

ORIGINAL ARTICLE

The performance of a component-based allergen microarray for the diagnosis of kiwifruit allergy

M. Bublin¹, S. Dennstedt¹, M. Buchegger¹, M. Antonietta Ciardiello², M. L. Bernardi³, L. Tuppo^{2,3}, C. Harwanegg⁴, C. Hafner^{1,5}, C. Ebner⁶, B. K. Ballmer-Weber⁷, A. Knulst⁸, K. Hoffmann-Sommergruber¹, C. Radauer¹, A. Mari³ and H. Breiteneder¹

¹Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria, ²Institute of Protein Biochemistry, CNR, Naples, Italy, ³Center for Molecular Allergy, IDI-IRCCS, Rome, Italy, ⁴Phadia Multiplexing Diagnostics, Vienna, Austria, ⁵Karl Landsteiner Institute for Dermatological Research, St Poelten, Austria, ⁶Allergy Clinic Reumannplatz, Vienna, Austria, ⁷Allergy Unit Department of Dermatology, University Hospital Zurich, Zurich, Switzerland and ⁸Department of Dermatology/Allergy, University Medical Center, Utrecht, The Netherlands

Clinical & Experimental Allergy

Summary

Background Allergy to kiwifruit is increasingly reported across Europe. Currently, the reliability of its diagnosis by the measurement of allergen-specific IgE with extracts or by skin testing with fresh fruits is unsatisfying.

Objective To evaluate the usefulness of a component-based allergen microarray for the diagnosis of kiwifruit allergy in a large group of patients.

Methods With an allergen microarray, we measured specific IgE and IgG4 levels to a panel of nine kiwifruit allergens in sera of 237 individuals with kiwifruit allergy. Sera from 198 allergic patients without kiwifruit allergy served as controls. Furthermore, we determined the extent of sensitization to latex.

Results The panel of kiwifruit allergens showed a diagnostic sensitivity of 66%, a specificity of 56% and a positive predictive value of 73%. Sera from kiwifruit-allergic patients contained significantly more frequently Act d 1-specific IgE than sera from control patients.

Furthermore, 51% of the positive sera contained IgE directed to a single allergen, namely Act d 1 (45%), Act d 9 (27%) or Act d 7 (13%). Within the control group, 36% sera recognized a single allergen. Out of those, 48% were positive to the cross-reactive glycoallergen Act d 7, 43% to the profilin Act d 9 and only 5% to Act d 1. Allergen-specific IgG4 levels did not differ between kiwifruit-allergic and -tolerant patients. Kiwifruit- and latex-allergic patients contained Hev b 11-specific IgE significantly more frequently than latex-allergic patients without kiwifruit allergy.

Conclusions Act d 1 can be considered a marker allergen for genuine sensitization to kiwifruit. We demonstrated that a component-based kiwifruit allergen microarray would improve the prognostic value of *in vitro* diagnostic tests.

Keywords allergens, component-resolved diagnosis, food allergy, kiwifruit, microarray

Submitted 2 February 2010; revised 3 August 2010; accepted 24 August 2010.

Correspondence:

Heimo Breiteneder, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Waehringer Guertel 18–20, 1090 Vienna, Austria. E-mail: heimo.breiteneder@meduniwien.ac.at

Introduction

Kiwifruit allergy has recently attracted the interest of allergologists because the number of cases significantly increased within the last two decades [1–3]. Kiwifruit allergy is often associated with birch and grass pollinosis [1, 4] as well as with latex allergy [5, 6]. Isolated allergy to kiwifruit is also relatively common and often severe [6–8].

At present, the diagnosis of kiwifruit allergy is unsatisfactory. *In vitro* and *in vivo* tests based on commer-

cially available kiwifruit extracts frequently fail to detect specific IgE, resulting in a low sensitivity [5, 7, 8]. Ripening stage, postharvest handling of kiwifruits and proteolytic degradation by the highly abundant protease actinidin significantly affect the relative amount of many proteins and subsequently the profile of IgE-binding components in a kiwifruit extract [9]. Prick-to-prick testing with fresh kiwifruit is often required to confirm a suggestive case history of kiwifruit allergy [6–8].

However, the high sensitivity of prick-to-prick testing [7, 8] is accompanied by its low specificity (31%) [8]. Further, the use of fresh kiwifruit as an allergen source cannot be standardized.

So far, 11 kiwifruit allergens have been included in the official list of the International Union of Immunological Societies Allergen Nomenclature Sub-Committee (<http://www.allergen.org>). Act d 1 (actinidin), a papain-like cysteine protease, which comprises about 50% of the total soluble fruit protein was described as the major kiwifruit allergen in previous studies [6, 7, 10]. Act d 2 (thaumatin-like protein) was established as an important *in vivo* reactive kiwifruit allergen [6, 11]. Act d 3, a 43 kDa glycoallergen, was recognized *in vitro* by 60% [6] and 33% [12] of sera from kiwifruit-allergic patients, respectively. This indicates that Act d 3 should be considered an important kiwifruit allergen as well. The IgE-binding properties of Act d 4 (phytolectin) and Act d 5 (kiwellin) have been reported [13, 14], and for both a sensitization prevalence of 20% was observed [12]. Act d 8 belongs to the pathogenesis-related 10 protein family with a sequence identity of 53% to Bet v 1, the major birch pollen allergen [15]. Act d 8 and Act d 9 (profilin) are important allergens in pollen-related kiwifruit allergy [12]. Additional kiwifruit allergens in the official list of allergens are the pectin methylesterase inhibitor Act d 6, the pectin methylesterase Act d 7, the non-specific lipid transfer protein Act d 10 and the ripening-related protein Act d 11. However, their importance in kiwifruit allergy is currently unknown [16, 17].

