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Analysis of the allergenic profile of patients hypersensitive to pollen pan-allergens living in two distinct areas of Northern Italy

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SUMMARY

Background: The allergenic profile of patients hypersensitive to pollen pan-allergens, profilin and polcalcin, has received little attention so far. **Objective:** To detect whether hypersensitivity to profilin and polcalcin follows sensitization to specific allergen sources or represents a primary phenomenon, and to examine the sensitization profiles of patients hypersensitive to pollen pan-allergens. **Methods:** IgE reactivity to markers of primary sensitization to different pollen species including grass, mugwort, ragweed, pellitory, birch, olive, and cypress was detected in sera from 106 pollen-allergic subjects, 86 sensitized to profilin and 29 to polcalcin living in two distinct areas of Northern Italy. **Results:** In profilin hypersensitive patients the primary sensitizer was detected in 24/86 (28%) cases: grass (n=15), ragweed (n=7), and birch (n=2). In 62 (72%) cases the primary sensitizing pollen was not detectable. In the polcalcin group the primary sensitizing pollen was detected in 8/29 (28%) cases: grass (n=6), ragweed and pellitory (1 each). All ragweed-allergic subjects were from the Milan area. In the 9 patients hypersensitive to both panallergens the primary sensitizing source could be identified in 2 (23%) cases (grass in both cases). **Conclusion:** A putative primary sensitizer to pollen pan-allergens can be detected only in 1/4 of cases, as most patients show IgE specific for > 1 pollen species. In these patients the prevalence of the primary sensitizer parallels the prevalence of clinical allergy to the different pollen sources in that specific geographic area. Most pollen sources are probably able to cause sensitization to cross-reacting pollen pan-allergens.

Introduction

Hypersensitivity to the largely cross-reacting pan-allergens, profilin, and polcalcins is quite common in patients with pollinosis. Polcalcins, a type of calcium-binding proteins, have recently emerged as cross-reacting pollen allergens, and may play a relevant clinical role in sensitized subjects (1-4). Profilin, a 12-15 kDa actin-binding protein present in all eukaryotic cells, is one of the main

causes of cross-reactivity between pollen and vegetable food (5-8), and its clinical allergenicity, albeit variable, is well recognized both in respiratory and food allergy (9, 10). Patients sensitized to either profilin and/or polcalcins almost invariably score positive for all allergenic sources on both skin tests and in-vitro tests performed using whole pollen extracts, and this poses a relevant diagnostic challenge for clinical allergologists willing to prescribe the proper allergen-specific immunotherapy.

The recent advances in molecular biology have eventually resulted in the detection, purification, cloning, and expression of an increasing number of recombinant and/or natural allergen proteins from different sources, many of which are now available for diagnostic purposes. This makes now possible to investigate whether hypersensitivity to pollen pan-allergens occurs as a consequence of sensitization to specific allergen sources (the so-called “primary sensitizer”) or if it represents a primary phenomenon. The present study examined the pollen sensitization profiles of patients hypersensitive to profilin and polcalcins living in two different zones of Northern Italy, an area where most seasonal allergen sources are present and can cause clinical allergy.

Methods

Patients

The study population was selected out from > 2700 adult subjects (age > 12 years) who spontaneously presented from April 1st, 2009 to May 31st, 2010 at the allergy outpatient clinics of Pordenone (in the extreme eastern part of Northern Italy) and Paderno Dugnano (very close to Milan). Patients with suspect airborne seasonal allergy (reporting a history of rhino-conjunctivitis with or without asthma for more than 1 month between February 15th and October 15th) were considered eligible for the study. SPT with a large panel of commercial pollen extracts including grass, ragweed, mugwort, birch, pellitory, cypress, olive, and plantain (Allergopharma, Reinbeck, Germany) were performed and read at 15 min following generally accepted criteria. As a part of their routine evaluation, subjects scoring positive on SPT with > 3 distinct pollen extracts underwent the detection of IgE to rBet v 2, the birch profilin, and to rPhl p 7, the grass polcalcin, by ImmunoCAP (Phadia, Uppsala, Sweden). Subjects seen in Paderno Dugnano underwent also SPT with both profilin- and polcalcin-enriched extracts of date palm pollen (kindly provided by Dr. Domingo Barber, ALK-Abellò, Madrid, Spain) as an in-vivo control of in-vitro tests results. Those scoring positive for 1 of the 2 pan-allergens were included in the study and further investigated in-vitro. Since all these in-vivo and in-vitro tests were performed within routine clinical activity no ethical committee approval was needed for the study; the IRBs approved the study.

Specific IgE measurements

Sera from all study patients underwent the detection of specific IgE to markers of primary sensitization to grass (Phl p 1 and Phl p 5), mugwort (Art v 1), ragweed (Amb a 1), pellitory (Par j 2), birch (Bet v 1), olive (Ole e 1), and cypress (Cup a 1). All measurements were carried out by ImmunoCAP (Phadia, Uppsala, Sweden) following manufacturer’s recommendations. Results were expressed in kU/L; values > 0.35 kU/L were considered positive. Since the aim of the study was to detect whether hypersensitivity to pollen pan-allergens is a primary or secondary phenomenon and, in the latter case, to detect the putative allergen sources responsible for sensitization, it was arbitrarily stated that a specific allergen source could be regarded as the possible cause of sensitization to polcalcin and/or profilin when IgE levels to specific allergens exceeded that to the pan-allergen. In patients showing reactivity to multiple marker allergens all exceeding that to the pan-allergen, the primary sensitizer was considered as “not detectable”.

