



Kiwifruit Allergies

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Abstract

While kiwifruit has a high nutritive and health value, a small proportion of the world's population appears to be allergic to the fruit. IgE-mediated kiwifruit allergy is often associated with birch and grass pollinosis as well as with latex allergy. Isolated allergy to kiwifruit is also relatively common and often severe.

Eleven green kiwifruit (*Actinidia deliciosa* cv. Hayward) allergens recognized to date are termed as Act d 1 through Act d 11. Bet v 1 homologue (Act d 8) and profilin (Act d 9) are important allergens in polysensitized subjects, whereas actinidin (Act d 1) is important in kiwifruit monosensitized subjects. Differences in allergenicity have been found among kiwifruit cultivars. Allergy sufferers might benefit from the selection and breeding of low-allergenic kiwifruit cultivars.



1. INTRODUCTION: FOOD ALLERGY AND ALLERGENS

Adverse food reactions (food hypersensitivities) include any abnormal reaction resulting from the ingestion of a food and might be the result of food intolerances (nonallergic food hypersensitivities) or food hypersensitivity (food allergy). Food intolerances are adverse responses caused by some unique physiological characteristic of the host, such as metabolic disorders (e.g., lactase deficiency). Food allergies are adverse immunological reactions that might be due to IgE- or non-IgE-mediated immune mechanisms (Sicherer & Sampson, 2009).

For the purpose of this chapter, allergy is defined as the clinical reaction resulting from the induction of an IgE-mediated immune response to a food protein. The most common food allergy sources, responsible for up to 90% of IgE-mediated food allergic reactions, are the proteins in cows' milk, eggs, peanuts, wheat, soy, fish, shellfish, and tree nuts (Hefle, Nordlee, & Taylor, 1996; Sicherer & Sampson, 2009). The spectrum of food allergy sources relevant to children differs from that for adults. Cows' milk, peanut, and eggs have a high impact as allergen sources in young children. In the adult population, most of the reactions are due to nuts, peanut, fruit, milk, and vegetables (Schafer et al., 2001). The prevalence of allergic sensitivities to specific foods obviously varies due to regional dietary habits, methods of food preparation, and the age of the subject when the food is introduced into the diet.

A protein with the potential to induce an IgE-mediated sensitization, which could subsequently provoke allergic reactions, is called an allergen. Sensitization to food allergens can occur in the gastrointestinal tract (class 1 food allergy or primary food allergy) or as a consequence of an allergic sensitization to inhalant allergens (class 2 food allergy or secondary food allergy). Allergens found in foods of both plant and animal origin that cause allergy as a result of oral sensitization seem generally to be characterized by two properties: abundance in the food and/or structural stability (Breiteneder & Clare Mills, 2005). The harsh conditions of the gastrointestinal tract (low pH, proteolytic enzymes and surfactants such as phospholipids and bile salts) favor rapid and extensive hydrolysis of proteins, yet food protein allergens retain sufficient structural integrity to be taken up by specialized epithelial cells in the gut and transferred to the mucosal immune system. These properties characterize the so-called complete allergens. On the other hand, incomplete allergens can trigger allergic reactions but usually are not able to

sensitize. Thus, they are readily soluble in physiological buffers, tend to be thermolabile, and are readily broken down by proteases (Aalberse, 2000). The immunological basis for class 2 food allergy is IgE cross-reactivity, due to a high level of homology between sensitizing allergens found in pollen and latex, and allergens in foods such as apple, celery, peaches banana, kiwifruit, etc. Thus, the terms “pollen-fruit allergy syndrome” and “latex-fruit allergy syndrome” have been established.



2. KIWIFRUIT ALLERGY

The first reported case of IgE-mediated kiwifruit allergy dates back to 1981 (Fine, 1981), and since 1995, several publications have shown that eating or peeling green-fleshed kiwifruits may elicit typical IgE-mediated allergic reactions in children and adults.