Our previous study showed that the use of single kiwifruit-allergen ImmunoCAPs resulted in a high diagnostic sensitivity compared with the extract and allowed the differentiation of subjects with primary and secondary kiwifruit allergy [12]. The aim of the present study was to evaluate the performance of purified kiwifruit allergens in a customized version of the ISAC assay (Immuno Solid-Phase Allergen Chip, Phadia Multiplexing Diagnostics, Vienna, Austria) for the *in vitro* diagnosis of kiwifruit allergy. As controls, sera from allergic subjects without clinical evidence of kiwifruit allergy were used. In addition to measurement of allergen-specific IgE, the measurement of allergen-specific IgG4 was performed to study the immunological response to individual kiwifruit allergens and whether the presence of allergen-specific IgG4 was associated with allergy or tolerance to kiwifruit.

We produced a panel of nine kiwifruit allergens either as natural (n) or recombinant (r) proteins: nAct d 1, nAct d 2, nAct d 4, nAct d 5, nAct d 6, nAct d 7, rAct d 8, rAct d 9 and nAct d 11. Instead of Act d 10, an nsLTP from peach (nPru p 3) was used. Further, we aimed to identify the allergens responsible for cross-reactivity between kiwifruit and latex by analysing the IgE reactivity of 46 sera from latex- and kiwifruit-sensitized patients to latex allergens (Hev b 1–Hev b 11).

Methods

Study population

Serum samples from 237 kiwifruit-allergic patients were included in the study (Table 1). Subjects were enrolled in four European countries (Austria, Italy, Switzerland and

Table 1. Characteristics of the study population

	Kiwifruit allergic	Kiwifruit tolerant
Number of tested sera	237	198
Age (years)		
Mean ($\pm$ SD)	33.4 ($\pm$ 15.9)	36.8 ($\pm$ 16.9)
Range	1–82	5–73
Sex		
Male, number (%)	86 (36%)	79 (40%)
Female, number (%)	151 (64%)	119 (60%)
Symptoms to kiwifruit		
OAS	204	0
Respiratory symptoms	23	0
Gastro-intestinal symptoms	15	0
Urticaria	12	0
DBPCFC		
Positive	30	
Negative		
Not done	207	198
Prick-to-prick test		
Positive	95	
Negative	15	
Not done	131	198
Skin-prick test		
Positive	37	
Negative	24	
Not done	176	198
Kiwifruit-specific IgE		
Positive	56	
Negative	77	
Not done	104	198
Sensitization to inhalant allergen sources		
Multiple pollen	43	16
Grass pollen	150	99
Birch pollen	86	64
Mugwort	4	37
Mites	103	84
Cat	73	31
Multiple fungi	25	3
Food allergy		
Peach	40	3
Apple	35	13
Nuts	33	25
Peanut	23	1
Tomato	22	8
Banana	18	17
Melon	9	3
Carrot	9	9
Fig	4	2
Pineapple	3	1
Latex sensitization	48	101

The Netherlands). Ethical approval was obtained by the respective institutional review boards and signed informed consent was obtained from the patients. Thirty subjects were included on the basis of a positive double-blind placebo-controlled food challenge (DBPCFC) to kiwifruit [12] and 207 subjects were included on a reliable clinical history of kiwifruit allergy. Skin tests were performed with either fresh kiwifruit (*Actinidia deliciosa* cv Hayward) using the prick-to-prick technique [18] (110 subjects) or commercial extracts (61 subjects) (Soluprick[®], ALK, Hørsholm, Denmark, and Allyostal, Stallergènes, France) according to the recommendation of the European Academy of Allergy and Clinical Immunology [19]. Specific IgE (ImmunoCAP, Phadia, Uppsala, Sweden) to kiwifruit was measured in 133 of the subjects. Among them, 96 patients underwent both skin test and kiwifruit-specific IgE measurement. As controls for specificity, 198 sera from patients with sensitization and/or allergy to at least one inhalant allergen source and/or latex but without clinical evidence of kiwifruit allergy were used. Food allergy was assessed on the basis of a clinically proven history to the offending food. The clinical data of the tested patients are summarized in Table 1.

The diagnosis of latex allergy was based on the clinical evidence of reactivity to latex exposure and specific IgE determination to latex performed with ImmunoCAP or skin prick test with latex. In 22 of 149 patients, latex allergy was confirmed by the glove use test.

Allergens

The natural kiwifruit allergens Act d 1, 2, 4 and 5 [12], as well as Act d 6 and 7 [20] and Act d 11 [21] were purified from fruit tissue. Recombinant Act d 8 [15] and Act d 9 [12] were produced and purified from an *E. coli* expression system as described previously. Purified allergens were demonstrated to be >95% pure as confirmed by denaturing SDS-PAGE and Coomassie staining (data not shown). The identities of the proteins were confirmed by N-terminal sequence analysis. CD spectroscopy analysis indicated that purified allergens were authentically folded (data not shown). IgE binding tested by ELISA also confirmed that they retained their immunological properties.

Latex allergens Hev b 1, Hev b 3 and Hev b 5–11 were purchased from Biomay (Vienna, Austria). Natural latex allergens Hev b 2 and Hev b 4 were purified from latex B-serum by gel filtration, as described previously [22–24]. Pru p 3 was purified as described by Gaier et al. [25].

