

Evaluation of 70 polysensitised allergic patients with skin prick test and an allergen microarray

Valutazione di 70 pazienti allergici polisensibilizzati con skin prick test e microarray allergenico

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Key words

Allergen • IgE • Allergen microarray

Parole chiave

Allergene • IgE • Microarray allergenico

Summary

Riassunto

Background

Diagnosis of type I allergy conducted with allergen extracts does not allow confirmation of cross-reactivity towards structurally-related components contained in other allergen sources. Recently a multi-allergen assay was developed which contains natural and recombinant allergens and takes advantage of micro-array technology.

Objective

The aim of the study was to obtain information through diagnostic testing with marker allergens in polysensitised patients.

Methods

We evaluated 70 allergic patients who resulted polysensitised with skin prick test. Sera of all patients were analysed for the presence of specific IgE to allergen components with an allergen-microarray assay.

Results

Of 70 patients, 52 (74.3%) were sensitised to major grass pollen allergens rPhl p 1; 51 (72.9%) to rLol p 1; 47 (67.1%) to rPhl p 5; and 36 (51.4%) to rPhl p 6. Sensitivity to birch pollen major allergen rBet v 1 was expressed in 36 patients (51.4%) and 35 patients (50%) were co-sensitised to olive tree pollen major allergen nOle e 1. Of the 36 patients with specific IgE to rBet v 1, 22 of them (61.1%) also had specific IgE to apple major allergen rMal d 1.8. Among 9 different profilins tested 29 out of 70 individuals (41.4%) resulted sensitised to rMer a 1. Among perennial allergens 26 (37.1%) subjects had specific IgE against mite major allergen nDer f 2 and 26 subjects (37.1%) had positive results for the cat dander major allergen rFel d 1. In the serum of 25 subjects (35.7%) specific IgE antibodies to *Alternaria alternata* major allergen, Alt a 1, were found. Five out of 70 (7.1%) allergic patients were sensitised to peach lipid transfer protein nPru p 3 in evaluating food allergens.

Conclusions

This data gives us reason to believe that testing patients for a broader number of allergen molecules enables today's allergist

Premessa

La diagnosi delle allergie di tipo I utilizzando estratti allergenici non permette di valutare possibili cross-reattività verso allergeni strutturalmente correlati presenti in altre fonti allergeniche. Recentemente, è stato messo a punto un test multi-allergenico contenente allergeni naturali e ricombinanti basato sulla tecnologia dei micro-array.

Obiettivo

Scopo del presente studio è quello di ottenere informazioni con l'utilizzo di marcatori allergenici di sensibilizzazione genuina in soggetti apparentemente polisensibilizzati.

Metodi

Abbiamo valutato 70 pazienti allergici che risultavano polisensibilizzati ai tests cutanei. I sieri di tutti i pazienti sono stati valutati per la presenza di IgE specifiche verso componenti allergenici utilizzando un allergen-microarray test.

Risultati

Di 70 pazienti, 52 (74,3%) erano sensibilizzati all'allergene maggiore delle graminacee Phl p 1; 51 (72,9%) al Lol p 1; 47 (67,1%) al Phl p 5; e 36 (51,4%) al Phl p 6. La percentuale di sensibilizzazione verso l'allergene maggiore della betulla è stata riscontrata in 36 pazienti (51,4%); 35 pazienti (50%) erano co-sensibilizzati all'allergene maggiore dell'olivo Ole e 1. Dei 36 pazienti con IgE specifiche per Bet v 1, 22 di questi (61,1%) avevano IgE specifiche per l'omologo allergene maggiore della mela rMal d 1. Tra i 9 allergeni di natura profilinica testata 29 pazienti su 70 (41,1%) sono risultati positivi per rMer a 1. Tra gli allergeni perenni 26 soggetti (37,1%) avevano IgE specifiche per l'allergene maggiore degli acari nDer f 2, e 26 soggetti (37,1%) presentavano positività per l'allergene maggiore del gatto Fel d 1. Nel siero di 25 soggetti (35,7%) sono state riscontrate IgE specifiche per l'allergene maggiore dell'*Alternaria alternata* Alt a 1. Nell'ambito degli allergeni alimentari, 5 soggetti su 70 (7,1%) sono risultati positivi alla proteina di trasferimento lipidico della pesca Pru p 3.

to better define the allergy IgE profile of a given patient in order to obtain broader information about patient sensitisation to cross reactive allergens or to identify a state of co-sensitization. A diagnostic approach which reflects a more specifically detailed IgE profile would more than likely pave the way toward improved immunotherapeutic intervention. The next step might be to enrich the panel with other allergens of epidemiologic significance.

