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Analysis of hypersensitivity to oleaceae pollen in an olive-free and ash-free area by commercial pollen extracts and recombinant allergens

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Summary

Background: *Olive pollen sensitization is surprisingly frequent in Milan, an area that is virtually both olive- and ash-free.* ***Objective:*** *To establish the prevalence of olive pollen sensitization north of Milan, and to investigate the allergens involved.* ***Methods:*** *300 pollen-allergic patients living in this area were randomly selected. Based on SPT results, olive pollen reactors were classified as multi-sensitized, oligo-sensitized, or mono-sensitized. IgE to markers of primary sensitization to olive pollen (Ole e 1), as well as to pollen pan-allergens such as profilin (Phl p 12) or polcalcin (Phl p 7) were measured. Further, the putative cross-reactivity between grass group XI allergen and Ole e 1 was investigated.* ***Results:*** *107 (36%) patients were sensitized to olive pollen; 67 (63%) were multi-sensitized, while only 4 (4%) were mono-sensitized. Specific IgE to Ole e 1 were found in 32/46 (70%) cases; 22 of them (69%) co-recognized pollen pan-allergens, as shown by IgE reactivity to Phl p 7 and/or Phl p 12. Sera from 14 (30%) patients did not react to Ole e 1; of these, 10 (71%) were pan-allergens reactors. No correlation was found between IgE levels to Phl p 11 and Ole e 1.* ***Conclusions:*** *A majority of olive pollen-sensitized subjects seen in the surroundings of Milan are truly allergic to Oleaceae. In the absence of both olive and ash trees exposure to privet pollen might represent the source of this kind of sensitization.*

Introduction

Oleaceae pollen is a relevant cause of respiratory allergy in Europe. Based on the geographical distribution of the various members of this botanical family, hypersensitivity *Oleaceae* pollen can occur at different latitudes. In the Mediterranean basin, olive (*Olea europaea*) pollen represents unquestionably a major cause of seasonal allergy (1-3); in contrast, in northern and central Europe, where *Oleaceae* pollen allergy represents a minor clinical problem, sensitization is mainly caused by ash (*Fraxinus excelsior*) pollen (4, 5). The pollen allergens of the different members of the *Oleaceae* family (olive, ash, privet) show a high degree of cross-reactivity, as demonstrated by RAST inhibition, isoelectric focusing, tandem CIE, as well as immunoblot inhibition (6-8); thus, it is generally accepted that Ole e 1, the major allergen of olive pollen, can be used as a diagnostic marker for sensitization to the whole family (9).

The area of Milan, in the very centre of the Po river flat, is virtually both olive- and ash-free (10).

Nonetheless, olive pollen sensitization is frequently observed in the daily practice in this zone. The present study

aimed to establish the prevalence of olive pollen sensitization, and to investigate the allergens involved by diagnostic methods based on the use of recombinant allergen proteins.

Methods

Prevalence of olive pollen sensitization

Starting from a population of about 4000 adult subjects (>12 years) with pollen allergy diagnosed during the last 7 years at the allergy department of the Clinica San Carlo, Paderno Dugnano, the recordings of 300 patients were randomly selected in order to establish the prevalence of olive pollen sensitization in this area. At the time of the first visit all patients had undergone a thorough interview aiming to establish their origin (birthplace, for how long they had been living in the area of Milan, and whether they spent time in areas where olive trees are present during the olive pollen season, that is May and June) as well as SPT with a large panel of commercial pollen extracts (Allergopharma, Reinbeck, Germany) including, grass, mugwort, ragweed, pellitory, plantain, birch, plane, cypress, and olive. Skin tests were performed and read after 15 min following established methods (11).
Based on the results of SPT, olive pollen reactors were classified as multi-sensitized (if skin reactivity to ≥ 4 pollen sources was found), oligo-sensitized (if skin reactivity to a total of 2-3 pollen sources was present) or mono-sensitized.

Specific IgE measurements

In order to detect whether olive pollen extract reactors showed a primary sensitization or if skin reactivity to olive pollen was the result of co-recognition of pollen pan-allergens, IgE to both markers of primary sensitization to olive pollen (Ole e 1), as well as to markers of hypersensitivity to pollen pan-allergens (Phl p 12 for profilin) and (Phl p 7 for polcalcin) were measured. In vitro tests were carried out by ImmmunoCAP (Phadia, Uppsala, Sweden) following manufacturer's recommendations. Results were expressed in kU/L; values > 0.35 kU/L were considered positive.

Analysis of the relationship between Ole e 1 and Phl p 11.

In view of previous studies reporting a high degree of homology (44%) between group XI grass pollen allergens (16 kDa glycoproteins belonging to soybean trypsin-inhibitor-related proteins) and Ole e 1 (12-14), 18 patients who had always lived in this area and had grass pollen allergy, as shown by clinical history, positive SPT with commercial grass pollen extract (50.000 PNU/ml; Allergopharma, Reinbeck, Germany), and IgE reactivity to specific grass-pollen allergens underwent the detection of IgE to rPhl p 11 as well as SPT with Olive pollen extract (Allergopharma; 5000 PNU/ml) and the detection of IgE to rOle e 1 (n= 12).

