ORIGINAL ARTICLE

Component-Resolved in Vitro Diagnosis in Peach-Allergic Patients

PM Gamboa,[1] ML Sanz,[2] M Lombardero,[3] D Barber,[3] R Sánchez-Monje,[4] MJ Goikoetxea,[2] I Antépara,[1] M Ferrer,[2] G Salcedo[4]

[1] Allergy Service, Basurto Hospital, Bilbao, Spain
[2] Department of Allergology and Clinical Immunology, University Clinic, University of Navarra, Pamplona, Spain
[3] Departmento I+D, ALK Abelló, Madrid, Spain
[4] Department of Biotechnology, ETS Ingenieros Agrónomos, UPM, Madrid, Spain

Abstract

Background: The in vitro diagnosis of pollen-related food allergy presents low specificity and reproducibility with many conventional extracts. This can be improved using natural purified allergens, recombinant purified allergens, or both.
Objective: We compared specific immunoglobulin (Ig) E determination (sIgE), the basophil activation test (BAT), the histamine release test (HRT), and the cellular allergen stimulation test (CAST) using natural and recombinant allergens in the diagnosis of peach allergy.
Methods: Thirty-two peach allergic patients were studied. Skin prick tests were performed with commercial peach and extract with Mal d 1, nPru p 3, and profilin (nPho d 2). sIgE, BAT, CAST, and HRT were determined using rPru p 3, rMal d 3, rBet v 1, rMal d 1, and rMal d 4.
Results: Agreement between the techniques was good with all the allergens, except HRT with rMal d 1 and rMal d 4. With rPru p 3, sIgE, CAST, BAT, and HRT showed sensitivity values of 88%, 81%, 72%, and 69% and specificity values of 100%, 93%, 97%, and 83%, respectively. In patients with systemic symptoms or contact urticaria, the values were 100%, 85%, 81%, and 81%. In patients with oral allergy syndrome, sensitivity to profilins or homologues of Bet v 1 was detected in 100% of the cases by all the techniques, except by HRT with rMal d 1, which detected 66% of the cases.
Conclusions: The use of single allergens in the in vitro diagnosis of peach allergy by specific IgE determination, BAT, and CAST offers high specificity and sensitivity, with better results than the HRT.

Key words: Peach allergy. Component-resolved in vitro diagnosis. Basophil Activation Test. Histamine Release Test. Sulfidoleukotriene determination.

Resumen

Antecedentes: El diagnóstico de alergia alimentaria relacionada con pólenes es poco específico y muestra una baja reproducibilidad entre los diferentes lotes de extractos convencionales utilizados para tal fin. Este problema puede minimizarse utilizando alérgenos purificados naturales y/o recombinantes.
Objetivos: En este estudio comparamos la fiabilidad diagnóstica de dichos alérgenos purificados naturales y/o recombinantes en la determinación de IgE específica, test de activación de basófilos (TAB), test de liberación de histamina (TLH), y en la producción antígeno específica de sulfidoleucotrienos (CAST), en el diagnóstico de alergia a melocotón.
Métodos: Se incluyeron en el estudio 32 pacientes alérgicos a melocotón a los que se realizaron pruebas cutáneas (prick test) con extracto comercial de melocotón y extracto enriquecido en Mal d 1, nPru p 3 y profilina (nPho d 2). Se realizaron IgE específica, TAB, CAST y TLH utilizando rPru p 3, rMal d 3, rBet v 1, rMal d 1 y rMal d 4.
Resultados: En cuanto a los resultados obtenidos la concordancia entre las diferentes técnicas fue buena con todos los alérgenos, excepto para el test de liberación de histamina con Mal d 1 y Mal d 4. Utilizando como alérgeno rPru p 3 la IgE específica, el CAST, TAB y TLH mostraron valores de sensibilidad de 88%, 81%, 72% y 69% con unas especificidades de 100%, 93%, 97% y 83% respectivamente. En pacientes con síntomas sistémicos o con urticaria de contacto los valores de sensibilidad de dichas técnicas fueron de 100%, 85%, 81% y 81%. En los pacientes con síndrome de alergia oral la sensibilidad de las técnicas con profilina o con homólogos de Bet v 1 fue del 100% para todas las técnicas, excepto para el TLH con Mal d1 que detecta el 66% de los casos.
Conclusiones: Mediante le determinación de IgE específica, TAB y CAST con alérgenos purificados obtenemos unos altos valores de sensibilidad y especificidad en el diagnóstico in vitro de alergia a melocotón, por encima de los observados mediante TLH.