Introduction

It is well known that diagnosis of type I allergy with allergen extract representative of a given allergen source does not provide a rational basis for prediction of cross-reactivity towards other allergen sources containing structurally-related components. An important consequence of the lack of component-resolved diagnosis is that it may lead to an unsuitable choice of allergen-specific immunotherapy. To paraphrase the German philosopher Georg W.F. Hegel, allergists often see "all cows as black simply because it is dark". *In vitro* diagnosis of type I allergy is based on the detection of patients' serum IgE antibodies directed to allergens. Presently a large number of allergen molecules as well as panels of recombinant and natural allergens (which assemble the epitope-complexity of natural allergen sources) are commercially produced and on the market. The evaluation of specific IgE towards these components allows to discriminate allergic patients on the basis of their sensitisation to those allergens eliciting symptoms which represent a valuable marker of genuine sensitisation to a given allergen source. Moreover, the assessment of an existing status of sensitisation to cross-reactive allergen contribute to exclude a given allergen source as cause of allergy¹. Recently a multi-allergen assay has been developed. This assay takes advantage of micro-array technology and contains 76 natural and recombinant allergens (for information about allergens consult the website www.allergome.org) that are printed onto a glass slide and can be tested with minute amounts of serum or other biological fluids². Recently, Bousquet et al. reported that a minority of symptomatic allergic patients are sensitised to a single allergen (monosensitised) whereas over 75% present IgE against several allergens (polysensitised)³. In this context the use of allergen molecules is mandatory for diagnostic purposes in order to target an allergen vaccine, the unique curative approach to respiratory allergic diseases. Therefore, a combination of two diagnostic tests both utilizing different reagents (allergen extract and allergen molecules) may represent an advantage in order to identify the allergen causative of symptoms. Hence, the aim of the present study is to compare standard allergen-extract based skin prick with an *in*

Conclusioni

Questi dati ci indicano che testare pazienti per un numero allargato di allergeni mette in grado l'allergologo di definire meglio il profilo delle IgE di un dato paziente allo scopo di ottenere informazioni più dettagliate sulla sensibilizzazione del paziente verso allergeni cross-reattivi o per accertare uno stato di co-sensibilizzazione. Un approccio diagnostico che rifletta più specificamente un dettagliato profilo delle IgE dovrebbe aprire la strada verso approcci immunoterapeutici più mirati. Il passo successivo sarà quello di integrare il pannello di allergeni con altri allergeni di significato epidemiologico.

in vitro allergen microarray assay equipped with 76 purified allergens⁴ in 70 oligo- and poly-sensitized allergic patients. It is not included in the aim of the present study to perform a sensitivity analysis of ISAC[®] given that the other term of comparison is represented by skin test which, at least in 30% of cases of polysensitization, provides unreliable informations.

Materials and methods

This retrospective study involved 70 patients, mean age 23.3 years, range 4-64 years, with a history of atopic dermatitis (n = 10); rhino-conjunctivitis (n = 68) and/or mild (n = 13) to moderate (n = 11) severe (n = 2) asthma; oral allergy syndrome (OAS) (n = 51); acute urticaria (n = 4); abdominal complaints (n = 3). All patients had positive skin test to at least a pollen extract. Patients' skin tests resulted positive to birch-, timothy-grass-, *Parietaria* ssp., mugwort-, olive-pollen, house-dust mites and other common allergen sources (cat and dog dander, *Alternaria alternata*, latex). Food allergen extracts were employed when patient's personal history suggested food allergy.

