R. Asero

Allergy to kiwi: is component-resolved diagnosis in routine clinical practice really impossible?

Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano (MI), Italy - E-mail: r.asero@libero.it

Key words

Food allergy, kiwi allergy, cross-reactivity, diagnosis, skin prick tests

Corresponding author
Dr Riccardo Asero
Ambulatorio di Allergologia
Clinica San Carlo
20037 Paderno Dugnano (MI), Italy
Phone +39 02 99038470
Fax +39 02 99038223
e-mail r.asero@libero.it

Summary

***Background**: Kiwi allergy is frequent and can be the result of sensitization to a number of allergens showing different physicochemical characteristics. Component-resolved diagnosis of kiwi allergy is still unavailable in routine clinical practice.* ***Objective:** To investigate whether component resolved-diagnosis of kiwi allergy can be, at least in part, carried out by a proper combination of routinely available diagnostic tools.* ***Methods:** 63 adults with plant food allergy were studied. 36 were kiwi-allergic while 27 were kiwi-tolerant and served as controls. Patients and controls underwent SPT with commercial peach and kiwi extracts, and with a profilin-enriched date palm pollen extract (all by ALK-Abellò), and the measurement of IgE to birch, kiwi, and natural rubber latex.* ***Results:** The in-vitro test showed poor sensitivity and specificity, as it scored positive in about 50% of patients and controls irrespective of clinical allergy to kiwi. The kiwi SPT showed overall poor sensitivity; however, it scored negative in all subjects with pollen food-allergy syndrome, was weakly positive in some lipid transfer protein-hypersensitive/kiwi tolerant subjects and in one latex-sensitized subject, and strongly positive in all subjects with primary kiwi sensitization.* ***Conclusion:** SPT with this commercial kiwi extract sensitively and specifically detects patients reacting to specific kiwi allergens. This can be useful to detect patients that are at risk of potentially severe reactions, particularly in case of co-sensitization to labile allergens, while we wait that the whole spectrum of kiwi allergens becomes available for routine in-vitro testing.*

Introduction

Since its massive introduction on the markets worldwide some 30 years ago kiwi has become one of the plant-derived foods most frequently implicated in allergic reactions. In a recent Italian survey, kiwi ranked at # 4 among individual foods causing type 1 food allergy after the lipid transfer protein group as a whole, hazelnut and walnut (1); kiwi caused systemic symptoms in 30% of sensitized individuals, although no case of anaphylaxis was recorded (2).
In sensitized individuals, kiwi-induced symptoms may vary from slight oral allergy syndrome to severe systemic symptoms, largely depending on the kiwi allergen protein involved in IgE-mediated reaction. Several allergens have been detected so far in kiwifruit (Tab. 1). First it was shown that kiwi frequently causes oral allergy syndrome in birch pollen-allergic patients (3, 4); the cross-reacting allergen was subsequently characterized and cloned, and is presently known as Act d 8 (5). Very recently a ripening-related protein, denominated Act d 11, displaying IgE co-recognition with allergens belonging to the PR-10 family has been described as well (6). Other allergens identified so far include actinidin

Table 1 - Kiwi allergens detected to date

Allergen	IUIS Name	Molecular weight
Actinidin	Act d 1	30 kDa
Thaumatin-like protein	Act d 2	24 kDa
?	Act d 3	45 kDa
Cystatin	Act d 4	11 kDa
Kiwellin	Act d 5	28 kDa
Pectin metylesterase inhibitor	Act d 6	16 kDa
Pectin methylesterase	Act d 7	50 kDa
PR-10	Act d 8	17 kDa
Profilin	Act d 9	14 kDa
Lipid transfer protein	Act d 10	10 kDa
Major Latex Protein	Act d 11	17 kDa
Chitinase	Act d chitinase	32 kDa
UDP glucose pyrophosphorylase	-	52 kDa