Palabras clave: Alergia a melocotón. Diagnóstico basado en componentes. Test de activación de basófilos. Determinación de sulfidoleucotrienos.

Introduction

In the diagnosis of allergy caused by fruits and vegetable food in general, skin tests with conventional total extracts present 2 drawbacks: low specificity [1,2] and low allergenic potency [3,4]. The use of fresh fruits in skin prick testing (SPT) is an alternative with low reproducibility problems due to variations in the allergenic content of the different species [5-9].

Of all the in vitro methods available today for the diagnosis of fruit allergy, specific immunoglobulin (Ig) E determination in whole extracts presents low specificity due partly to the high content of glycoproteins with IgE-binding capacity, and to the presence of cross-reacting allergens.

Natural or recombinant purified food allergens have proven useful in the diagnosis of food allergy, by overcoming the limitations of commercial extracts and fresh foods [10,11].

Different authors have validated the reliability of these allergens for in vivo diagnosis by SPT [12-16]. In the present study, we compared the reliability of specific IgE determination (sIgE) and 3 cellular techniques—the basophil activation test (BAT), the cellular allergen stimulation test (CAST), and the histamine release test (HRT)—in the diagnosis of peach allergy. We used a wide panel of recombinant allergens that include the 3 families of allergens involved in peach allergy (Southern Europe, lipid transfer protein [LTP]; Central Europe; homologues of Bet v 1; and profilins), rPru p 3, rMald 3, rBet v1, rMal d 1, and rMal d 4. We also compared them with peach commercial extracts.

Material and Methods

Patients and Challenge Tests

Thirty-two patients were studied (12 men, 20 women, age [SD] 26.8 [7 years]). They had all visited the allergy service of the Basurto Hospital in Bilbao, Spain, or the University Clinic of Navarre in Pamplona, Spain because of a potential peach allergy. All the patients presented symptoms compatible with peach allergy (oral allergy syndrome [OAS], contact urticaria, and/or systemic symptoms). A rubbing test with peach peel and skin tests with different allergens were performed on all patients [17]. Dermographism was ruled out in all the patients.

An open oral challenge test was performed on 26 patients starting with 1/8 of the peach weight for ethical reasons. It was not performed on patients with a recent history (< 1 year) of anaphylaxis after peach ingestion and with a positive SPT result and specific IgE result to peach for ethical reasons. Anaphylaxis was defined according the clinical criteria of Sampson [18].

The 6 patients who presented OAS underwent a double-blind oral challenge test. The allergen was prepared and weighed, and administered at an amount equivalent to 1/8 of the peach weight; each dose was duplicated every 20 minutes until the equivalent of a whole peach was reached.

The patients were classified into 3 groups according to their clinical history and oral challenge test result, as follows:

Group I: 6 peach-allergic patients with OAS only.

Group II: 20 peach-allergic patients with systemic symptoms (anaphylaxis, urticaria/angioedema).

Group III: 6 patients with peach-induced contact urticaria. The patients presented hives after contact with peach peel and all of them had a positive rubbing test result with peach peel. Within the group of OAS patients, 5 were allergic to grass pollen and 1 of them also to birch pollen.

Thirty control subjects were also selected (16 men and 14 women): 10 were healthy subjects and 20 were pollen-allergic patients (mean age 30.6 years). Among the pollen allergic patients, 16 were monosensitized to grass pollen and 3 were sensitized to grass and birch pollen.

All control subjects underwent the same in vivo and in vitro determinations as the patients—an open oral challenge test and rubbing test—and the results of both assays were negative.

Prick Test

All the patients and controls underwent prick testing following the usual technique with the most frequent inhalant allergens in our environment and peach extracts (ALK-Abelló SA, Madrid, Spain). In addition, we performed SPT with natural purified Pru p 3 (20 µg/mL), palm tree pollen profilin (nPho d 2) (50 µg/mL), and Mal d 1-enriched golden apple extract [9]. Histamine hydrochloride (10 mg/mL) and physiological serum were used as positive and negative controls, respectively. The SPT result was considered positive when the mean wheal diameter was 3 mm or greater, compared with that produced by the negative control.