IN VIVO TESTS

The oligo- and polysensitisation status was established via skin test and ranged from 3 to 14 positive results. Skin prick tests were performed with commercial extracts from *Phleum pratense* pollen, containing 60 µg/ml of Phl p 5, *P. judaica* pollen (6 µg/ml of Par j 1), birch (45 µg/ml of Bet v 1), olive tree (60 µg/ml of Ole e 1), mugwort (135 µg/ml of Art v 1), cat dander (60 µg/ml of Fel d 1) and mites (40 µg/ml of Der p 1 and Der f 1; 20 µg/ml of Der p 2 and Der f 2) (Alk-Abellò, Lainate, Milan, Italy). Some food allergens extract were utilized in those patients in which food allergy was suspected. No recombinant allergens were utilized for *in vivo* diagnosis.

IN VITRO TESTS

Sera of all patients were analysed for the presence of specific IgE to allergen components by the allergen-microarray (Immune-Solid Phase Allergen Chip ISAC[®] version CRD-76, VBC-GENOMICS, Vienna, Austria)

according to manufacturer's guidelines. Different groups of seventy-six allergens are spotted on the allergen chip: tree pollen allergens (i.e. rAln g 1, rBet v 1a, rBet v 1d, rBet v 2, rCor a 1, nCup a 1, nOle e 1, nOle e 2, rPho d 2, nPla a 1, nPla a 2); grass pollen (i.e., rPhl p 1, 2, 5, 6, 7, 12, rCyn d 12, nLol p 1); weeds (i.e. rPar j 1, rPar j 2, rPar j 3, rAmb a 1, rMer a 1, rHel a 2, nArt v 1); arthropods (i.e. nDer p 1, nDer p 2, rDer p 10, nDer f 1, nDer f 2, rBla g 2, rBla g 5, rPer a 7); other animals (i.e. nCan f 1, nCan f 3, nFel d 1, rHel a s 1, rAni s 3); moulds (i.e. rAlt a 1, rAlt a 2, nAsp f 1); latex (i.e. rHev b 1, 3, 5, 6, 7, 8, 9, 10, 11); and foods: i.e. Ana c 2, Bos d 4 (a-Lactalbumin), Bos d 5 (β-Lactoglobulin A), Bos d 5 (β-Lactoglobulin B), Bos d 7 (IgG bovine), Bos d 8 (Casein), Alpha-Casein, Beta-Casein, Kappa-Casein, Bos d Lactoferrin, Bos d 6 (Bovine serum albumin), Gal d 1 (Ovomucoid), Gal d 2 (Ovalbumin), Gal d 3 (Conalbumin), Gal d 4 (Lysozym), rDau c 1, rApi g 1, nPru p 3, rMal d 1.8, Alpha-Amylase, Beta-Amylase, nPen i 1, nPen m 1, Tri a 18 (Lectin/Agglutinin), Tri a 19 (Gliadin).

The allergen-microarray contained either highly purified natural allergen components or recombinant allergen components and the assays were performed as described by Deinhofer et al.⁴. Briefly, the microarray slides were washed in TBS-T buffer (150 mM sodium chloride, 10 mM Tris base, and 0.5% Tween 20, pH 8.0) for 120 min to remove non-covalently bound allergens from the microarray surface; 20 µl of undiluted patient serum was applied directly to the individual reaction sites of the microarray, generated by a pre-affixed mask of Teflon®. Following incubation in a humid chamber for 180 min, excess serum was removed by a washing step in TBS-T buffer and 20 µl of fluorescence-labelled anti-human IgE detection antibody was applied. After a 60 minute staining reaction and a second washing step to remove unbound detection antibody, the slides were scanned in an Affymetrix 428 microarray scanner (Affymetrix, Santa Clara, CA, USA) with double-laser power settings to achieve a maximum dynamic range across the IgE reactivities of the serum samples. Both images of each spot were analysed using GenePix image analysis software (Version 3.0.6.89; Axon Instruments, Union City, CA, USA).

The specific IgE values were then spread to the full range of the RAST classes¹⁻⁶ in order to enable a reliable fluorescence intensity (FI) value normalization. Linear extrapolation of FI log values obtained from the calibration serum against the log of the determined IgE titres of the individual allergens was performed. The corresponding regression parameters (slope, axis intercept), were calculated by using MS Excels or Sigma Plots linear regression functions. The coefficient of correlation (R^2) obtained by this procedure was at least 0.9. Using the regression parameters calculated above, the IgE titres (normalized values) for all of the allergen spots contained in the ISAC® microarray can be determined by using the following formula:

FI = calculated allergen fluorescence intensity

a = slope

b = axis intercept of the regression curve

kUA/l = kilo Units Allergen/litre

inv log = inverse logarithm

IgE titre (kUA/l) = $\text{inv log} [\log \text{FI} \times a + b]$

The individual values can be arranged into the following ISAC® classes:

Negative values (≤ 0 kUA/l) are allocated to Negative (0).