(Act d 1)(7, 8) that has been considered a marker of genuine sensitization to kiwi (9) but whose role as the major kiwi allergen has been recently tackled (10), cystatin (Act d 4)(11), kiwellin (Act d 5)(12), and thaumatin-like protein (Act d 2)(13). Further allergens have recently joined this already long list, including a 38 kDa protein other than actinidin (10) and a 40 kDa protein denominated Act c 3 (14). Moreover, patients sensitized to the plant pan-allergen profilin may cross-react to kiwi profilin (Act d 9) and have oral allergy syndrome following the ingestion of kiwifruit (7,15-18). Kiwi allergy has been reported also as an offending food within the so-called latex-fruit allergy syndrome; several allergen proteins may be involved in latex-kiwi cross-reactivity, including hevein (19), and UDP glucose pyrophosphorylase (20,21). Finally, kiwi allergy has been reported in patients monosensitized to lipid transfer protein, suggesting some extent of cross-reactivity between peach and kiwi LTP, Act d 10 (22,23) although clinical allergy to kiwi LTP seems very rare (24). The clinical relevance of most allergens listed above is ill-defined due to paucity of monosensitized patients.

Although most studies of kiwi allergy conclude highlighting the importance of the use of single kiwifruit allergens (17), carrying out a component-resolved diagnosis of kiwi allergy in the clinical practice is presently a problem. In fact, only some of the kiwi allergen proteins are available for diagnostic purposes, and all of them are present only on the ISAC microarray, an expensive platform that is available only in few settings. This study aimed to investigate whether an at least partial component resolved-diagnosis of kiwi allergy can be accomplished by a proper combination of routinely available diagnostic tools, while we wait that the whole spectrum of single kiwi allergens become available on the market for routine diagnosis in-vitro by ImmunoCAP.

Patients and methods

Patients

Thirty-six kiwi-allergic adults (M/F 7/29; mean age 42,5, range 19-67) were studied. All had a clear-cut history of several episodes of kiwi-induced oral allergy syndrome (n=35) or urticaria (n=1) and a positive skin reactivity to fresh kiwi by the prick-prick test. This test has been reported to show 100% sensitivity (25) although its specificity may be limited (26).

Twenty-seven adults allergic to various plant-derived foods but tolerant to kiwi served as controls.

Methods

Both patients and controls underwent a series of standard tests including:

a) SPT with a commercial kiwi extract (5% w/v, 150 μg protein/ml; ALK-Abellò Madrid, Spain).
b) SPT with a commercial peach extract (ALK-Abellò) lacking labile allergens (PR-10 and profilin) and containing 30 μg/ml of lipid transfer protein.
c) SPT with a commercial profilin-enriched date palm pollen extract (profilin, Pho d 2, 50 μg/ml; ALK-Abellò).
d) Measurement of IgE specific for Birch pollen, whole kiwi, and natural rubber latex by ImmunoCAP (Phadia, Uppsala, Sweden).

SPT were performed and read following established methods (27). IgE levels exceeding 0.35 KU/l were considered positive.

Statistics

Proportions were compared by the chi-square test with Yates' correction. Correlation coefficients were assessed after Pearson. Probability values < 5% were considered statistically significant.

Results

Results in kiwi-allergic patients are shown in Table 2. Skin tests with commercial kiwi extract, peach extract, and date palm profilin scored positive in 6 (17%), 3 (8%), and 10 (28%) patients, respectively. IgE specific for birch pollen, kiwi extract, and natural rubber latex were detected in 29 (81%), 20 (55%), and 11 (31%) patients, respectively.

The commercial kiwi SPT was positive in 1/29 (3%) birch pollen reactors, 0/10 (0%) profilin reactors, 0/3 (0%) peach reactors, and 2/11 (18%) latex reactors. Of the 6 patients showing skin reactivity to kiwi extract, only 1 (17%) showed circulating birch-specific IgE vs 28/30 (93%) patients who scored negative on kiwi SPT ($p< 0.001$). Five/6 (83%) patients positive on kiwi SPT did not show any reactivity to birch pollen, profilin, peach lipid transfer protein or natural rubber latex.

Table 2 - Clinical features of kiwi-allergic patients and results of diagnostic tests