In vitro tests

Allergens. We used the following recombinant allergens: rPru p 3, rMal d3, rMal d 4, rBet v 1, and rMal d. These allergens were obtained, purified, and characterized as described elsewhere [19-21].

Specific IgE determination. Total and specific IgE determinations to apple, peach, *Lolium perenne*, *Betula verrucosa*, *Artemisa vulgaris*, rBet v 1, rBet v 2, and rPhl p 12 were determined by CAP (Phadia, Uppsala, Sweden) following the manufacturer's instructions. In addition, specific IgE to rBet v 1, rMal d 1, rMal d 3, rMal d 4 and rPru p 3 was also determined using the ADVIA-Centaur platform (Bayer Diagnostics, Barcelona, Spain).

BAT. BAT was performed as previously described [15,22,23]. Briefly, blood was collected in 6-mL EDTA tubes and resuspended in 100-µg HEPES calcium buffer containing interleukin (IL) 3 (10 ng/mL).

In the cellular stimulation phase, and simultaneously with CAST, 2 final concentrations of the tested samples were assayed as follows: 2 and 0.5 mg/mL for peach peel, 2 and 0.3 mg/mL for apple peel, and 0.3 and 0.1 µg/mL for the purified recombinant allergens rPru p 3, rMal d 3, rBet v 1, rMal d 1, and rMal d 4. As a positive control, a monoclonal anti-IgE receptor antibody (Bühlmann Laboratories, Allschwil, Switzerland) at a concentration of 1 µg/mL was used.

In order to evaluate baseline values without stimulation, 50 µL of stimulation buffer was added to another well and 50 µL of cell suspension was added to all wells. Soon afterwards, plates were centrifuged at 1000*g* for 5 min at 4°C and 100 µL of supernatant was pipetted and saved for the sulphidoleukotriene (sLT) analysis by CAST–enzyme-