Monosensitization to kiwifruit has been reported (Aleman et al., 2004; Bublin et al., 2010; Garcia, de la Cuesta, Santos, Feliu, & Cordoba, 1989; Nölle & Schapowal, 2000; Steurich & Feyerabend, 1996), but the largest group of kiwifruit-allergic subjects comprises subjects with a pollen-fruit syndrome (Aleman et al., 2004; Bublin et al., 2010; Gavrovic-Jankulovic, Cirkovic, Burazer, Vuckovic, & Jankov, 2002; Pastorello et al., 1996). About 70% of all birch pollen-allergic individuals display allergic symptoms toward various fruits like apple, pear, hazelnut, stone-fruits, celery, carrot, potato, and tomato (reviewed in Vieths, Scheurer, & Ballmer-Weber, 2002). A similar cross-reactivity is observed between kiwifruit and grass pollen allergy (Aleman et al., 2004; Gavrovic-Jankulovic, Cirkovic, Burazer, et al., 2002; Pastorello et al., 1996). Kiwifruit also plays a prominent role in the so-called latex-fruit syndrome. About half of latex-allergic patients show an allergy toward avocado, banana, chestnut, kiwifruit, and many other fruits (Lavaud, Sabouraud, Deschamps, & Perdu, 1997). In addition, allergy to kiwifruit was shown to occur in patients allergic to fig fruits and to the latex of the ornamental plant *Ficus benjamina*, but without rubber latex allergy (Diez-Gomez, Quirce, Aragonese, & Cuevas, 1998; Focke, Hemmer, Wohrl, Gotz, & Jarisch, 2003).

2.1. Epidemiology

There are no studies on the prevalence of kiwifruit allergy in the general population, but it can be estimated from the incidence among birch pollen-allergic individuals, the largest group of kiwifruit-allergic patients.

About 35–40% of birch pollen-allergic patients show symptoms of allergy to kiwifruit (Eriksson et al., 2003; Voitenko et al., 1997). As the prevalence of birch pollen allergy in the general population of Central Europe is about 5%, the proportion of kiwifruit-allergic individuals can be estimated to be at least 1.75–2% of the population. Approximately 12–17% of individuals who are allergic to natural rubber latex appear to be kiwifruit allergic (Beezhold, Sussman, Liss, & Chang, 1996; Blanco, Carrillo, Castillo, Quiralte, & Cuevas, 1994; Kim & Hussain, 1999). A questionnaire-based survey among schoolchildren conducted in France found that, out of 2716 questionnaires returned, 192 reported a food allergy. In those schoolchildren reporting allergy, the main foods reported as causing adverse reactions were cows' milk (12%), eggs (9.4%), kiwifruit (9%), peanut (8.2%), fish (7.8%), tree nuts (7.8%), and shrimps (5.3%) (Rance, Grandmottet, & Grandjean, 2005).

2.2. Symptoms

The majority of allergic reactions to kiwifruit occur within minutes of consuming a kiwifruit or kiwifruit-containing food products.

Oral allergy syndrome is most common in adults and is especially frequent among patients with pollen allergy, as it is provoked by sensitization to pollen allergens homologous to kiwifruit proteins. The symptoms of 65–72% of kiwifruit-allergic subjects are confined to the oral mucosa (Aleman et al., 2004; Bublin et al., 2010; Lucas, Grimshaw, Collins, Warner, & Hourihane, 2004; Moller et al., 1997; Voitenko et al., 1997). Symptoms involved in oral allergy syndrome are swelling and itching of the lips, tongue, and pharynx, and pruritis of the eyes, ears, tongue, pharynx, and mouth. More severe symptoms such as urticaria, abdominal pain, moderate dyspnea, rhinitis, cyanosis, or anaphylaxis occur in 18–46% of kiwifruit-allergic patients (Aleman et al., 2004; Lucas et al., 2004). Young children (<5 years) were statistically significantly more likely than adults (>15 years) to react on their first known exposure and to report severe symptoms (Lucas et al., 2004). Severe symptoms of allergy to kiwifruit are frequently reported in patients not allergic to pollen (Aleman et al., 2004; Bublin et al., 2010).

Allergy in the form of contact dermatitis has been reported, usually associated with handling the fruit or from contact with the vine (Fine, 1981; Rademaker, 1996; Veraldi & Schianchi-Veraldi, 1990; Zina & Bundino, 1983). Additionally, a case of acute pancreatitis has been described (Gastaminza, Bernaola, & Camino, 1998).

2.3. Diagnosis

The diagnosis of kiwifruit allergy is based on a combination of case history and demonstration of allergen-specific sensitization. Allergen-specific sensitization can be diagnosed by *in vivo* tests such as double-blind and placebo-controlled food challenge (DBPCFC) and skin tests and *in vitro* tests such as measurement of allergen-specific IgE in a patient's serum using ImmunoCAP or allergen microarray (ISAC, Phadia Multiplexing Diagnostics). The standard ("gold standard") in food allergy diagnosis is a properly performed DBPCFC but this cannot always be undertaken. The test is time-consuming, and as there is a lack of uniform challenge protocols, DBPCFC is only performed in specialized centers.