Extrapolated values lying within RAST 1-2 ($0.35 \leq 3.5$ kUA/l) are allocated to Low (1).

IgE values between 3.5 and ≤ 50.0 kUA/l are classified Intermediate (2).

Higher values (> 50.0 kUA/l) are categorised High (3).

Results

The Table I represents, even in its complexity, a reliable graphic representation of allergen distribution, in which upwards IgE profiles to aeroallergens are represented as well as specific IgE antibodies to food allergens at the bottom. The whole of the patients had positive skin test results to at least one pollen extract (Fig. 1). Of these 61/70 (87.1%) individuals had a skin test positive to *P. pratense*, 52 (74.3%) and positive ISAC results to major grass pollen allergens rPhl p 1, 51 (72.9%) to rLol p 1, 47 (67.1%) to rPhl p 5, and 36 (51.4%) to rPhl p 6 (Fig. 1). Six patients of 70 (8.6%) with pollenosis had positive ISAC® results to the unique calcium binding (rPhl p 7) present in the ISAC®-panel and all suffered from asthma. Of 43 (61.4%) patients with positive skin test to birch extract 36 patients (51.4%) resulted sensitised to major birch pollen allergen rBet v 1. Of 47 (67.1%) subjects with positive test to olive tree pollen extract 35 (50%) had positive ISAC results towards major allergen nOle e 1. Other data relative to tree pollen allergen sensitisation are reported in Figure 2. Among patients allergic to weeds, we documented differences between skin test and ISAC® results referred to ragweed, mugwort and pellitory (Fig. 3). Of 9 different profilins tested 29 (41.4%) individuals resulted sensitised to rMer a 1. Among 29 patients sensitised to at least to one profilin 22 (75.9%) suffering from OAS eating vegetable and fruits. Percentage of sensitization to remaining profilins are reported in Figure 4. Among perennial allergens, 26 (37.1%) subjects had specific IgE against mite major allergen nDer f 2 and 26 (37.1%) had positive results for the cat dander major allergen rFel d 1. In the serum of twenty-five subjects (35.7%) specific IgE antibodies to *Alternaria alternata*, Alt a 1, major allergen were found. The comparison of the percentage of sensitization to perennial allergens

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524
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Fig. 1. Percentage of sensitisation to allergen markers of grass pollen in 70 polysensitised allergic patients. Phl p E, *Phleum pratense* extract.

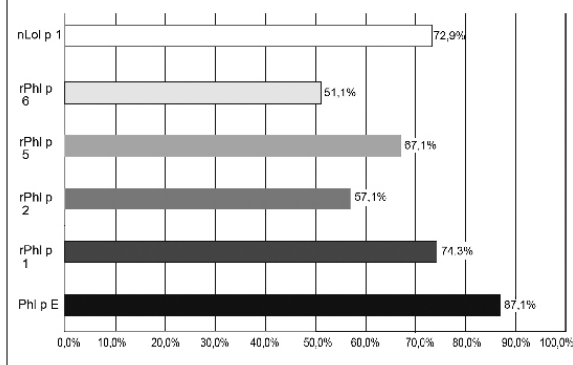


Fig. 2. Percentage of sensitisation to allergen markers of tree pollen in 70 polysensitised allergic patients. Cup a E, *Cupressus arizonica* extract; Pla a E, *Platanus acerifolia*; Bet v E, *Betula verrucosa* extract; Aln g, *Alnus glutinosa* extract; Cor a, *Corylus avellana* extract.