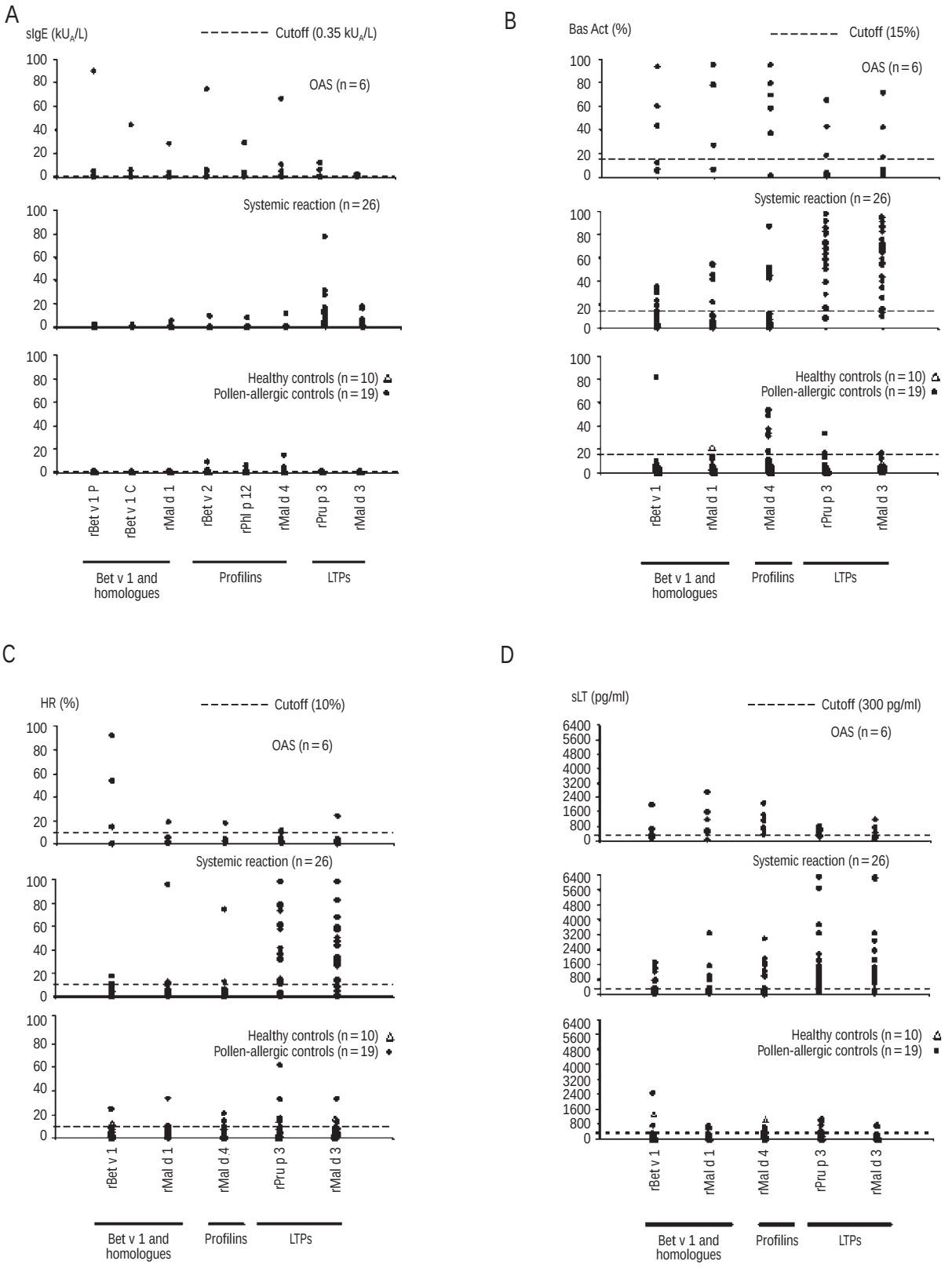

Figure. Individual results of specific IgE determination (A), basophil activation test (B), histamine release (C), and antigen-specific sulfidoleukotriene production (D) to purified and/or recombinant proteins in peach allergic patients with systemic reactions and OAS, and in pollen-allergic or healthy controls.

Table 1. Summary of the In Vitro Results in Patients and Controls[a]

	LTP							Bet v 1 Homologues						Profilin			
	Commercial peach extract				Pru p 3				Bet v 1				Mal d 4				
					Mal d 3				Mal d 1								
	sIgE	BAT	CAST	HRT	Prick test nPru p 3	sIgE	BAT	CAST	HRT	Prick test Mal d 1[b]	sIgE	BAT	CAST	HRT	Prick test nPho d 2	sIgE	BAT	CAST	HRT
OAS	4/6	6/6	6/6	4/6	3/6	2/6	2/6	4/6	1/6	3/6	3/6	3/6	3/6	3/6	4/6	4/6	5/6	6/6	1/6
						2/6	2/6	3/6	1/6		3/6	4/6	5/6	1/6					
Systemic patients	26/26	21/26	22/26	19/26	26/26	26/26	21/26	22/26	21/26	2/26	1/26	3/26	4/26	3/26	2/26	1/26	5/26	6/26	3/26
						23/26	22/26	22/26	19/26		1/26	3/26	4/26	4/26					
Pollinic controls	4/20	10/20	7/20	2/20	Not done	0/20	1/20	2/20	4/20	ND	0/20	1/20	1/20	1/20	ND	8/20	7/20	5/20	7/20
						0/20	0/20	0/20	2/20		1/20	0/20	0/20	2/20					
Healthy controls	0/10	0/10	0/10	1/10	Not done	0/10	0/10	0/10	1/10	ND	0/10	0/10	0/10	0/10	ND	0/10	0/10	0/10	0/10
						0/10	0/10	0/10	1/10		0/10	1/10	1/10	0/10					

Abbreviations: BAT, basophil activation test; CAST, cellular allergen stimulation test; HRT, histamine release test; OAS, oral allergy syndrome; sIgE, specific immunoglobulin E.
[a] Positive results/number of patients or controls
[b] Mal d 1–enriched golden apple extract.

linked immunosorbent assay (ELISA) (see below). The basophils from the cell pellet were double labelled by adding 20 µL of anti-CD63 PE–labelled antibody diluted at 1:80 and 20 µL of anti-IgE FITC–labelled antibody. Flow cytometric analysis was performed at 488 nm on a FACScan flow cytometer (BD Biosciences, San Jose, California, USA). The results were analyzed with the CellQuest software program, as described elsewhere [22].