No.	Kiwi symptoms	Kiwi SPT	Peach SPT	Profilin SPT	Birch IgE	Kiwi IgE	Latex IgE
2	OAS	Neg	Neg	+++	100	7,52	29
9	OAS	Neg	Neg	++++	1,13	0	0
10	OAS	Neg	Neg	Neg	3,18	0	0
16	OAS	Neg	Neg	Neg	11,4	0	0
26	OAS	Neg	+++	+++	2,89	0	1,12
30	OAS	Neg	Neg	++++	7,1	0	0
31	OAS	Neg	Neg	Neg	11,6	0	0
33	OAS	Neg	Neg	Neg	100	2,17	0
36	OAS	Neg	Neg	Neg	48,3	0,54	0
38	OAS	Neg	++++	Neg	0	0	0
41	OAS	Neg	Neg	Neg	48,2	1,73	1,62
42	OAS	Neg	Neg	Neg	80,5	1,66	0
48	OAS	Neg	Neg	++++	20,9	0,61	1,33
49	OAS	Neg	Neg	++++	100	18,5	1,39
51	OAS	Neg	Neg	Neg	41,7	0	0
53	OAS	Neg	Neg	Neg	4,24	0	0
54	OAS	Neg	Neg	++++	18,5	1,49	1,68
57	OAS	Neg	Neg	Neg	11,2	0	0
61	OAS	Neg	Neg	++++	30,6	5,38	9,65
64	OAS	Neg	Neg	Neg	5,04	0,4	0
67	OAS	Neg	Neg	Neg	34,9	0,63	0
68	OAS	Neg	Neg	Neg	56,5	0	0
69	OAS	Neg	Neg	Neg	3,4	0	0
72	SOA	Neg	Neg	Neg	24	0	0
80	SOA	Neg	Neg	+++	1,68	0,41	1,57
83	SOA	Neg	Neg	Neg	49,8	0,98	0
87	SOA	Neg	Neg	Neg	17,3	0	0
94	SOA	Neg	Neg	Neg	15,4	0	0
96	SOA	Neg	++++	++++	14,1	0,38	0,44
21	OAS	+++	neg	neg	0	0,35	0
73	OAS	++++	neg	neg	0	8,74	0
85	OAS	++++	neg	neg	0	2,92	0
88	OAS + asthma	++++	neg	neg	0	6,71	0
91	OAS	++++	neg	neg	100	38,3	0,51
119	Urticaria	+++	neg	neg	0	4,12	0
82	OAS	Neg	Neg	neg	0	0	14,4

Positive skin tests are expressed by comparison with a SPT with histamine 10 mg/ml.
IgE are expressed as kU/l (Positive if > 0.35)

Kiwi IgE were detected in sera from 20/36 (56%) patients. IgE levels ranged from 0 to 38.3 kU/l and were significantly higher in patients showing a positive SPT with kiwi extract than in SPT-negative ones ($p< 0.005$); further, 6/6 (100%) patients positive on kiwi SPT showed a positive kiwi ImmunoCAP vs 14/30 (47%) SPT-negative ones ($p <0.05$). In birch pollen-sensitized patients the sensitivity of kiwi ImmunoCAP was poor (52%; 15/29) and kiwi-specific IgE levels were correlated to birch pollen IgE levels ($r= 0,64$; $p< 0.001$). Kiwi-specific IgE were detected in 9/11 patients showing latex-specific IgE; 8/9 (89%) were profilin reactors, as were most latex-sensitized patients (and, hence, probably Hev b 8 reactors).

Results in control subjects are shown in Table 3. Again, no patient with pollen-food allergy syndrome scored positive on kiwi SPT (lines 1-15, Table 3). In contrast, (and as a difference from the patients group) 5/9 LTP-allergic controls showed a weak skin reactivity to kiwi (lines 16-24, table 3), as did 1/3 latex-allergic individuals (lines 25-27). Sixteen/27 (59%) control subjects showed detectable IgE to kiwi, equally distributed between the 3 subgroups. The proportion of subjects scoring positive on kiwi ImmunoCAP did not differ statistically between patients and controls (p= NS).

Discussion

This study shows that it is possible to carry out a partial component-resolved diagnosis of kiwi allergy by the proper use and interpretation of available routine tests. Using 3 in-vivo and 3 in-vitro tests, 4 of which (birch, latex, peach, profilin) as markers of sensitization to potentially cross-reacting allergens, it was possible to understand the characteristics of the 2 kiwi tests employed. Theoretically (and ideally) an allergen extract should contain all the relevant proteins present in a certain allergen source. As a consequence, its sensitivity should be near 100%. This was not the case with our kiwi tests. The kiwi ImmunoCAP showed a reduced sensitivity in birch pollen-allergic