Quantification of allergen-specific immunoglobulin E and immunoglobulin G4 using a microarray-based diagnostic test

Sera of all patients were analysed for the presence of allergen-specific IgE and IgG4 by an allergen microarray

assay (ISAC, Phadia Multiplexing Diagnostics). The quantification of allergen-specific IgE was performed as described previously [26]. Allergen-specific IgG4 was determined according to the manufacturer's recommendations (Directions for Use ImmunoCAP ISAC[®] Assay Kit IgG4, Phadia). We used a customized version of the microarray containing the following nine purified kiwifruit allergens: natural (n) Act d 1, nAct d 2, nAct d 4, nAct d 5, nAct d 6, nAct d 7 and nAct d 11 and recombinant (r) Act d 8 and rAct d 9. Furthermore, the array contained 11 latex allergens (Hev b 1–Hev b 11). Natural Pru p 3 was included as a marker for sensitization to nsLTP.

Statistical data analysis

Sensitivity and specificity as well as positive predictive value (PPV) and negative predictive value (NPV) were calculated as follows: sensitivity = TP/(TP+FN), specificity = TN/(TN+FP), PPV = TP/(TP+FP) and NPV = TN/(TN+FN). TP is the number of true positives (patients with kiwifruit allergy and positive ISAC results). FN is the number of false negatives (patients with kiwifruit allergy and negative ISAC results). A true negative (TN) result is defined as a patient without reported kiwifruit allergy and negative ISAC results and a false positive (FP) without kiwifruit allergy but with positive ISAC. Data analysis was performed using SPSS 14 (SPSS, Chicago, IL, USA). A χ^2 -test was used for comparisons of frequencies. Paired correlations were calculated by the Spearman rank test. *P*-values <0.05 were considered significant.

Results

Evaluation of the allergen microarray for the diagnosis of kiwifruit allergy

Specific IgE was detected against at least one of the nine tested kiwifruit allergens in 156 of 237 (66%) sera of patients with kiwifruit allergy and in 172 (73%) when sera containing Pru p 3-specific IgE were included as a marker for sensitization to nsLTPs.

Inclusion of nPru p 3 increased the sensitivity of the panel but decreased the specificity from 56% to 51%. In an initial screening, measurement of specific IgE levels using the commercial kiwifruit ImmunoCAP yielded positive results with 56 (42%) of 133 patients (Table 1), whereas microarray-based measurement for these patients resulted in a diagnostic sensitivity of 75% (100/133) and 79% (105/133) with Pru p 3 included. Ninety-five (86%) of 110 tested patients had a positive prick-to-prick test to kiwifruit, and 37 (61%) of 61 showed a positive skin test with kiwifruit extract. The sensitivity of the microarray for those two groups were 65% (72/110) and 59% (36/61), respectively. Including Pru p 3, sensitivity increased to 74% (81/110) for the first group and to 70% (43/61) for the second group.

The sensitivity of the microarray for the 30 patients with a positive DBPCFC was 56%.

Ninety-six of 172 (62%) positive sera contained specific IgE directed exclusively against one of the tested allergens, predominantly against Act d 1 (35 sera) followed by Act d 9 (21 sera), Pru p 3 (18 sera) and Act d 7 (10 sera). Forty-nine (28%) sera contained IgE specific for two allergens, 18 (10%) for three allergens and 9 (5%) sera for more than three allergens. In the control group, 88 of 198 (44%) of sera from atopic patients without kiwifruit allergy contained specific IgE against at least one of the tested allergens (Pru p 3 included). Seventy-two of 198 (36%) were positive to only one of the allergens, where Act d 7 was the allergen with the highest frequency of recognition (30 of 72 sera), followed by Act d 9 (27 sera) and Pru p 3 (nine sera). Sera from kiwifruit-allergic patients showed higher IgE levels (Fig. 1a) and contained significantly more frequently ($P < 0.001$) Act d 1-specific IgE (Table 2) than sera from kiwifruit-tolerant patients. Diagnostic sensitivity, specificity, PPV and NPV of the tests were calculated to evaluate the potential of the individual kiwifruit allergens as *in vitro* diagnostic reagents (Table 2). A diagnostic specificity of $> 95\%$ was observed for the allergens not related to pollen or latex allergens (nAct d 1, nAct d 2, nAct d 5 and nAct d 11) with nAct d 1 showing the highest sensitivity (28%) followed by nAct d 11 (10%) and nAct d 2 (8%). The diagnostic sensitivity of nAct d 4 and nAct d 5 was only 2% and 3%, respectively. None of the patients' sera contained nAct d 6-specific IgE. The diagnostic specificity of the highly cross-reactive allergens nAct d 7 (glycoallergen) and rAct d 9 (profilin) was lower than 90%, and the sensitivities were 22% and 18%, respectively.

The panel of kiwifruit allergens (at least one positive) showed a diagnostic sensitivity of 66% and a specificity of 56% (Table 2). When the pectin methylesterase Act d 7 and the profilin rAct d 9 were excluded, the diagnostic sensitivity was reduced from 66% to 48% but the diagnostic specificity increased from 56% to 89% and resulted in the PPV and NPV of 84% and 59%, respectively (Table 2).