The optimal cut off point, calculated by receiver operating characteristic (ROC) curves, combines sensitivity and specificity. We used this to determine the cutoff point by preferentially selecting the optimal specificity values over sensitivity, since peach allergy is a low prevalence condition. On that basis, results indicating a percentage of basophil activation greater than 20% with a stimulation index (SI: test value/background value) higher than 2 were considered positive for BAT.

sLT assay by CAST-ELISA. SLTs were analyzed by CAST-ELISA (Bülhmann Laboratories, Allschwil, Switzerland).

The assay measures the amount of sLT (LTC4, LTD4, LTE4) produced by blood leukocytes after in vitro stimulation by allergens according to the manufacturer's instructions [24].

As in BAT, the optimal cut off point was calculated using ROC curves.

A leukotriene production greater than 300 pg/mL with an SI (response to antigen/basal response) equal to or greater than 3 was considered positive.

HRT. We followed the method described by Shore et al [25], which was automated by Siraganian [26] using an autoanalyzer Technicon II Analyzer (Technicon Instrument Corp, Terrytown, New York, USA). The technique has been described elsewhere [27,28] using the following final concentrations: peach peel (0.8375 mg/mL), apple peel (0.8375 mg/mL), rPru p 3 (0.5 and 0.25 µg/mL), rMal d 3 (0.5 and 0.25 µg/mL), rBet v 1 (0.5 and 0.25 µg/mL), rMal d 1 (0.5 and 0.25 µg/mL), and rMal d 4 (0.5 and 0.25 µg/mL).

The results were interpreted according to the following formulae: % of histamine release = (antigen specific histamine release minus spontaneous or basal release) divided by (total histamine release minus spontaneous or basal release). Basal: spontaneous release of histamine without stimulation, Total: histamine release after 10% perchloric acid cell treatment.

A result was considered positive when a histamine release equal to or greater than 10% was produced.

Statistical Analysis

The data were analyzed with the statistical program SPSS 13.0 (SPSS Inc, Chicago, Illinois, USA). Qualitative variables were compared using the chi-square or Fisher exact test. A *P* value of less than .05 was considered statistically significant.

The agreement between the 4 diagnostic techniques used was analyzed using the κ statistic. Variables were expressed dichotomously as positive-negative in order to simplify the interpretation of the results.

Results

The results of BAT and sIgE for rPru p 3, rMal d 3, rBet v 1, rMal d 1, rMal d 4, rBet v 2, and rPhl p 12 in 30 of these patients have already been published [29]. Given the similarity of the results obtained in groups II and III, and the lack of significant differences in all the in vivo and in vitro parameters in these groups, we decided to merge both groups into one.

The results are summarized in the Figure and in Table 1.

Skin Tests

Of the 32 patients studied, 30 presented a positive response with the peach commercial extract and 29 with nPru p 3 (the 3 negative patients belonged to the OAS group). Five patients had a positive SPT result with Mal d 1–enriched golden apple extract. The OAS group showed positive results in 3 out of 6 patients, and clear differences with the systemic symptoms group, where the SPT result was positive in 2 out of 26 patients. Six patients had positive SPT results with palm tree pollen profilin (nPho d 2), 4 of them belonging to the OAS group and 2 to the systemic symptoms group.

In Vitro Tests

Commercial extract. The sensitivity of the 4 techniques studied ranged from 94% for specific IgE determination to 72% for HRT. Specificity data was close to 100% in the healthy controls for all the techniques. Nevertheless, in the pollen-allergic controls, specificity ranged from 50% in BAT to 90% in HRT.

LTP. Specific IgE to rPru p 3 showed the best sensitivity (28/32, 88%) and specificity (100%) results of the 4 techniques. Sensitivity increased to 100% when only the patients with systemic symptoms were considered.

Table 2. Agreement Between the 4 In Vitro Techniques for the Different Allergens Studied