Table 3 - Results of diagnostic tests in control subjects

No.	Kiwi SPT	Peach SPT	Profilin SPT	Birch IgE	Kiwi IgE	Latex IgE
3	neg	neg	++++	17,6	10,4	18,8
6	neg	neg	++++	13,1	0	0,61
8	neg	neg	neg	21,4	0	0
11	neg	neg	++++	0,43	0	0,38
13	neg	neg	neg	9,79	1,8	0
15	neg	neg	++++	27,2	0,87	1,72
27	neg	neg	neg	53,9	0,98	0
28	neg	neg	++++	28,2	0,52	2,25
29	neg	neg	+++	11,8	0	0
32	neg	neg	neg	15	0,87	0,48
35	neg	neg	neg	93,6	1,42	0
37	neg	neg	neg	16,8	0	0,65
40	neg	neg	neg	16,6	0	0
43	neg	neg	neg	26,4	1,45	0
50	neg	neg	neg	6,8	0	0
1	++	++++	Neg	0	0,41	0
17	++	++++	Neg	0	1,1	0
18	++	+++	neg	0	0,76	0
19	neg	++++	neg	0	0	0
22	++	+++	neg	0	0,68	0
24	neg	++++	neg	0	0	0
25	++	+++	neg	0	0,83	0
44	neg	+++	neg	0	0	0
46	neg	+++	neg	0	0	0
14	neg	neg	neg	0,92	2,16	32,6
63	+	neg	neg	0	2	51,3
84	neg	neg	neg	0	0,62	38

subjects (just above 50%), and also a reduced specificity as shown by the findings in kiwi-tolerant controls. This observation is in keeping with recent observations of the poor diagnostic usefulness of Act c 8, the kiwi Bet v 1-homologue protein, in ISAC microarray immunoassay (28). Altogether, the in-vitro test with kiwi extract was unable to provide any meaningful information about the allergen(s) causing kiwi hypersensitivity. In contrast, the commercial Abellò kiwi SPT proved much more useful in this sense. It scored negative in all patients sensitized to birch pollen or profilin but one, was weakly positive in one latex-allergic individual and some lipid transfer protein-hypersensitive subjects, possibly as a consequence of cross-reactivity, and scored strongly positive in 5/5 birch/latex/LTP- negative patients. This suggests that this kiwi extract for SPT was able to discriminate those patients reacting to genuine kiwi allergens. Although the workup carried out here leads to a partial component-resolved diagnosis only, it is nonetheless useful to know that SPT-positive patients are at risk of potentially severe reactions whereas those who are not are most likely to have only slight oral symptoms. The usefulness of this information is maximal in patients that are co-sensitized to cross-reacting pollen allergens and primary kiwi allergens, as appears to be patient #91 (Tab. 2) who showed an elevated level of both birch and kiwi IgE, although monoreactivity to Act d 8 cannot theoretically be ruled out since an immunoblot analysis was not performed. Thus, as has been the case with a commercial peach extract for SPT (also by ALK-Abellò) that has been a cheap and easily available means to diagnose LTP hypersensitivity for years before the introduction of Pru p 3 for in-vitro testing, the commercial kiwi SPT by the same producer is an equally cheap and easy means to detect primary kiwi sensitization while we wait for the introduction of single natural or recombinant kiwi allergens to be used in-vitro in the routine practice. Whether these findings apply also to kiwi extracts from other producers remains to be established. Altogether, this study shows that in some instances "old" methods still work sufficiently well for our practical current needs.