Allergen-specific immunoglobulin G4

Allergen-specific IgG4 levels were not different in kiwifruit-allergic patients compared with kiwifruit-tolerant patients (Fig. 1b). While higher allergen-specific IgE mean values for all allergens with the exception of Act d 8 could be seen in the group of kiwifruit-allergic patients compared with the group of kiwifruit-tolerant patients, the differences of allergen-specific IgG4 between the two groups were allergen related (Table 3 and Fig. 1). Higher allergen-specific IgG4 mean values were measured for the allergens Act d 2, Act d 5, Act d 8, Act d 11 and Pru p 3 in the group of kiwifruit-allergic patients compared with the

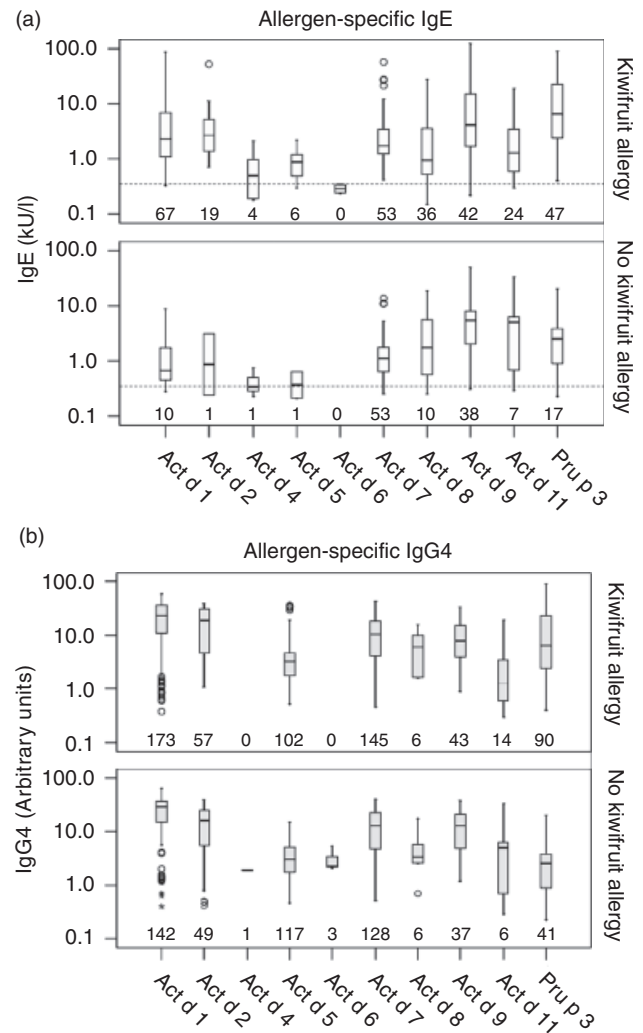


Fig. 1. Concentrations of specific IgE (a) and IgG4 (b) to individual kiwifruit allergens and Pru p 3 among kiwifruit-allergic subjects and kiwifruit-tolerant atopic controls. Mean of specific IgE and of fluorescence intensities for IgG4 are summarized in box plots. Each box represents the interquartile range containing 50% of the data. The line across the box indicates the median. Significances were calculated using the Spearman rank test ($*P < 0.05$). The dotted line indicates the 0.35 kU_A/L cut-off. The number of positive sera for each allergen is indicated below the boxes.

group of kiwifruit-tolerant patients. In contrast, lower IgG4 mean values were identified for the allergens Act d 1, Act d 4, Act d 7 and Act d 9 (Table 3). The number of subjects containing allergen-specific IgG4 in the group of kiwifruit-allergic patients was slightly higher than in the group of kiwifruit-tolerant subjects for the allergens Act d 1, Act d 2, Act d 7, Act d 9, Act d 11 and Pru p 3 (Fig. 1).

Sensitization to latex allergens

In order to determine the association between sensitization to kiwifruit and latex allergens, the IgE reactivity to

Table 2. Diagnostic sensitivity, specificity, PPV and NPV determination of allergen-specific IgE by ISAC using single kiwifruit allergens

Allergens	Number of positive reactions			P-value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	All (n = 433)	Kiwifruit allergy (n = 237)	Kiwifruit tolerant (n = 198)					
Act d 1	77 (18%)	67 (28%)	10 (5%)	<0.001*	28	96	87	52
Act d 2	20 (5%)	19 (8%)	1 (0.5%)	<0.001*	8	100	95	47
Act d 4	5 (1%)	4 (2%)	1 (0.5%)	0.254	2	100	80	46
Act d 5	7 (2%)	6 (3%)	1 (0.5%)	0.097	3	100	86	46
Act d 6	0 (0%)	0 (0%)	0 (0%)	–	–	–	–	–
Act d 7	106 (25%)	53 (22%)	53 (27%)	0.260	22	73	50	44
Act d 8	46 (11%)	36 (15%)	10 (5%)	0.001*	15	95	78	48
Act d 9	80 (18%)	42 (18%)	38 (19%)	0.657	18	81	53	45
Act d 11	31 (7%)	24 (10%)	7 (4%)	0.008*	10	96	77	47
All	242 (56%)	156 (66%)	86 (43%)	<0.001*	66	56	73	58
All w/o Act d 7 and 9	136 (31%)	114 (48%)	22 (11%)	<0.001*	48	89	84	59
Pru p 3	64 (15%)	47 (20%)	17 (9%)	0.001*	20	91	73	49
All+Pru p 3	269 (62%)	173 (73%)	96 (48%)	<0.001*	73	51	64	60

*Significant (P -value < 0.05).