In vitro and *in vivo* tests based on commercially available kiwifruit extracts frequently fail to detect specific IgE, resulting in false-negative diagnosis due to low sensitivity (Aleman et al., 2004; Brehler, Theissen, Mohr, & Luger, 1997; Bublin et al., 2010; Lucas et al., 2004). The reduced specificity of fresh kiwifruit for skin testing was observed in pollen- as well as in latex-allergic subjects (Beezhould et al., 1996; Gall, Kalveram, Forck, & Sterry, 1994; Latasa et al., 1995). A diagnostic sensitivity of *in vitro* IgE tests of 13% (Brehler et al., 1997) to 50% (Aleman et al., 2004) and 60% (Lucas et al., 2004) has been reported. Ripening stage, postharvest handling of kiwifruits, and proteolytic degradation by the highly abundant protease actinidin can all significantly affect the relative amounts of many proteins and subsequently the profile of IgE-binding components in a kiwifruit extract (Ciardiello et al., 2009). Thus, proper *in vivo* diagnosis of kiwifruit allergy requires the use of fresh fruit, for example, in the prick-prick technique. However, two studies showed that prick-prick testing resulted in 95% (Lucas et al., 2004) or 100% (Aleman et al., 2004) sensitivity, but was accompanied by a low specificity (31%) resulting in false-positive diagnosis. Accurate diagnosis of food allergy is the first step to improve the patient's quality of life. In recent years, great effort has been taken to overcome shortcomings in diagnostic approaches based on crude protein extracts of the allergen sources involved. The concept of component-resolved diagnosis has gained more attention in the recent past. Such tests utilize natural or recombinant individual allergens instead of protein extracts. Measuring IgE specific to single kiwifruit allergens results in a high diagnostic sensitivity and allows the differentiation of subjects with primary kiwifruit allergy and subjects with kiwifruit allergy associated with pollinosis (Bublin et al., 2011, 2010). It has been demonstrated that Bet v 1 homologue and profilin are important allergens in

pollen-related kiwifruit allergy, whereas actinidin is important in mono-allergy to kiwifruit, in which symptoms are often more severe.

2.4. Treatment

Currently, avoidance of kiwifruit by those who are allergic is the approach of choice, as there is, as yet, no cure for food allergy. Hence, the focus of kiwifruit allergy management is to educate afflicted subjects to avoid products containing kiwifruit, recognize early symptoms due to unintended ingestion, and administer self-injectable epinephrine when indicated.

Specific immunotherapy to inhalant allergens has been shown to reduce clinical symptoms (Bousquet & Demoly, 2006; Wilson, Lima, & Durham, 2005). This immunotherapeutic approach involves subcutaneous injection or sublingual application of progressively higher doses of allergen to induce immune tolerance. Immune tolerance is defined as redirecting the T-cell immune response from a Th2- to a Th1-type response. However, specific immunotherapy against food allergy in humans is currently not viable because of the high risk of the adverse reactions including anaphylaxis, limited efficacy, and lack of standardized protocols. Nevertheless, one isolated report has suggested that such approaches appear to be effective in inducing a persistent state of tolerance to kiwifruit. A 29-year-old woman with several episodes of severe anaphylaxis after ingestion of kiwifruit was subsequently treated using a sublingual immunotherapy protocol, enabling her to subsequently ingest substantial amounts of kiwifruit without symptoms (Kerzl, Simonowa, Ring, Ollert, & Mempel, 2007; Mempel, Rakoski, Ring, & Ollert, 2003).



3. KIWIFRUIT ALLERGENS

So far, 11 allergens from *Actinidia deliciosa* cv. 'Hayward' (green kiwifruit) have been included in the official list of the International Union of Immunological Societies Allergen Nomenclature Subcommittee (IUIS, <http://www.allergen.org>) and are termed as Act d 1 through Act d 11 (Table 18.1). To date, three allergens from *Actinidia chinensis* cv. 'Hort 16A' (gold kiwifruit) have been recognized officially: Act c 5, Act c 8, and Act c 10.

