

Sensitization to the Pollen Pan-Allergen Profilin. Is the Detection of Immunoglobulin E to Multiple Homologous Proteins From Different Sources Clinically Useful?

D Villalta,¹ R Asero²

¹Allergologia e Immunologia Clinica, Dipartimento di Medicina di Laboratorio, A.O. "S. Maria degli Angeli", Pordenone, Italy

²Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano (MI), Italy

■ Abstract

Background: Profilin is a highly conserved protein regarded as a pan-allergen in pollen and vegetable food. Homologous proteins from different sources are highly cross-reactive.

Objective: To assess whether detecting immunoglobulin (Ig) E to multiple profilins from different sources is clinically more useful than detecting IgE to a single representative profilin.

Methods: Sera from 43 subjects sensitized to profilin selected in 2 allergy centers in Northern Italy showing a different pollen exposure profile were studied for their IgE reactivity to 5 profilins (Bet v 2, Ole e 2, Hev b 8, Mer a 1, and Phl p 12) using a commercial allergen microarray immunoassay.

Results: All 43 patients (100%) scored positive to at least 1 profilin on ISAC, although reactivity to all 5 profilins was observed in only 37 cases (86%). In approximately half of the reactors, IgE reactivity to Ole e 2 was much weaker than that to other profilins irrespective of the primary sensitizing allergen source, suggesting a low sensitivity of this allergen. Much discrepancy in IgE to Bet v 2 measured by ISAC microarray and ImmunoCAP was observed.

Conclusion: Detecting IgE reactivity to a single marker protein (eg, Bet v 2) is sufficient to diagnose or exclude sensitization to profilin. Detecting IgE to multiple homologous, cross-reacting allergen proteins is not clinically more informative and increases the risk of confusion and misinterpretation.

Key words: Profilin. Pan-allergens. Cross-reactivity. IgE. Microarray.

■ Resumen

Antecedentes: La profilina es una proteína altamente conservada considerada un panalérgeno en el polen y los alimentos vegetales. Las proteínas homólogas de origen diverso presentan una gran reactividad cruzada.

Objetivo: Evaluar si la detección de inmunoglobulina (Ig) E frente a múltiples profilinas de origen diverso es más útil clínicamente que la detección de IgE frente a una única profilina representativa.

Métodos: Se analizó el suero de 43 pacientes sensibilizados a la profilina, seleccionados en dos centros de alergología del norte de Italia, que presentaban un perfil diferente de exposición al polen, con el objeto de determinar la reactividad IgE frente a 5 profilinas (Bet v 2, Ole e 2, Hev b 8, Mer a 1 y Phl p 12) empleando un inmunoensayo de micromatrices de alérgenos comercial (ISAC).

Resultados: Los 43 pacientes (100%) dieron positivo como mínimo a 1 profilina en el inmunoensayo ISAC, pese a que solo se observó reactividad frente a las 5 profilinas en 37 casos (86%). En aproximadamente la mitad de los pacientes que dieron positivo, la reactividad IgE frente a Ole e 2 fue mucho más débil que frente al resto de profilinas, independientemente del origen del principal alérgeno sensibilizante, lo que indica una baja sensibilidad de dicho alérgeno. Se observaron mayores discrepancias en la detección de IgE frente a Bet v 2 mediante micromatriz ISAC e ImmunoCAP.

Conclusión: Detectar la reactividad IgE frente a una única proteína marcador (p. ej., Bet v 2) es suficiente para diagnosticar o descartar la sensibilización a la profilina. La detección de IgE frente a múltiples proteínas homólogas de alérgenos con reactividad cruzada no resulta más informativa clínicamente y aumenta el riesgo de confusión o interpretación errónea.

Palabras clave: profilina; panalérgenos; reactividad cruzada; IgE; micromatriz.