Act d 1, actinidin; Act d 2, thaumatin-like protein; Act d 4, phytocystatin; Act d 5, kiwellin; Act d 6, pectin methylesterase inhibitor; Act d 7, pectin methylesterase [20]; Act d 8, Bet v 1-homologue; Act d 9, profilin; Act d 11, ripening-related protein; Pru p 3, non-specific lipid-transfer protein from peach.

PPV, positive predictive value; NPV, negative predictive value.

11 individual natural and recombinant latex allergens (Hev b 1–11) was compared between the group of patients with kiwifruit and latex allergy ($n = 48$) and the group of latex allergic patients without kiwifruit allergy ($n = 101$) (Table 4). Interestingly, patients with kiwifruit and latex allergy contained Hev b 11-specific IgE significantly more frequently ($P < 0.001$) than latex-allergic patients without kiwifruit allergy (Table 4). All Hev b 11-sensitized patients showed concomitant sensitization to Hev b 6. None of the sera from kiwifruit-allergic patients without latex allergy contained Hev b 11-specific IgE.

While the IgE reactivities to Hev b 4, Hev b 5, Hev b 6 and Hev b 8 showed no significant differences between the groups of latex-allergic patients with and without kiwifruit allergy (Table 4), the latter one reacted significantly more frequently with Hev b 2 ($P = 0.002$). The IgE reactivity to the individual kiwifruit allergens was similar in both patients groups (data not shown).

Discussion

Diagnostic tests using fruit extracts to determine specific IgE show low sensitivity and low specificity [27]. Because such extracts display batch-to-batch variation and may lack relevant food allergens due to intrinsic enzymatic activities, component-resolved diagnosis (CRD) has gained more attention in the recent past [13, 28, 29]. The microarray technology allows to determine allergic patients' IgE reactivity profiles towards a large number of allergens in a single step using minute amounts of serum [30, 31].

Our aim was to evaluate the performance of microarray-based CRD for the diagnosis of kiwifruit allergy. The results from the microarray show that kiwifruit allergens can be identified which are highly specific for kiwifruit-allergic patients, whereas others represent cross-reactive allergens in pollen and food allergy. These results are in agreement with our previous study, where specific IgE for individual kiwifruit allergens was measured by ImmunoCAP using sera from 30 patients with a positive double-blind placebo-controlled food challenge to kiwifruit [13].

Both IgE reactivity and monosensitization to Act d 1 were significantly more common among kiwifruit allergies than among atopic patients without kiwifruit allergy. Therefore, Act d 1 can be considered a marker allergen for genuine sensitization to kiwifruit (Fig. 1a and Table 2). In addition, three other individual kiwifruit allergens, Act d 2, Act d 8 and Act d 11, were significantly more often recognized by patients suffering from allergy to kiwifruit than by kiwifruit-tolerant atopic patients (Table 2).

In contrast, the presence of IgE specific for kiwifruit profilin, Act d 9, and pectin methylesterase, Act d 7, did not differ between kiwifruit-allergic and kiwifruit-tolerant patients. We observed that all patients' sera positive to profilin were cross-reactive to Bet v 2 (birch pollen profilin), Phl p 12 (timothy grass profilin) and Hev b 8 (latex profilin) (data not shown). Similarly, all patients' sera positive to Act d 7 were also positive to Api g 5, Ana c 2 and Hev b 4 (data not shown), which are known as allergens containing cross-reactive carbohydrate determinants (CCDs) [32]. This can be explained by the high cross-reactivity of profilins and CCDs from different allergen

Table 3. The number of positive subjects and levels (median, mean and range among positive sera) of allergen-specific antibodies in kiwifruit-allergic and kiwifruit-tolerant subjects

	Kiwifruit allergic				Kiwifruit tolerant			
	Positive (%)	Median	Mean	Range	Positive (%)	Median	Mean	Range
Act d 1								
IgE	67 (28%)	2.3	7.2	0.37–86.68	10 (5%)	0.78	1.9	0.37–8.78
IgG4	173 (70%)	22.56	23.7	0.38–58.28	145 (72%)	29.55	29.25	0.4–62.62
Act d 2								
IgE	19 (8%)	2.7	6.1	0.71–52.22	1 (0.5%)	3.1	3.1	3.1–3.1
IgG4	57 (24%)	18.8	19.1	1–39.2	49 (29%)	16.4	16.8	0.42–39.21
Act d 4								
IgE	4 (2%)	0.81	1.03	0.38–2.11	1 (0.5%)	0.75	0.75	0.75–0.75
IgG4	0	–	–	–	1 (0.5%)	1.9	1.9	1.9
Act d 5								
IgE	6 (3%)	0.95	1.081	0.47–2.2	1 (0.5%)	0.64	0.64	0.64–0.64
IgG4	102 (43%)	3.2	4.8	0.52–35.99	117 (60%)	3.01	3.85	0.46–15.22
Act d 6								
IgE	0	–	–	–	0	–	–	–
IgG4	0	–	–	–	3 (2%)	2.25	3.24	2.07–5.39
Act d 7								
IgE	53 (22%)	1.72	4.77	0.42–57.07	53 (27%)	1.19	2.1	0.38–13.62
IgG4	145 (61%)	10.4	12.9	0.46–42.88	128 (65)	12.6	14.8	0.5–39.98
Act d 8								
IgE	36 (15%)	1.09	3.6	0.38–27.67	10 (5%)	2.3	4.9	0.55–18.8
IgG4	6 (3%)	6.2	6.8	1.6–15.45	6 (3%)	3.5	5.6	0.7–17.67
Act d 9								
IgE	42 (18%)	4.2	11.7	0.42–124.2	38 (19%)	5.5	8.4	0.42–50.2
IgG4	43 (18%)	7.97	10.5	0.88–33.79	37 (19%)	12.57	13.5	1.2–37.5
Act d 11								
IgE	24 (10%)	1.4	3.5	0.36–18.84	7 (4%)	5.3	8.4	0.68–33.1
IgG4	14 (6%)	2.4	4.7	0.25–20.34	6 (3%)	2.6	4.2	0.3–15.06
Pru p 3								
IgE	47 (20%)	6.5	15.98	0.4–89.66	17 (9%)	2.8	3.69	0.37–20.42
IgG4	90 (38%)	19.4	18.05	0.4–43.24	53 (27%)	7.8	11.27	0.59–36.96