Table 18.1 Kiwifruit allergens

Allergen	Accession number (UniProt or GenBank)	SDS-PAGE (kDa)	Protein family/ biochemical designation	Cross-reactivity to birch and grass pollen and latex
<i>A. deliciosa</i>				
Act d 1	P00785	27	Actinidin	Unknown
Act d 2	Q5ND92	24	Thaumatin-like protein	Unknown
Act d 3	P85063	42	Glycoprotein of unknown function	Birch and grass pollen, latex
Act d 4	Q6TPK4	11	Phytocystatin	Unknown
Act d 5	P84527	26	Kiwellin	Unknown
Act d 6	P83326		Pectin methylesterase inhibitor	Unknown
Act d 7	P85076	35	Pectin methylesterase	Birch and grass pollen, latex
Act d 8	D1YSM5	17	Pathogenesis- related protein 10 family	Birch pollen
Act d 9	EMBL/ GenBank FG438715	14	Profilin	Birch and grass pollen, latex
Act d 10	P85205	9	Nonspecific lipid-transfer protein	Unknown
Act d 11	P85524	17	Major latex protein	Birch pollen
<i>A. chinensis</i>				
Act c 5	P85261	26	Kiwellin	Unknown
Act c 8	D1YSM4	17	Pathogenesis- related protein 10 family	Birch pollen
Act c10	P85204	9	Nonspecific lipid-transfer protein	Unknown

3.1. Act d 1 (actinidin)

Actinidin was the first green kiwifruit allergen to be identified and assigned the official designation Act c 1 by the WHO/IUIS Allergen Nomenclature Subcommittee (Fahlbusch et al., 1998; Pastorello et al., 1998). However, since 1986, the accepted botanical names of kiwifruit species are *A. deliciosa* for stiff-haired variants (which include the 'Hayward' cultivar) and *A. chinensis* for soft-haired variants (which include the 'Hort 16A' cultivar) (Liang & Ferguson, 1986). Thus, the IUIS Allergen Nomenclature Subcommittee has corrected the kiwifruit allergen nomenclature and Act c 1 has been renamed to Act d 1. In papers published before 2004, the allergen names such as Act c actually refer to proteins from *A. deliciosa*.

Act d 1 is well established as a major kiwifruit allergen and causes sensitization ranging from 19% to 100% of subjects with kiwifruit allergy, depending on the population group studied (Gavrovic-Jankulovic, Cirkovic, Burazer, et al., 2002; Möller, Kayma, Steinhart, & Paschke, 1997; Pastorello et al., 1996). Act d 1 had been proposed as a marker for genuine sensitization to kiwifruit as it was more frequently recognized by patients without pollen sensitization (Bublin et al., 2011, 2010; Palacin et al., 2008). In addition, it has emerged that only Act d 1 was associated with severity of symptoms: patients with severe symptoms were more commonly sensitized to Act d 1 compared to patients with only mild symptoms. The stability of Act d 1 regarding thermal processing and gastrointestinal digestion might explain the association with severe symptoms. Even though actinidin undergoes irreversible unfolding when heated, it preserves IgE reactivity *in vitro* (Bublin et al., 2008). One out of 20 children, with a history of allergic reactions to fresh kiwifruit, also reacted to cooked kiwifruit (Fiocchi et al., 2004).

Stability of Act d 1 to gastrointestinal enzymes was assessed using an *in vitro* digestion system that approximates physiological conditions with respect to the passage of food through the stomach into the duodenum. Act d 1 precipitated in simulated gastric fluid at pH 2 and digestion of the aggregated protein proceeded slowly. The residual precipitate redissolved completely in simulated duodenal fluid at pH 6.5 and was partially digested, implying that Act d 1 acts to sensitize individuals via the gastrointestinal tract (Bublin et al., 2008).

Actinidin belongs to a large, ubiquitous protein family, the papain-like cysteine proteases also designated as protease family C1A (Barrett & Rawlings, 2001; Berti & Storer, 1995). This family comprises similar proteins from

other plants, for example, papain from papaya, ficin from fig, and bromelain from pineapple, as well as group I house dust mite allergens homologous to Der p 1 and the mammalian lysosomal proteases cathepsin B and cathepsin L. There is evidence that the protease activity of papain and Der p 1 is important in their allergenicity. Der p 1 can cleave human IgE receptor, CD23, and thereby ablate the feedback mechanism that normally regulates IgE synthesis (Hewitt, Brown, Hart, & Pritchard, 1995). Papain potentially induces human eosinophils to degranulate and to produce superoxide anion. It is likely that human eosinophils are equipped with machineries that recognize and respond to cysteine proteases, such as those found at allergic inflammation sites (Miike & Kita, 2003). This may also be true of actinidin.