	Pru p 3				Mal d 3			Mal d 1				Bet v 1			Mal d 4			
	Prick Test	BAT	sIgE	CAST	BAT	sIgE	CAST	Prick Test Mal d 1[a]	BAT	sIgE	CAST	BAT	sIgE	CAST	Prick Test nPho d 2	BAT	sIgE	CAST
HRT	(25/29)[d] 25/32	29/30c 29/32p k = 0.8[b]	25/30c 26/32p k = 0.6[b]	27/30c 26/32p k = 0.7	27/30c 26/32p k = 0.7	27/30c 24/32p k = 0.6[b]	27/30c 25/32p k = 0.7[b]	22/30 (1/5)[d]	27/30c 24/32p k = 0.2	27/30c 25/32p k = 0.1	27/30c 21/32p k = 0.1	27/30c 28/32p k = 0.5[b]	28/30c 30/32p k = 0.6[b]	27/30c 26/32p k = 0.4	24/30 (2/6)[d]	23/30c 20/32p k = 0.1	24/30c 25/32p k = 0.3[b]	25/30c 19/32p k = 0.1
CAST	(23/29)[d] 25/32	29/30c 26/32p k = 0.9[b]	28/30c 26/32p k = 0.7[b]		30/30c 29/32p k = 0.9[b]	30/30c 25/32p k = 0.8[b]		26/30 (4/5)[d]	29/30c 29/32p k = 0.8[b]	28/30c 24/32p k = 0.3[b]		30/30c 28/32p k = 0.8[b]	29/30c 28/32p k = 0.6[b]		23/30c (5/6)[d]	26/30c 31/32p k = 0.8[b]	25/30c 26/32p k = 0.5[b]	
sIgE	(28/29)[d] 31/32	29/30c 27/32p k = 0.8[b]			30/30c 28/32p k = 0.9[b]			27/30 (3/5)[d]	28/30c 27/32p k = 0.4[b]			29/30c 30/32p k = 0.7[b]			28/30 (4/6)[d]	29/30c 27/32p k = 0.7[b]		
BAT	(26/29)[d] 26/32							26/30 (4/5)[d]							25/30 (5/6)[d]			

Abbreviations: BAT, basophil activation test; c, controls; CAST, cellular allergen stimulation test; HRT, histamine release test; p, patients; sIgE, specific immunoglobulin E.

[a] Mal d1 enriched golden apple extract

[b] $P < .001$

[c] $P < .001$

[d] The numbers in parenthesis represent the number of cases with positive in vitro tests/number of cases with positive skin tests.

In the other 3 techniques, the sensitivity in the global study group reached 70%-80%, which increased to 80%-85% in the systemic symptoms group. The specificity of the 3 techniques, as well as CAP, was near 100% for both LTPs except in HRT with Pru p 3, which had a value of 83%. In the group of pollen-allergic controls, the specificity of specific IgE, BAT, and CAST with LTP was greater than that with peach commercial extract ($P < .05$).

Profilin. HRT with Mal d 4 was only positive in 1 OAS patient. Nevertheless, sIgE (4 patients), BAT (5 patients), and CAST (6 patients) showed a totally different frequency of sensitization to profilin compared with the patients with systemic symptoms (with a maximum of 23% detected by CAST with Mal d 4).

Sensitizations to profilin in the control subjects were only detected with all 4 techniques in the pollinic group.

Bet v 1 homologues. The 4 techniques detected sensitization to rBet v 1 in approximately 50% of the patients with OAS. Nevertheless, as with Mal d 4, HRT only detected 1 case of sensitization to rMal d 1 in that group, whereas the other 3 techniques detected sensitization in 3-5 patients out of 6.

Specific IgE to rBet v 1 and rMal d 1 was positive in only 1 of the 26 patients with systemic symptoms, whereas in the OAS group, 3 out of 6 were positive, and they all had positive SPT results with golden apple. Only one control patient, who was allergic to birch pollen, showed positive results to rMal d 1.

Sensitization to these allergens in the pollen-allergic control group was very low (5%), as determined by any of the 4 techniques.

Agreement Between In Vitro Allergy Tests

The results are summarized in Table 2. Agreement was good and shows statistical significance between the 4 techniques used, particularly for LTP. Only 2 antigens, rMal d1 and rMal d 4, behaved differently, with no significant agreement between HRT and the other in vitro tests. This is mainly due to the low sensitivity of HRT in the OAS group when using these allergens.

The agreements are particularly good between sIgE, SLT, and BAT for all the allergens tested.

Discussion

Our results show that the sensitivity of the 4 in vitro diagnostic techniques used is good, especially in the identification of patients who are sensitized to Pru p 3, which is the main allergen of peach-allergic patients in southern Europe [30].

The specificity of the 4 techniques using this allergen is also excellent, with values near 100% in sIgE, CAST, and BAT. Their specificity is greater using rPru p 3 and rMal d 3 than with the commercial peach extract, which shows low specificity in the pollen-allergic control group for all the techniques used, except HRT.