sources [33, 34]. Data obtained for Pru p 3 (Tables 2 and 3), a representative member of the nsLTP family, will have to be evaluated by using the kiwi nsLTP Act d 10, which was recently been purified by M. A. Ciardiello and A. Mari (unpublished results).

For Act d 4 and Act d 5, only weak signals were obtained with sensitivities as low as 2% and 3%, respectively. None of the sera positive in ImmunoCAP in a previous study [12] reacted with microrrayed Act d 4 and Act d 5. Act d 6 was recognized on the microarray neither by IgE nor by IgG4 from any tested serum, although a prevalence of 72% was reported when the sera of subjects with a convincing history of allergy to kiwi fruit were tested by IgE immunoblotting (<http://www.allergen.org/viewallergen.php?aid=12>). These results indicate that the immobilization procedure may not be suitable for Act d 4, Act d 5 and Act d 6. Consequently, the immobilization protocol must be optimized before incorporation to routine use and the relevant test parameters (e.g., sensitivity, reproducibility) must be validated to ensure the accuracy of the results for these allergens.

The panel of kiwifruit allergens (at least one positive) showed a diagnostic sensitivity of 66%, which is an improvement over the commercial kiwifruit ImmunoCAP (47%) and approached that of the component-resolved ImmunoCAP analysis (77%) [12]. In parallel, the specificity increased (56% vs. 30%). Furthermore, comparing the specific IgE profiles of 30 patients with a positive DBPCFC to kiwifruit obtained by ImmunoCAP in the previous study to the ISAC results, a high degree of correlation could be found in the case of allergens applied in both studies, with a particular high correlation coefficient in the case of Act d 1, Act d 2 and rAct d 9. Less correlation between the two methods was detected for rAct d 8.

The lower sensitivity of rAct d 8 (15% microarray vs. 43% ImmunoCAP) was accompanied by a higher specificity (95% microarray vs. 50% ImmunoCAP). The lower sensitivity could be the consequence of insufficient coupling yield or denaturation of the protein during the coupling process. This also explains the lower sensitivity of the microarray compared with ImmunoCAP (57% vs. 77%) for these 30 patients.

Table 4. IgE-reactivity to the 11 latex allergens in latex-allergic patients with kiwifruit allergy and kiwifruit-tolerant latex-allergic patients

Latex allergens	Kiwifruit- and latex-allergic patients (<i>n</i> = 48)	Latex-allergic patients without kiwifruit allergy (<i>n</i> = 101)
Hev b 1	1 (2%)	3 (3%)
Hev b 2	16 (33%)	61 (60%)*
Hev b 3	0 (0%)	2 (2%)
Hev b 4	18 (38%)	35 (35%)
Hev b 5	5 (10%)	17 (17%)
Hev b 6.02	12 (25%)	30 (30%)
Hev b 7	0 (0%)	1 (1%)
Hev b 8	10 (21%)	19 (19%)
Hev b 9	1 (2%)	0 (0%)
Hev b 10	0 (0%)	1 (1%)
Hev b 11	10 (21%)*	9 (9%)

*Significant (*P*-value < 0.05).

Hev b 1, rubber elongation factor; Hev b 2, β -1,3-glucanase; Hev b 3, small rubber particle protein; Hev b 4, lecithinase homologue; Hev b 5, acidic protein; Hev b 6, hevein; Hev b 7, patatin-like protein; Hev b 8, profilin; Hev b 9, enolase; Hev b 10, superoxide dismutase; Hev b 11, class I chitinase.

From these results, we conclude that the microarray-based allergen-specific *in vitro* diagnosis may not only be useful to discriminate among kiwifruit-allergic and kiwifruit-tolerant patients, it can additionally provide patient-tailored diagnosis and information about cross-reactivity patterns.

In the present study, we also evaluated whether the presence of allergen-specific IgG4 was associated with allergy or tolerance to kiwifruit. The role of IgG4 in the diagnosis and therapy of IgE-dependent food allergies is controversial. An EAACI position statement did not endorse testing for IgG4 against foods as a diagnostic tool [35]. High levels of IgG4 to animal foods in infancy were shown to be associated with tolerance to the respective food later in life [36]. There are also indications that the presence of serum IgG4 could be used to rule out food allergy [37]. The present study demonstrated that the presence of allergen-specific IgG4 was neither associated with allergy nor tolerance, thus we do not recommend its measurement for the diagnosis of kiwifruit allergy (Fig. 1b and Table 3).