3.1.1 Cross-reactivity

Cross-reactivity among cysteine proteases from plants may be in part responsible for the clinical correlation of allergy to *F. benjamina*, figs, and other fruits including kiwifruit (Diez-Gomez et al., 1998). Moreover, cross-reactivity between cysteine proteases from house dust mites and plants was shown by ELISA inhibition experiments with an IgE-inhibiting anti-Der p 1 antibody as well as by comparative molecular modeling (Furmonaviciene, Sewell, & Shakib, 2000; Greene & Thomas, 1992). However, the clinical relevance of these *in vitro* data is yet to be proved. Experiments showing cross-reactivity between papain and the important *Hevea* latex allergen Hev b 1, a protein without sequence homology to cysteine proteases, suggest a contribution of these proteins to the latex-fruit syndrome (Baur, Chen, Rozynek, Duser, & Raulf-Heimsoth, 1995).

3.2. Act d 2 (thaumatin-like protein)

A thaumatin-like protein (TLP) from kiwifruit was first isolated in 1999 (Wurms, Greenwood, Sharrock, & Long, 1999) and later characterized as a major kiwifruit allergen and received the official designation Act d 2 (Gavrovic-Jankulovic, Cirkovic, Vuckovic, et al., 2002). Sensitization rates for Act d 2 of 8–88% have been described (Bublin et al., 2011; Gavrovic-Jankulovic, Cirkovic, Vuckovic, et al., 2002; Palacin et al., 2008).

TLPs have been characterized as allergens from apple, sweet cherry, bell pepper, and grape, but also in pollen from the mountain cedar (*Juniperus ashei*) and *Cupressus arizonica* pollen (reviewed in Breiteneder, 2004).

The family of TLPs, also designated as PR-5, is one of the 17 plant families classified as pathogenesis-related proteins (van Loon, Rep, & Pieterse, 2006). Act d 2 possesses antifungal activity against *Botrytis cinerea* and some suppressive effects on *Mycosphaerella arachidicola*, *Coprinus comatus*, *Saccharomyces carlsbergensis*, and *Candida albicans* (Gavrovic-Jankulovic, Cirkovic, Vuckovic, et al., 2002; Wang & Ng, 2002). TLP from kiwifruit did not appear to be directly toxic to *Cryptosporiopsis actinidiae* and *Phomopsis* spp., the causal agents of ripe rots of gold kiwifruit, but it may be useful as a marker of resistance, given the temporal correlation between decreased TLP and increased ripe rots (Wurms et al., 2011).

The majority of PR-5 proteins have molecular masses of ~ 24 kDa, mainly consist of antiparallel β -sheets, and are stabilized by eight disulfide bonds (reviewed in van Loon et al., 2006). This highly stabilized structure can profoundly affect the processing and stability of allergens (Bublin et al., 2008; Polovic et al., 2007). Thermal denaturation under acidic conditions (such as those found in juice) reversibly unfolded TLP from kiwifruit. Consequently, immunoblotting of extracts prepared from kiwifruit jam and whey showed that Act d 2 was not removed and its allergenic activity was not decreased by technological treatments. Moreover, 40% of Act d 2 remained intact during gastric digestion and was cleaved by duodenal proteases into large fragments covalently linked by disulfide bonds. The digested allergen sample displayed nearly unchanged IgE-binding ability (Bublin et al., 2008). The stability of Act d 2 provides one explanation for its allergenic potency.

3.2.1 Cross-reactivity

No cross-reactivity between Act d 2 and other TLPs from fruits or *Cupressaceae* pollen has been described. The wide range of sequence similarities between Act d 2 and other allergenic TLPs may give rise to highly species-specific patterns of IgE cross-reactivity. The sequence of the cDNA (accession number AJ871175) encoding a kiwifruit TLP contained an open reading frame of 603 base pairs coding for a protein of 201 amino acid residues with a molecular weight of 21.6 kDa and with a calculated isoelectric point of 7.9. Sequence alignments with allergenic TLPs from other fruits showed identities of 35% with Mal d 2 from apple, 38% with the major allergen Pruv av 2 from cherry, 71% with Cap a 1 from bell pepper, and 76% with an osmotin-like protein from grape.