Introduction

Cross-reactivity to distinct allergen sources due to sensitization to phylogenetically highly conserved proteins of vegetable origin is one of the most intriguing fields in allergology. One of these allergen proteins, profilin, is so widely distributed in the vegetal kingdom that it is regarded as a pan-allergen. 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 [1-4], and its clinical allergenicity, albeit variable, is well recognized both in respiratory and food allergy [5,6]. The recent enormous advances in molecular biology have led to the detection, purification, cloning, and expression of an increasing number of recombinant and/or natural allergen proteins from different sources, and many of them are now available for diagnostic purposes. The ISAC multiplex allergen microarray (Phadia, Uppsala, Sweden), a recently marketed method for the detection of specific immunoglobulin (Ig) E, is presently able to detect IgE reactivity to more than 100 allergen proteins in a single assay. The currently available panel includes 5 profilins from different sources (birch pollen [Bet v 2], olive pollen [Ole e 2], natural rubber latex [Hev b 8], *Mercurialis annua* pollen [Mer a 1], and grass pollen [Phl p 12]). Being able to detect IgE to specific allergen components is certainly clinically essential when deciding which specific immunotherapy should be prescribed to multisensitized patients, and it is also the only means able to provide a global picture of sensitization in a single patient. However, the clinical usefulness of measuring IgE specific for a number of homologous, cross-reacting proteins is more uncertain. The aim of the present study was to assess whether the detection of IgE to multiple homologous, cross-reacting allergen proteins is clinically more useful than the detection of IgE to a single marker pan-allergen.

Methods

Patients

The study population was selected from a large number of individuals who spontaneously presented at 2 allergy outpatient clinics located in Pordenone and Paderno Dugnano from April 1 to December 31, 2009. Both of these places are located in the Po Valley in Northern Italy, where a large spectrum of pollens including grass, mugwort, ragweed, birch, cypress, olive, *Parietaria*, and plantain are present. However, while a high concentration of grass pollen is present throughout Northern Italy, marked differences in other seasonal allergens exist between the 2 study areas. In fact, birch pollen is a major allergen in Pordenone while mugwort is an intermediate allergen, and ragweed and *Parietaria* are virtually absent. In contrast, ragweed is a major allergen in Paderno Dugnano [7], while birch is an intermediate seasonal allergen and pellitory and mugwort are infrequent causes of respiratory allergy. Cypress and olive pollen are minor causes of respiratory allergy in both areas.

Patients with suspect airborne seasonal allergy (history of rhinoconjunctivitis with or without asthma for more than 1 month between February 15 and October 15) were considered

eligible for the study. They underwent skin prick testing (SPT) with a large panel of commercial pollen extracts including grass, ragweed, mugwort, birch, pellitory, cypress, olive, and plantain (Allergopharma, Reinbeck, Germany). Readings were taken at 15 minutes following generally accepted criteria. As a part of routine evaluation, those scoring positive on SPT with more than 3 distinct pollen extracts underwent detection of IgE to Bet v 2, the birch profilin, by ImmunoCAP (Phadia, Uppsala, Sweden). In Paderno Dugnano, patients also underwent SPT with a commercial profilin-enriched date palm pollen extract (ALK-Abelló, Madrid, Spain; Phod 2 50 µg/mL) [5,6], which scored strongly positive in all cases.

Forty-three profilin reactors were finally investigated. Since all the in vivo and in vitro tests were performed within routine clinical activity no ethical committee approval was needed for the study.

Specific IgE Measurements by Multiplex Allergen Microarray

Sera from all patients were screened for the detection of specific IgE using a commercial allergen microarray immunoassay (ISAC; Phadia, Uppsala, Sweden) following the manufacturer's recommendations. Five profilins were assayed: Bet v 2, Ole e 2, Hev b 8, Mer a 1, Phl p 12 from birch, olive, natural rubber latex, goosefoot, and grass pollen, respectively. Reactions sites were incubated with 20 µL of patients' serum for 2 hours. After rinsing, washing, and drying, allergen-specific IgE complexes were stained with fluorescence-labeled anti-human IgE for 1 hour. After further washings, a laser scanner was used to take fluorescence readings and results were transformed into numerical data by comparison with a reference serum standardized against ImmunoCAP IgE. As a consequence the results, expressed as ISAC standardized units (ISU/L), are indirectly linked to the World Health Organization International Reference Preparation of Human Serum IgE 75/502. Levels higher than 0.3 ISU/L were regarded as positive, following the manufacturer's recommendations.