Kiwifruit allergy plays a prominent role in the so-called latex-fruit syndrome. About half of latex-allergic patients have been reported to show an allergy towards avocado, banana, chestnut, kiwi and many other fruits [38]. The reported prevalence of kiwifruit allergy among latex-allergic individuals is between 17% and 25% [5, 6]. Because neither kiwifruit allergens nor latex allergens involved in latex-associated kiwifruit allergy have been identified, we aimed to elucidate the allergens responsible for the cross-reactivity. Plant class I chitinases containing N-terminal domains with high sequence similarities to the

major latex allergen Hev b 6.02 seem to be implicated in the case of avocado [39], banana [40] and chestnut [41]. While the rate of sensitization to Hev b 6.02 in our study was similar in the groups of latex-allergic patients with and without kiwifruit allergy, we found a significant difference in the sensitization to Hev b 11, the latex class I chitinase. Twenty-one per cent of sera from patients suffering from latex-kiwifruit allergy were positive to Hev b 11, whereas only 9% of latex-allergic patients without kiwifruit allergy and none of the sera from kiwifruit-allergic patients without latex sensitization contained Hev b 11-specific IgE. This result supports the hypothesis that class I chitinases from plant foods do not play a specific role in latex allergy without sensitization to fruits [42]. Additionally, the finding that all Hev b 11-sensitized sera showed concomitant sensitization to Hev b 6.02 supports the hypothesis that sensitization to class I chitinases occurs via hevein [38]. Hev b 11 can be a useful tool to improve the diagnosis and eventually the future immunotherapy of patients suffering from the latex-kiwifruit allergy.

In conclusion, our data suggest that CRD based on individual allergens improves sensitivity and PPV of kiwifruit allergy diagnosis. Secondly, we found that actinidin can act as a marker for genuine sensitization to kiwifruit.

Acknowledgements

This study was supported by grant SFB-F01802 from the Austrian Science Fund and by Italian Ministry of Health, Current research funding 2006–2007. The authors thank Nina Balazs for her excellent technical assistance.

References

- 1 Eriksson NE, Werner S, Foucard T *et al.* Self-reported hypersensitivity to exotic fruit in birch pollen-allergic patients. *Allergol Int* 2003; 52:199–206.
- 2 Osterballe M, Mortz CG, Hansen TK, Andersen KE, Bindslev-Jensen C. The prevalence of food hypersensitivity in young adults. *Pediatr Allergy Immunol* 2009; 20:686–92.
- 3 Rance F, Grandmottet X, Grandjean H. Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France. *Clin Exp Allergy* 2005; 35:167–72.
- 4 Pastorello EA, Pravettoni V, Ispano M *et al.* Identification of the allergenic components of kiwi fruit and evaluation of their cross-reactivity with timothy and birch pollens. *J Allergy Clin Immunol* 1996; 98:601–10.
- 5 Brehler R, Theissen U, Mohr C, Luger T. "Latex-fruit syndrome": frequency of cross-reacting IgE antibodies. *Allergy* 1997; 52:404–10.
- 6 Palacin A, Rodriguez J, Blanco C *et al.* Immunoglobulin E recognition patterns to purified Kiwifruit (*Actinidia deliciosa*) allergens in patients sensitized to kiwi with different clinical symptoms. *Clin Exp Allergy* 2008; 38:1220–8.