These results support the use of single allergens, and help us solve one of the main problems of the conventional extracts in daily clinical practice, that is, its low specificity [1,2], particularly when these techniques are applied to pollen-allergic patients, who are the most difficult to diagnose properly [31-33].

It is worth noting that, in our study, agreement between skin prick testing and the different in vitro techniques carried out with rPru p 3 was good, as was agreement between the in vitro techniques. Therefore, the 4 in vitro techniques can be considered reliable when detecting sensitization to LTP in our peach-allergic patients.

These values are comparable to those obtained in a multicenter European study carried out in cherry-allergic patients [12]. The authors showed that the agreement between SPT and sIgE was higher than 90% in patients sensitized to cherry LTP (rPru av 3).

In patients with OAS, sensitization to profilin, the predominant allergen in our environment [13,14], was identified in 80%-100% of cases by all the techniques studied except HRT. Agreement between the 4 in vitro tests is similar to those obtained with LTPs and to those of Reuter et al [12], who established agreement of almost 100% between skin testing and sIgE with rPru av 4 in cherry-allergic patients. In short, and despite the small number of patients sensitized to profilins in our series, which does not enable us to obtain conclusive results, it seems that, except for HRT, all the other techniques present similar diagnostic reliability.

Regarding the results obtained with rBet v 1 and rMal d 1, the usefulness of the 4 in vitro techniques to detect sensitization is similar to that of profilins. In our study, HRT with rMal d 1 did not show significant agreement with the other techniques, and its sensitivity is particularly low in the group of patients with OAS. This result is not consistent with the findings of Purohit et al [34] in Central Europe. These authors obtained positive results with HRT to rBet v 1 in all the patients studied with OAS caused by apple allergy, although with a low correlation between HRT and specific IgE determination.

Osterballe et al [35] studied 10 patients with OAS by apple allergy, obtaining 70% sensitivity using HRT with Mal d 1. The small number of patients included in our study prevents us from comparing results. One possible explanation could be a lower degree of sensitivity among southern European patients, or a higher concentration of rBet v 1 needed to obtain a similar histamine release level in patients with OAS [35]. Nevertheless, our results in BAT are similar to those of other authors. Ebo et al [32] obtained high sensitivity and specificity values (80%) in apple-allergic patients from central Europe using BAT and an apple extract with a high Mal d 1 content. Similarly, Erdmann et al found 75% sensitivity and 80% specificity in central European birch pollen–allergic patients with OAS caused by different foods [36].

In our series, BAT detected 87% of patients with positive SPT results using Mal d 1–enriched apple extract. This result was similar to the values obtained with sIgE (90%). Osterballe et al [35] obtained 100% sensitivity with sIgE to rMal d 1 in central European patients with OAS to apple. Purohit et al [34] found 100% sensitivity with sIgE to rBet v 1 in central European birch-allergic patients with OAS by Rosaceae. These results are similar to ours in the few patients sensitized to homologues of Bet v 1.

No published studies use recombinant or native antigens in the diagnosis of food allergy with CAST. In the small number of publications in the literature [37,38], high values of sensitivity and specificity are obtained, although the authors used commercial food extracts.

The use of allergenic molecular components in the in vivo diagnosis of food allergy will avoid the problems caused by the lack of fresh foods, the difficulty in obtaining lyophilized preparations that are less sensitive than SPT with fresh fruits [3,4], and the variation in the contents of major allergens between different food strains [5,9,39]. Moreover, it will facilitate diagnosis in patients with local reactions (OAS) by sensitization to profilin or Bet v 1 homologues and in patients at risk of presenting systemic symptoms by LTP sensitization [40].

In conclusion, although our sample presented low percentages of sensitization to profilin and Bet v 1 homologues, sIgE, BAT, and CAST show good sensitivity and specificity values that enable a correct diagnosis of sensitization to Pru p 3, the major allergen in our peach-allergic patients.

Allergenic molecular components (purified natural o recombinant allergens) will provide important advantages in the diagnosis of food allergy.

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María L. Sanz

Department of Allergology and Clinical Immunology
University Clinic of Navarra
Apartado 4209
31080 Pamplona, Spain
E-mail: mlsanzlar@unav.es