References

1. Asero R, Antonicelli L, Arena A, et al. EpidemAAITO: Features of food allergy in Italian adults attending allergy clinics: a multicentre study. Clin Exp Allergy 2009; 39: 547-55.
2. Asero R, Antonicelli L, Arena A, et al. Causes of food-induced anaphylaxis in Italian adults: a multi-centre study. Int Arch Allergy Immunol 2009; 150: 271-7.
3. Gall H, Kalveram KJ, Forck G, Sterry W. Kiwi fruit allergy: a new birch pollen-associated food allergy. J Allergy Clin Immunol 1994; 94: 70-6.
4. Voitenko V, Poulsen LK, Nielsen L, Norgaard A, Bindslev-Jensen C, Stahl Skov P. Allergenic properties of kiwi fruit extract: cross-reactivity between kiwi fruit and birch pollen allergens. Allergy 1997; 52: 136-43.
5. Oberhuber C, Bulley SM, Ballmer-Weber B, 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): 230-40.
6. D'Avino R, Bernardi ML, Wallner M, et al. Kiwifruit Act d 11 is the first member of the ripening-related protein family identified as an allergen. 2011; 66: 870-7.
7. 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.
8. 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.
9. Bublin M, Dennstedt S, Buchegger M, et al. The performance of a component-based allergen microarray for the diagnosis of kiwifruit allergy. Clin Exp Allergy 2011; 41: 129-36
10. Lucas JSA, Nieuwenhuizen NJ, Atkinson RG, et al. Kiwifruit allergy: actinidin is not a major allergen in the United Kingdom. Clin Exp Allergy 2007; 37: 1340-8
11. Bublin M, Mari A, Ebner C, et al. IgE sensitization profiles toward green and gold kiwifruits differ among patients allergic to kiwifruit from 3 European countries. J Allergy Clin Immunol 2004; 114: 1169-75.
12. Tuppo L, Giangrieco I, Palazzo P, et al. Kiwellin, a modular mrotein from green and gold kiwi fruits: evidence of in vivo and in vitro processing and IgE binding J Agric Food Chem 2008; 56: 3812-7
13. Gavrovic-Jankulovic M, Cirkovic T, et al. Isolation and biochemical characterization of a thaumatin-like kiwi allergen J Allergy Clin Immunol 2002; 110: 805-10.
14. Palacin A, Rodriguez J, Blanco C, et al. Immunoglobulin E recognition patterns to purified Kiwifruit (Actinidinia deliciosa) allergens in patients sensitized to Kiwi with different clinical symptoms. Clin Exp Allergy 2008; 38: 1220-8.
15. Van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. Int Arch Allergy Immunol 1992; 98: 97-104.
16. Asero R, Monsalve R, Barber D. Profilin sensitization detected in the office by skin prik test: a study of prevalence and clinical relevance of profilin as a plant food allergen. Clin Exp Allergy 2008; 38: 1033-7.
17. 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.
18. Santos A, van Ree R. Profilins: mimickers of allergy or relevant allergens? Int Arch Allergy Immunol 2011; 155: 191-204.
19. Salcedo G, Diaz-Perales A, Sanchez-Monge R. The role of plant panallergens in sensitization to natural rubber latex. Curr opin Allergy Clin Immunol 2001; 1: 177-83.

20. Asero R, Mistrello G, Roncarolo D, Amato S, Falagiani P. Detection of novel latex allergens associated with clinically relevant allergy to plant-derived foods. J Allergy Clin Immunol 2005; 115: 1312-4.
21. Conti A, Giuffrida MG, Hoffmann-Sommergruber K, et al. Identification of a Latex UDP glucose pyrophosphorylase (Hev b UDPGP) as a novel cause of latex fruit allergy syndrome. Eur Ann Allergy Clin Immunol 2007; 39: 116-8.
22. Asero R. Lipid transfer protein cross-reactivity assessed in-vivo and in-vitro in the office: pros and cons. J Investig Allergol Clin Immunol 2011; 21: 129-36.
23. Egger M, Hauser M, Mari A, Ferreira F, Gadermaier G. The role of lipid transfer proteins in allergic diseases. Curr Allergy Asthma Rep 2010; 10: 326-35.
24. Asero R, Mistrello G, Roncarolo D, et al. Immunological cross-reactivity between lipid transfer proteins from botanically unrelated plant-derived foods: a clinical study. Allergy 2002; 57: 900-6.
25. 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.
26. Lucas JS, Grimshaw KE, Collins K, Warner JO, Hourihane JO. Kiwi fruit is a significant allergen and is associated with differing patternof reactivityin children and adults.Clin Exp Allergy 2004; 34: 1115-21.
27. Dreborg S, Frew A. Allergen standardization and skin tests. EAACI position paper. Allergy 1993; 48: 49-75.
28. Asero R, Villata D. Is the detection of IgE to multiple Bet v 1-homologous food allergens by means of allergen microarray clinically useful? J Allergy Clin Immunol 2010; 125: 1158-61.
29. Asero R. Detection and clinical characterization of patients with oral allergy syndrome caused by stable allergens in Rosaceae and nuts. Ann Allergy Asthma Immunol. 1999; 83: 377-83.
30. Asero R, Mistrello G, Roncarolo D, Casarini M, Falagiani P. Allergy to nonspecific lipid transfer proteins in Rosaceae: a comparative study of different in vivo diagnostic methods. Ann Allergy Asthma Immunol. 2001; 87: 68-71.