- 7 Aleman A, Sastre J, Quirce S *et al*. Allergy to kiwi: a double-blind, placebo-controlled food challenge study in patients from a birch-free area. *J Allergy Clin Immunol* 2004; 113:543–50.
- 8 Lucas JS, Grimshaw KE, Collins K, Warner JO, Hourihane JO. Kiwi fruit is a significant allergen and is associated with differing patterns of reactivity in children and adults. *Clin Exp Allergy* 2004; 34:1115–21.
- 9 Ciardiello MA, Giangrieco I, Tuppo L *et al*. Influence of the natural ripening stage, cold storage, and ethylene treatment on the protein and IgE-binding profiles of green and gold kiwi fruit extracts. *J Agric Food Chem* 2009; 57:1565–71.
- 10 Pastorello EA, Conti A, Pravettoni V *et al*. Identification of actinidin as the major allergen of kiwi fruit. *J Allergy Clin Immunol* 1998; 101:531–7.
- 11 Gavrovic-Jankulovic M, Cirkovic T, Vuckovic O *et al*. Isolation and biochemical characterization of a thaumatin-like kiwi allergen. *J Allergy Clin Immunol* 2002; 110:805–10.
- 12 Bublin M, Pfister M, Radauer C *et al*. Component-resolved diagnosis of kiwifruit allergy with purified natural and recombinant kiwifruit allergens. *J Allergy Clin Immunol* 2010; 125:687–94.
- 13 Bublin M, Mari A, Ebner C *et al*. IgE sensitisation profiles toward green and gold kiwifruits differ among patients allergic to kiwifruit from 3 European countries. *J Allergy Clin Immunol* 2004; 114:1169–75.
- 14 Tamburrini M, Cerasuolo I, Carratore V *et al*. Kiwellin, a novel protein from kiwi fruit. Purification, biochemical characterization and identification as an allergen. *Protein J* 2005; 24:423–9.
- 15 Oberhuber C, Bulley SM, Ballmer-Weber BK *et al*. Characterization of Bet v 1-related allergens from kiwifruit relevant for patients with combined kiwifruit and birch pollen allergy. *Mol Nutr Food Res* 2008; 52 (Suppl. 2):S230–40.
- 16 Lucas JS, Atkinson RG. What is a food allergen? *Clin Exp Allergy* 2008; 38:1095–99.
- 17 Mari A. When does a protein become an allergen? Searching for a dynamic definition based on most advanced technology tools. *Clin Exp Allergy* 2008; 38:1089–94.
- 18 Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983; 38:167–72.
- 19 Dreborg S, Frew A. Position paper – allergen standardization and skin-tests. *Allergy* 1993; 48:49–82.
- 20 Ciardiello MA, D'Avino R, Amoresano A *et al*. The peculiar structural features of kiwi fruit pectin methylesterase: amino acid sequence, oligosaccharides structure, and modeling of the interaction with its natural proteinaceous inhibitor. *Proteins: Struct Funct Bioinf* 2008; 71:195–206.
- 21 D'Avino R, Bernardi ML, Palazzo P *et al*. Act d 11, a new kiwifruit allergenic protein belonging to the Ripening-related protein family: biochemical and immunological characterisation. *Allergy* 2010; 65:184–5.
- 22 Seifert U, Wagner S, Bublin M *et al*. The role of glycosylation of nHev b 2, the beta-1,3-glucanase from *Hevea brasiliensis* latex, in IgE recognition. *J Allergy Clin Immunol* 2006; 117:S132.
- 23 Yeang HY, Chow K-S. DNA cloning of Hev b 2 (Hevea latex β -1,3-glucanase) and IgE reactivity of the recombinant protein. *J Rubber Res* 2004; 7:180–7.
- 24 Sunderasan E, Hamzah S., Hamid S., Ward MA, Yeang HY, Cardosa MJ. Latex B-serum β -1,3-glucanase (Hev b II) and a component of the microhelix (Hev b IV) are major latex allergens. *J Nat Rubber Res* 1995; 10:82–99.
- 25 Gaier S, Marsh J, Oberhuber C *et al*. Purification and structural stability of the peach allergens Pru p 1 and Pru p 3. *Mol Nutr Food Res* 2008; 52 (Suppl. 2):S220–9.
- 26 Deinhofer K, Sevcik H, Balic N *et al*. Microarrayed allergens for IgE profiling. *Methods* 2004; 32:249–54.
- 27 Steckelbroeck S, Ballmer-Weber BK, Vieths S. Potential, pitfalls, and prospects of food allergy diagnostics with recombinant allergens or synthetic sequential epitopes. *J Allergy Clin Immunol* 2008; 121:1323–30.
- 28 Hansen KS, Ballmer-Weber BK, Sastre J *et al*. Component-resolved in vitro diagnosis of hazelnut allergy in Europe. *J Allergy Clin Immunol* 2009; 123:1134–41.
- 29 Ballmer-Weber BK, Wangorsch A, Bohle B *et al*. Component-resolved in vitro diagnosis in carrot allergy: does the use of recombinant carrot allergens improve the reliability of the diagnostic procedure? *Clin Exp Allergy* 2005; 35:970–8.
- 30 Constantin C, Quirce S, Poorafshar M *et al*. Micro-arrayed wheat seed and grass pollen allergens for component-resolved diagnosis. *Allergy* 2009; 64:1030–7.
- 31 Hiller R, Laffer S, Harwanegg C *et al*. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J* 2002; 16:414–6.
- 32 Altmann F. The role of protein glycosylation in allergy. *Int Arch Allergy Immunol* 2007; 142:99–115.
- 33 Radauer C, Willeroider M, Fuchs H *et al*. Cross-reactive and species-specific immunoglobulin E epitopes of plant profilins: an experimental and structure-based analysis. *Clin Exp Allergy* 2006; 36:920–9.
- 34 Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. *Int Arch Allergy Immunol* 2002; 129:286–95.
- 35 Stapel SO, Asero R, Ballmer-Weber BK *et al*. Testing for IgG4 against foods is not recommended as a diagnostic tool: EAACI task force report. *Allergy* 2008; 63:793–6.
- 36 Tomicic S, Norrman G, Falth-Magnusson K, Jenmalm MC, Devenney I, Bottcher MF. High levels of IgG4 antibodies to foods during infancy are associated with tolerance to corresponding foods later in life. *Pediatr Allergy Immunol* 2009; 20:35–41.
- 37 Bernardi D, Borghesan F, Faggian D *et al*. Time to reconsider the clinical value of immunoglobulin G4 to foods? *Clin Chem Lab Med* 2008; 46:687–90.
- 38 Blanco C. Latex-fruit syndrome. *Curr Allergy Asthma Rep* 2003; 3:47–53.
- 39 Chen Z, Posch A, Cremer R, Raulf-Heimsoth M, Baur X. Identification of hevein (Hev b 6.02) in Hevea latex as a major cross-reacting allergen with avocado fruit in patients with latex allergy. *J Allergy Clin Immunol* 1998; 102:476–81.
- 40 Mikkola JH, Alenius H, Kalkkinen N, Turjanmaa K, Palosuo T, Reunala T. Hevein-like protein domains as a possible cause for allergen cross-reactivity between latex and banana. *J Allergy Clin Immunol* 1998; 102:1005–12.
- 41 Diaz-Perales A, Sanchez-Monge R, Blanco C, Lombardero M, Carillo T, Salcedo G. What is the role of the hevein-like domain of fruit class I chitinases in their allergenic capacity? *Clin Exp Allergy* 2002; 32:448–54.
- 42 Diaz-Perales A, Collada C, Blanco C *et al*. Cross-reactions in the latex-fruit syndrome: a relevant role of chitinases but not of complex asparagine-linked glycans. *J Allergy Clin Immunol* 1999; 104:681–7.