of peach-specific IgE clearly influenced the likelihood of a co-recognition of pollen LTP, a situation that is virtually identical to the one observed for the cross-reactivity between plant-derived foods in LTP allergic patients some years ago (17). Moreover, it cannot be excluded that some plane, mugwort, or olive hypersensitive patients reacted to unique pollen allergens other than LTP, which would further disregard the relevance of a putative cross-reactivity. Allergy to LTP is common in Southern Europe and very rare in the North. The reasons for this strange geographical distribution are still unclear. In previous studies we suggested the possibility that sensitisation to peach LTP may occur through the skin (18). Further, we and others reported primary sensitisation to this protein via the airways, although this was in all cases induced by peach-derived particles (19,20). Altogether, although some previous works concluded that exposure to peach and mugwort or plane pollen could facilitate the development of LTP allergy in Spain (21), the present study confirms the absence of association between olive pollen and peach LTP observed in the same Country (12), and suggests that plane or mugwort pollen as well represent an unlikely source of LTP sensitisation in Northern Italy.

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