In order to determine whether differences in IgE reactivity to the 5 assayed profilins were associated with allergy to a specific primary sensitizing pollen, the data were also analyzed after dividing patients on the basis of their IgE reactivity to the major pollen-specific allergens Phl p 1, Bet v 1, Amb a 1, Art v 1, Par j 2, Cup a 1, and Ole e 1 used as markers of primary hypersensitivity to grass, birch, ragweed, mugwort, pellitory, cypress, and olive pollen, respectively. In this analysis, single major specific allergens were not considered as a likely primary cause of profilin cosensitization when profilin IgE levels exceeded those of IgE to the putative primary sensitizer. Patients showing strong IgE reactivity to 3 or more major specific allergens (exceeding that of profilin) were considered to be multisensitized; the primary sensitizer to profilin could not be established in these patients.

Results

Results are shown in Table 1. All 43 patients (100%) scored positive to at least 1 profilin on ISAC. However, reactivity to

all 5 profilins investigated was found in only 37 cases (86%). The sera from the remaining 6 patients did not show reactivity to 1 profilin (n= 2, Phl p 12 in both cases), 2 profilins (n= 3; Ole e 2 and Hev b 8 in 1 case, Ole e 2 and Phl p 12 in 2 cases),

or 4 profilins (n=1, this serum showed IgE reactivity to Phl p 12 only). Altogether, on ISAC microarray, sera from profilin-hypersensitive patients did not react to Phl p 12, Ole e 2, Hev b 8, Mer a 1, or Bet v 2 in 4 (9%), 4 (9%), 2 (5%), 1 (2%),

Table 1. Specific Immunoglobulin (Ig) E Levels^a in Patients Sensitized to Profilin

Patient	Bet v 2 (CAP)	ISAC				
		Bet v 2	Ole e 2	Hev b 2	Mer a 1	Phl p 12
1	5.7	8	11.4	8.9	8.9	2
2	0.45	0.3	0	0	0.9	0.7
3	1.03	0.8	1	0.8	1.2	1
4	0.76	0.9	1.3	1	1	0
5	1.63	2.4	2.2	2	1.9	2
6	0.4	1.4	0.7	1.1	1.2	0.2
7	3.97	3.8	3.4	4.5	4.8	2.5
8	13.5	6.9	1.2	9.6	10.3	9.1
9	1.98	2.4	0.7	2.5	3.6	2.2
10	3.48	4.8	0.8	4.2	5.1	3
11	4.9	11.6	4.3	11.6	9.7	4
12	5.8	4.8	4.5	5.4	5.2	4.7
13	3.3	4.8	5.6	6.7	6	0.8
14	43.9	25	26.8	43	41.2	29.4
15	6.18	6.2	2.2	8	11.6	5.8
16	15.7	6.3	1.7	5.4	11.8	4.6
17	22.7	10.8	2.2	13.4	17.7	10.9
18	4.9	3.7	1.1	2.9	6.2	3.9
19	17.5	27.6	17.5	33.6	36.5	34.3
20	18.1	51.3	20.8	44	30.6	30.1
21	1.19	4.7	2.3	3.9	6.5	3.9
22	27.3	38.1	28.4	36.1	42.6	31.5
23	1.67	2.7	0.9	2.5	4	2.5
24	1.47	2.5	0.5	1.9	2	1.2
25	10.9	30.6	23.7	20.8	20	15.5
26	33.6	39.3	6.3	23	39.1	29.4
27	1.77	5.3	1.1	3.3	4.8	1.8
28	41.7	5	9.6	27.7	49.2	44.8
29	8.6	9.2	1.7	6.8	10.9	7.2
30	1.72	3.6	0.5	2.6	3.4	2.4
31	10.4	14.4	2.2	5.1	8.5	1.9
32	5.31	9.9	5.3	9.5	8.9	8.1
33	2.56	2.8	2.1	2.1	3.1	3.6
34	21.7	42.1	22.1	32.8	29.7	17.4
35	28.2	52.5	47.7	48.2	54.7	22.8
36	1.2	0	0	0	0	1.4
37	1.6	1	0	0.7	1.7	0
38	4.5	6	2.8	6.9	5.7	15.8
39	4.5	3.4	1.9	3.9	3.6	3
40	6.4	9.8	1.8	8.2	7.1	5.3
41	1.4	0.8	0	0.4	0.6	0
42	8.2	7	3.7	9.6	9.8	3.2
43	12.5	18.8	4.4	12.6	22.6	11.1

^aIgE levels are expressed as kU/L (CAP), or ISU/L (ISAC).