Results

Prevalence of olive pollen sensitization and patients' characteristics

107/300 (36%) pollen-allergic patients scored positive on SPT with commercial olive extract. The majority of them (67/107 [63%]) were multi-sensitized. Thirty-six/107 (34%) were oligo-sensitized (skin reactivity to olive pollen was found associated to grass, ragweed, or birch pollen hypersensitivity in 18, 12, and 6 cases, respectively), whereas only 4/107 (4%) were mono-sensitized to olive. All patients had been living in the area of Milan for more than 10 years, and none of them spent time in olive tree-rich areas during the olive pollen season (from the beginning of May to the end of June). Many of them spent their summer holidays in Southern Italy, Tuscany, or Liguria in a period ranging from mid-July to early September, when the olive pollen season is over. The few patients mono-sensitized to olive tree pollen reported slight respiratory symptoms in May and June.

Specific IgE

Specific IgE to Ole e 1 were measured in 46 patients positive on SPT with olive pollen extract.
The in-vitro test scored positive in 32 (70%) cases with IgE levels ranging between 0.36 kU/L and 40.3 kU/L; 22/32 (69%) patients showed a co-recognition of pollen pan-allergens, as shown by IgE reactivity to Phl p 7 (Grass polcalcin) and/or Phl p 12 (Grass profilin), whereas 10/32 did not react to pollen panallergens.
Sera from 14 (30%) patients did not show IgE reactivity to Ole e 1; of these, 10 (71%) were pan-allergens reactors.

Analysis of the relationship between Ole e 1 and Phl p 12 (Table 1).

Of 18 patients studied SPT with olive pollen extract scored positive 16, and IgE to Ole e 1 were found in sera from 12/12 patients; olive pollen SPT and IgE to Ole e 1

were in keeping in all cases. IgE to Phl p 11 were detected in 6/18 sera; 5/6 were positive on SPT with olive pollen. Of 12 Phl p 11-negative patients 11 were olive pollen reactors in vivo; in 7/7 cases they reacted to rOle e 1 in-vitro. No correlation was found between IgE levels to Phl p 11 and Ole e 1.

Discussion

Ole e 1, the major olive pollen allergen, binds IgE from 90% of olive pollen allergic patients' sera (9,15). In view of previous studies showing a high degree of homology between group XI grass allergens and Ole e 1, further corroborated by reports of cross-reactivity between olive and grass pollen in-vitro (16,17), olive pollen sensitizations found in areas where olive trees are absent have been always considered as the result of cross-reactivity to pollen pan-allergens or to grass pollen allergens. The recent advances in molecular biology have resulted in the detection, purification, cloning, and expression of an increasing number of recombinant and/or natural allergen proteins from different sources, which makes it now possible to elucidate these points on a molecular basis.

The present study found that about 1/3 of pollen allergic patients living in this olive- and ash-free area score positive on SPT with commercial olive pollen extract. However, only a small minority (4/300; 1%) were mono-sensitized, most sensitizations being found in patients reacting to other seasonal allergen sources. The majority of olive pollen reactors were multi-sensitized to pollen sources (thus suggesting co-recognition of plant pan-allergens); it was, therefore rather surprising to find that most (70%) patients studies were in effect hypersensitive to the major olive pollen allergen Ole e 1, a marker of genuine allergy.

Since most Ole e 1 reactors were co-sensitized to different sources showing the same pollen season (e.g., grass, pellitory, *Fagales*) it is very difficult to estimate the clinical relevance of olive pollen allergy in this area. The few mono-sensitized subjects reported very slight nasal symptoms in late springtime. As a difference from previous reports (18,19) no patient hypersensitive to olive pollen re-

Table 1 - IgE to grass and olive pollen allergens and skin reactivity to olive pollen extract in 18 grass-pollen-allergic patients.

Patient	Phl p 1	Phl p 4	Phl p 5	Phl p 11	Ole e 1	SPT olive
1	13	4,12	Neg	0		pos
2	72,9	52,4	64,6	0		pos
3	0,62		0	0	0,39	pos
4	0	6,43		0		neg
5	3,38	0,87	1,8	0		pos
6	>100	79,3	80,80	0		pos
7	>100		>100	22		neg
8	14,9	11,3	1,01	0	1,68	pos
9	1,58		0	0	2,25	pos
10	2,43		0	0	1,97	pos
11	16,2		14,3	2,36	4,26	pos
12	14,9		7,33	0	2,95	pos
13	>100		>100	>100	0,59	pos
14	59,5		49,5	0	14,10	pos
15	57,4		38,4	31,2	19,70	pos
16	95,6		>100	58,9	9,17	pos
17	29,5		22,7	0,42	2,76	pos
18	1,05		0	0	3,47	pos

IgE levels are expressed as KU/l (negative if < 0.35 KU/l)

ported perennial symptoms. Further, it is unclear why patients mono-sensitized to olive pollen are so rare in this area. One possible explanation might be that sensitization to other pollen that are more relevant in northern Italy represents a facilitating factor for sensitization to a weak allergen source such as *Oleaceae* in the Po basin.

Another interesting question is how all these patients got sensitized to olive pollen. In areas where olive trees are missing ash pollen has been considered as the main allergen source (5), but this is certainly not the case in the surroundings of Milan where no ash trees are present as well. One alternative explanation might be exposure to privet pollen. Privet has been considered as a weak allergen source ever since; however, the peak concentrations of its pollen may exceed 100 granules per cube meter in this area (10). It is therefore possible that privet pollen is responsible for the high prevalence of pollen sensitization in the area of Milan, although its allergenic power is too low to induce significant clinical allergies.

In conclusion, a majority of olive pollen-sensitized subjects seen in this area show a true allergy to this allergen source

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