Results

A total of 106 patients were studied; 86 were profilin reactors and 29 were sensitized to polcalcins; 9 patients were sensitized to both profilin and polcalcin. All patients had been living in the areas of Milan and Pordenone, respectively, for at least 5 years before the start of the study. SPT with profilin and polcalcin-enriched date palm pollen extracts performed in the patients group from Paderno Dugnano showed overlapping results with in-vitro assays.

In the profilin group, a putative primary sensitizing pollen source was detected in 24/86 (28%) cases: 15 of these were allergic to grass pollen, 7 were allergic to ragweed, and 2 to birch. Looking at differences between the 2 participating centers it turned out that all ragweed-allergic subjects were from Paderno Dugnano and all birch pollen-allergic patients from Pordenone, whereas grass pollen-allergic patients were similarly distributed between the two centers. In 62 (72%) cases it was not possible to detect the primary sensitizing pollen as these patients showed IgE reactivity to > 1 allergen source. These patients reacted to 2, 3, 4, and > 4 distinct allergen sources in 27 (43%), 26 (42%), 8 (13%), and 1 (2%) case, respectively. The different combinations included ragweed in most patients recruited in Paderno Dugnano (38/42

[90%]), whereas ragweed was totally absent from multiple sensitizations detected in Pordenone.

In the polcalcin group the primary sensitizing pollen source was detected in 8/29 (28%) cases; in 6/8 cases grass pollen was responsible for polcalcin sensitization, whereas the remaining 2 patients were allergic to ragweed and pellitory, respectively. Again the only ragweed-allergic subject was from Paderno Dugnano. Twenty patients were sensitized to > 1 allergen source, and reacted to 2, 3, and 4 markers of primary sensitization in 8, 9, and 3 cases, respectively; 1 polcalcin reactor did not show any IgE reactivity to primary sensitizers. Again, the different combinations included ragweed in 6/10 (60%) patients recruited in Paderno Dugnano, but in no patient from Pordenone. Grass pollen allergy was largely prevalent in patients from both areas.

In the 9 patients hypersensitive to both panallergens the primary sensitizing source could be identified in 2 (23%) cases (grass pollen in both cases); the remaining patient reacted to 2 (n= 2), 3 (n= 3) or 4 (n=2) primary sensitizing sources.

Discussion

This is one of the first studies investigating IgE reactivity to different pollen sources in patients hypersensitive to pollen pan-allergens aiming to detect the possible primary cause of sensitization. One of the most interesting findings in this work is that a primary sensitizing source could be identified only in about one fourth of cases. Such proportion might have been even smaller if other specific markers such as Phl p 2, Phl p 4 or Phl p 11 (for grass pollen), Pla a 1 (for *Platanus*), or Sal k 1 (for *Salsola*) had been measured as well. However, some of these allergens were not available in the ImmunoCAP series at the time when the study was performed, whereas the group 2, 4, and 11 grass pollen allergens were not tested in view of previous studies showing that Phl p 1 plus Phl p 5 are able to detect the large majority of grass pollen allergic individuals (11). Further, at least theoretically, it cannot be excluded that profilin sensitization is caused by plant-derived foods. Interestingly enough, one patient was apparently monosensitized to polcalcin; although some specific allergen sources were not investigated (see above) this might suggest that in some (rare) cases pan-allergens might themselves behave as primary sensitizers. The large majority of patients showed sensitization to more than one pollen, and sometimes reacted up to more than four different allergenic sources. This suggests that, at

least in this geographic area, most profilin and polcalcin reactors are characterized not only by the co-recognition of a cross-reacting allergen but also by genuine co-sensitization to different seasonal airborne allergens, a fact that may make clinical decisions about immunotherapy prescription particularly complex even in the presence of a component-resolved diagnosis. In these cases, clinical experience, patient's history (preferably along with recordings of symptom severity on a VAS) and data about local airborne pollen levels still remain essential tools to take correct decisions.

Another relevant aspect is that when a putative primary sensitizing pollen source could be identified its prevalence reflected that of allergy to the different pollen sources in that specific geographic area. This suggests that a specific pollen inducing more frequently hypersensitivity to profilin or polcalcin probably doesn't exist, and all pollen sources are probably able to do so although previous studies suggest that profilins from certain sources, such as pellitory or cypress don't show the same high degree of cross-reactivity as profilins from other sources (10,12). A survey in areas where olive, mugwort, pellitory or cypress pollen are particularly abundant and are a common cause of seasonal respiratory allergy might be able to confirm these observations.

In conclusion, most patients hypersensitive to pollen pan-allergens are truly multi-sensitized to distinct allergen sources. A tailored allergen specific immunotherapy would probably represent the correct outcome of the refined diagnostic workup that is now available, but this is unfortunately not the case.

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