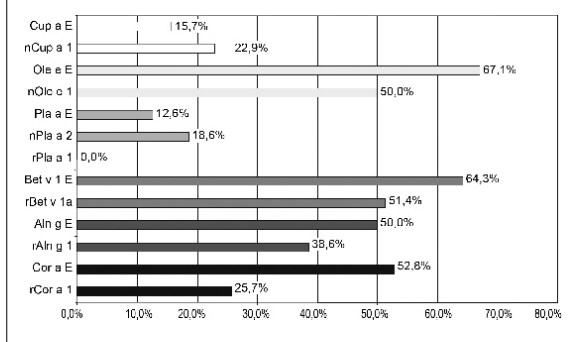
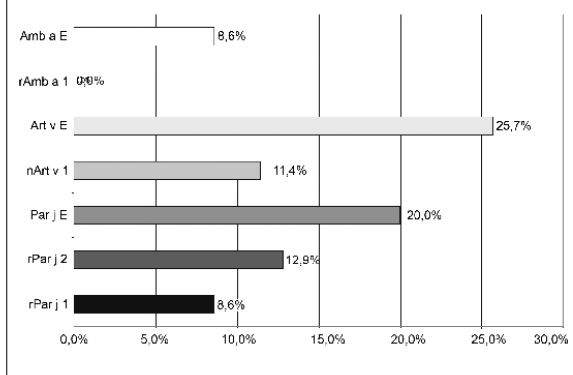


Fig. 3. Percentage of sensitisation to allergen markers of weed pollen in 70 polysensitised allergic patients. Amb a E, *Ambrosia artemisiifolia* extract; Art v E, *Artemisia vulgaris* extract; Par j E, *Parietaria judaica* extract.



between skin prick test and ISAC results are reported in Figure 5. Of 36 patients with specific IgE to rBet v 1, 22 (61.1%) also had specific IgE to apple major allergen rMal d 1.8 of which 24 (66.6%) subjects

Fig. 4. Prevalence of sensitisation to pollen profilins from different sources (*Phleum pratense*, *Cynodon dactylon*, *Parietaria judaica*, *Betula verrucosa*, *Phoenix dactylifera*, *Helianthus annuus*, *Olea europea*, *Hevea brasiliensis*, *Mercurialis annua*) in 70 polysensitised allergic patients.

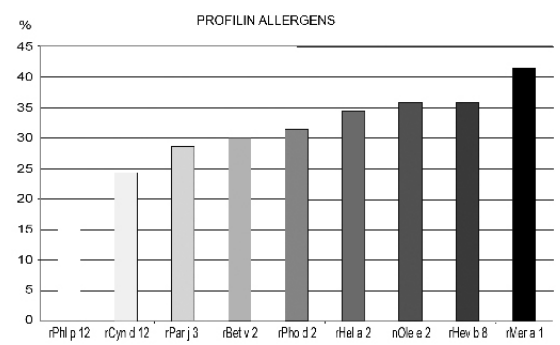
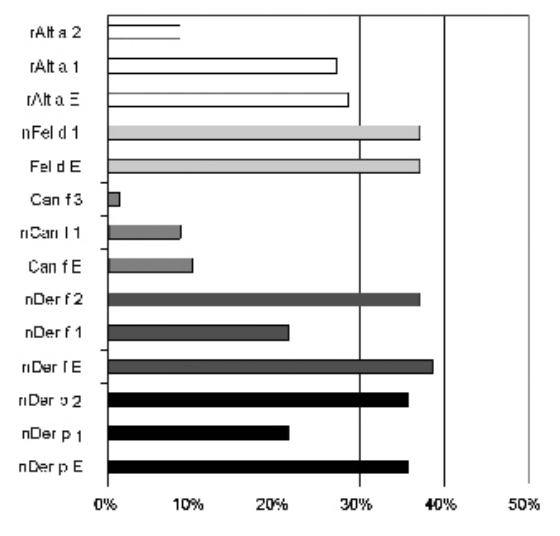
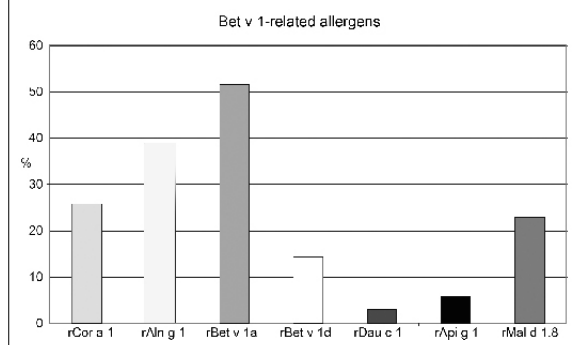