Table 2. No. of Primary Sensitizing Pollens in the 2 Participating Centers

	Paderno Dugnano	Pordenone
Grass	3	4
Birch	0	4
Olive	0	0
Cypress	0	0
Ragweed	6	1
Grass and ragweed	3	0
Birch and grass	1	4
Birch and ragweed	4	0
Birch and cypress	0	2
Multiple pollens	10	1

and 1 (2%) patients, respectively. Further, in about half of the reactors, IgE reactivity to Ole e 2 was markedly less intense than that to other profilins, suggesting a lower sensitivity of this allergen. Furthermore, although performed using the same serum samples, in many cases CAP and ISAC showed marked differences in Bet v 2 reactivity. Particularly, reactivity was significantly stronger on CAP in patients 2, 8, 14, 16, and 17. In contrast, reactivity was stronger on ISAC in patients 11, 19, 20, 22, 25, 27, 32, 34, and 35 (Table 1).

On analyzing data by recruitment center (Table 2), differences in the primary sensitizing pollen were evident, with ragweed and birch pollen markedly prevalent in Paderno Dugnano and Pordenone, respectively. On grouping patients on the basis of primary sensitizing pollens (Table 3), it was seen that a weak or negative response to Ole e 2 was equally distributed between the different subgroups whereas negative results with Phl p 12 were all associated with birch pollen allergy, although in 3 out of 4 cases, the patients were primarily sensitized to grass pollen as well.

Discussion

This study aimed to investigate whether in patients sensitized to the pollen pan-allergen profilin, detecting IgE reactivity to multiple, homologous proteins from different sources using a commercial multiplex allergen microarray offered some clinical advantage over testing a single marker of sensitization to this specific allergen. The results of our investigations suggest that it does not. Based on our data, it seems that detecting IgE only for Bet v 2 is sufficient to diagnose or exclude profilin sensitization. Since current diagnostic recombinant allergens consist of only a single isoform per species, the theoretical risk of false negative results caused by slight epitopic differences might be overcome by using a mixture of several isoforms of the same allergen or, alternatively, a mixture of homologous recombinant allergens from different species. This conclusion reflects what happens with many other highly cross-reacting allergens, such as grass pollen, *Fagales* pollen, or *Oleaceae* pollen, where the detection of IgE specific for few marker allergens (eg, Phl p 1 and Phl

Table 3. Analysis of ISAC Results^a Based on Primary Sensitizing pollen

Primary Sensitizing Pollen	No.	Weak/Negative Ole e 2	Negative Phl p 12
Grass	7	6	0
Ragweed	7	3	0
Birch	4	1	1
Grass + Birch	5	3	3
Grass + Ragweed	3	3	0
Birch + Ragweed	4	3	0
Birch + Cypress	2	1	0
Multisensitized	11	6	0

^aResults are expressed as no. of patients.

p 5 for grass, Bet v 1 for *Fagales*, Ole e 1 for *Oleaceae*, etc) is generally considered sufficient to diagnose sensitization to a whole species of allergens [8-10]. Interestingly, of the 5 profilins currently present in the ISAC microarray, the olive pollen profilin, Ole e 2, showed reduced sensitivity when challenged with sera from our patients. This defect was independent of the primary sensitizing agent (birch, grass, or ragweed alone or in different combinations). Since primary sensitization to olive pollen is rare in our area, we do not know whether this would also occur in true olive-allergic individuals such as those frequently seen in Southern Europe [11]. However, the SPT with profilin-enriched date palm pollen extract (an allergen source that is obviously absent in Northern Italy) scored strongly positive in all tested patients, suggesting that sensitivity of single profilins might not be only a matter of the primary sensitizing allergen.

The allergen microarray immunoassay is a potentially useful and powerful diagnostic instrument although up to now its introduction as a routine method has been hampered by its high cost and rigidity (one is forced to test over 100 allergens even if only a few of them are of interest in a certain patient). In keeping with data from previous structure-based analyses [12], this study shows that in the case of the pan-allergen profilin, detecting IgE to multiple homologous, cross-reacting allergen proteins is clinically not more informative than detecting IgE to a single representative marker that might serve equally well while reducing the risk of confusion and misinterpretation.

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■ **Riccardo Asero**

Ambulatorio di Allergologia
Clinica San Carlo
Via Ospedale 21
20037 Paderno Dugnano
Milano, Italy
E-mail: r.asero@libero.it