Fig. 5. Prevalence of sensitisation to perennial allergen extracts evaluated by skin test (Alt a E, *Alternaria alternata* extract; Fel d E, *Felix domesticus* extract; Can f E, *Canis familiaris* extract; Der p E, *Dermatophagoides pteronissinus* extract; Der f E, *Dermatophagoides farinae* extract) and their relative allergens evaluated by ISAC. Note that the percentage of sensitisation between crude allergen extracts and their major allergen is roughly equivalent.



had OAS after eating apple. Among 2 Dau c positive subjects one had OAS after ingestion of carrot. Three patients of 4 with positive results to Api g 1 suffered from OAS after eating celery (Fig. 6). Specific IgE antibodies to Ana c 2, used to detect specific IgE directed towards carbohydrate side chains of glycoproteins, were found in 10 out of 70 subjects (14.3%). Evaluating other food allergens, five out of 70 (7.1%) allergic patients were sensitised to peach lipid transfer protein nPru p 3. Three of 5 patients with

Fig. 6. Prevalence of sensitization to Bet v 1-related allergens from different pollen (*Corylus avellana*, *Alnus glutinosa*, *Betula verrucosa*) and plant food sources (*Daucus carota*, *Apium graveolens*, *Malus domestica*).



positive results for the lipid transfer protein also had positive results with the prick test performed with a commercial peach extract and experienced abdominal complaints eating fruit belonging to Rosaceae (2 subjects also suffered from urticaria eating peaches). The percentage of sensitization to another relevant food allergens, such as tropomyosins from crustaceans and mollusks, involved three (4.3%) allergic patients. All these patients had documented systemic reactions after ingestion of crustaceans. Finally, 8 patients (11.4%) were sensitised to at least one cow-milk allergen without apparent symptoms after ingesting milks and derivative but two.

Discussion

The main findings of this study are the identification, by allergen markers contained in the ISAC®, of a large number of genuine sensitisations toward allergens from different allergen sources (grass, weeds, trees, mites). In addition, we have ascertained that an apparent polysensitisation status of subjects in the study was caused by sensitisation to pan-allergens (mainly profilins and calcium binding proteins).

In vitro allergy diagnosis requires new sample-processing systems that are quick, reliable, and efficient and that avoid the use of expensive equipment. The clinical, epidemiologic, and economic concerns imply that these systems must maintain acceptable assay sensitivity and brief wait time for results. As demonstrated in previous studies^{2,4,8} large scale use of the allergen microarray could result in decreased costs coming down. The major advantage of the chip-based assay is the simultaneous detection of allergen-specific IgE to a large number of different allergens. The existence of highly cross-reactive allergens (such as profilins, calcium-binding proteins, tropomyosins) included in crude allergen extracts currently utilized as diagnostics may lead to

unresolved problems, particularly in cases of frequent polysensitisation skin pictures³. It is noteworthy that the patients in our study who were sensitised to Phl p 7 suffered from moderate/severe asthma, confirming anecdotal findings that sensitisation to calcium binding proteins may represent a marker of disease severity (Mari, personal communication). Another interesting finding was the high prevalence of sensitisation to the profilin Mer a 1 that involved more than 40% of allergic patients. In practical terms, this means that many cases of polysensitisation actually are genuinely allergic at most to one or two allergen sources, and this may have therapeutic implications (i.e. choice of the appropriate allergen vaccine). Moreover, given the variability of the percentage of sensitisations to different profilins ranging from 15.7% (Phl p 12) to 41.4% (Mer a 1) as documented here (Fig. 4), the opportunity to utilise a diagnostic assay which contains profilins from different allergen sources, as in the case of ISAC®, represents an advantage. Regarding the prevalence of sensitization to rBet v 2 (profilin) the data obtained by ISAC® (Bet v 2 = 30%) differ from those obtained in our previous study of subjects from the same geographic area using the CAP-system (Bet v 2 = 40%)⁵. Similar conclusions may be made for rPhl p 12 (15.7%, ISAC®; 35.1%, CAP-System)⁶. These findings may be due to biochemical and immunologic differences between the allergens employed in the two tests, i.e. lack of sugar side chains crucial for IgE binding. When perennial allergen sources are considered, the prevalence of sensitisation to the crude extract, evaluated with the skin test, seems to coincide with sensitisation prevalence to their corresponding allergens evaluated by allergen microarray. Furthermore, in a large percentage of subjects allergic to pollens, there is a substantial difference in the prevalence of sensitisation to purified allergens with respect to crude extracts. In particular, in the present study we have confirmed our previous observation⁷ of a great difference in the percentage of sensitisation towards *Parietaria judaica* extract with respect to its major allergen Par j 1 and Par j 2. We found analogous results for other weeds such as ragweed and mugwort. These findings may be explained through the existence of cross-reactive allergens, such as calcium binding proteins, profilins, and carbohydrate determinants, all of which are largely distributed within pollens and may cause misleading results in allergen extract-based diagnosis; this does not seem to be the case in component-resolved diagnosis. Low percentage of sensitisation to Art v 1 reported by ISAC® with respect to corresponding allergen extract evaluated by skin test may be due to immunological characteristics of Art v 1 utilised in the ISAC® test which result in reduced sensitivity, as previously reported⁸. In particular it can be speculated that the Art a 1 molecule in the ISAC® assay does not have an optimal epitope distribution. Within perennial allergen sensitisation to major

allergens (i.e. mite groups 1 and 2, or cat Fel d 1) the difference in the percentage of sensitisation between allergen extract- and allergen-specific IgE is small when compared to pollen sensitisation. This is due to a high degree of cross-reactivity among pollen allergen and consequently makes diagnosis easier in the case of perennial allergens. In other words, the prevalence of sensitisation to mite extract roughly corresponds to the sum of sensitisation toward the sum of mite major allergens. Moreover, in the case of sensitisation to non-pollen allergens, the prevalence of sensitisation to related cross-reactive allergens (i.e. tropomyosins) is low and therefore does not cause interference. Among the birch group 1 allergen molecules, rBet v 1 represents a gate-picker marker for Fagaceae, to which most allergic patients are genuinely sensitised to with respect to other Bet v 1-related allergens such as rCor a 1 and rAln g 1 (where the percentage of sensitisation is lower). In addition, in our study about 50% of Bet v 1-sensitive patients resulted positive for IgE antibodies against Mal d 1 apple major allergen (Bet v 1-homologous) causing OAS. Only a minority of Bet v 1 positive subjects have specific IgE towards Mal d 1, as well as to homologous Dau c 1 (carrot major allergen) and Api g 1 (celery major allergen). The differences of percentage of sensitisation against Mal d 1, Dau c 1 and Api g 1 reflect molecular differences that may influence specific IgE production (Mal d 1 is an incomplete allergen because it is extremely inefficient in inducing IgE antibodies, but it is able to provoke symptoms due to its ability to trigger mast cells loaded with specific IgE against Bet v 1)⁹. On the other hand, this minor sensitivity to Mal

d 1, Dau c 1, and Api g 1 may reflect a minor degree of exposure to the allergen source.

In our study of 70 patients who were polysensitized 14% had specific IgE antibodies to bromelain, which contains an oligosaccharide with two structural features, i.e. core α 1,3-fucose and xylose, which are responsible for *in vitro* reactivity of patients' sera to a wide variety of allergens due to cross-reactive carbohydrate determinants (CCD)¹⁰. In a CAP essay Mari¹¹ observed that 23% of sera from 1,831 patients contained IgE antibodies to bromelain and demonstrated that anti-CCD IgE led to false positive *in vitro* results whenever crude allergen extracts were used.

With respect to food allergies in our subjects, positive ISAC[®] results to LTP Pru p 3 (n = 5) and tropomyosins (n = 3) corresponded to clinical reactions in patients ingesting food sources with those allergens. Contrariwise, only two of the eight patients with positive results for milk allergens suffered from ingestion of milk and its derivatives.

This data gives us reason to believe that this allergen microarray represents a useful tool in allergy diagnosis, permitting physicians to more accurately establish the root causes of patient suffering. Clearly this is only the beginning in what should be seen as an important phase in allergology. The next step would be to enrich the panel with other allergens with epidemiologic significance. It is, however, of notable importance that the present panel include cross-reactive allergens which can facilitate diagnosis in cases of unresolved polysensitisation, whether genuine or apparent.

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