3.3. Act d 3

Act d 3, a 43-kDa glycoallergen, was recognized *in vitro* by 60% (Palacin et al., 2008) and 33% (Bublin et al., 2010) of sera from kiwifruit-allergic patients, indicating that Act d 3 should be considered an important kiwifruit allergen. Fifty percent of the atopic control subjects, however, were also sensitized to Act d 3, indicating that IgE from control subjects with pollen or pollen/latex allergy recognize cross-reactive carbohydrate determinates on Act d 3. Thus, IgE binding to highly cross-reactive Act d 3 is not a clinically specific marker for kiwifruit allergy in the presence of pollen or pollen/latex sensitization (Bublin et al., 2010). The protein was identified by N-terminal sequencings (accession no. P85063) as a glycoprotein with a Pfam profile PF04862 but with unknown function. Carbohydrate analysis of the allergen showed the existence of glycans carrying fucosyl and xylosyl residues, structures previously shown to bind IgE (Palacin et al., 2008).

3.4. Act d 4 (phytocystatin)

Allergens belonging to the phytocystatins are found in green kiwifruit (*A. deliciosa*) and gold kiwifruit (*A. chinensis*). The allergen from green kiwifruit designated as Act d 4 is a minor allergen recognized by 2–20% sera from kiwifruit-allergic patients (Bublin et al., 2011, 2010). It is a first evidence of the involvement of this plant protein family in food allergy. A cystatin from short ragweed (*Ambrosia artemisiifolia*) (Rogers, Pollock, Klapper, & Griffith, 1993), a cat cystatin (Fel d 3), and a cystatin from herring worm (*Anisakis simplex*) have also been described as allergens (Ichikawa, Vailes, Pomes, & Chapman, 2001).

Phytocystatins are cysteine proteinase inhibitors that are involved in plant defense mechanisms against fungi and insects as well as in controlling germination by means of inhibition of endogenous cysteine proteases (Arai, Matsumoto, Emori, & Abe, 2002). Consequently, there has been increasing interest in constructing transgenic plants, expressing phytocystatins, which may confer resistance to a variety of pathogens (Soares-Costa, Beltrami, Thiemann, & Henrique-Silva, 2002; Urwin, Lilley, McPherson, & Atkinson, 1997). The expression of these new proteins causes concern about the resulting allergenic potential of the genetically modified foods. Assessment of potential allergenicity of genetically engineered foods should start with evaluating amino acid sequence similarities to known allergens (Goodman et al., 2008). Therefore, the identification of novel allergens assists the

assessment of the allergenicity of products of biotechnology, which is important in the novel food age.

3.5. Act d 5 (kiwellin)

Kiwellin, Act d 5, is a modular cysteine-rich protein with two domains belonging to the expansin, N-terminal domain family. Serological tests showed that Act d 5 is recognized by 3–20% of kiwifruit-allergic patients (Bublin et al., 2011, 2010) and skin tests showed reactivity to Act d 5 in 38% of the subjects (Bernardi et al., 2010). The Act d 5 may undergo pH- and polarity-driven conformational changes that may affect the pattern of exposed IgE epitopes. Some of the tested subjects had a positive reaction only when Act d 5 was applied to the skin in acidic solution (Bernardi et al., 2010). Kiwellin, Act c 5, has also been identified as the most abundant protein in gold kiwifruit showing similar IgE-binding capacity to that of the green species (Tuppo et al., 2008).

Kiwellin has a molecular weight of 20 kDa calculated on the basis of the amino acid sequence (UniProt: P84527), although in SDS-PAGE it shows a band at 26–28 kDa. It was shown that kiwellin undergoes endoproteolytic processing by actinidin, which results in the production of a 4-kDa peptide named kissper and a 16-kDa C-terminal fragment named KiTH. When tested with sera recognizing kiwellin, KiTH showed similar IgE binding to that of kiwellin. KiTH and kissper were not detected in gold kiwifruit (*A. chinensis* ‘Hort 16A’) protein extract, possibly due to the very low amount of actinidin present in this cultivar (Tuppo et al., 2008).

3.6. Act d 6 (pectin methylesterase inhibitor)

A further kiwifruit allergen included in the official list of allergens is the pectin methylesterase inhibitor Act d 6. Its importance in kiwifruit allergy is currently unknown. Act d 6 immobilized on the microarray was not recognized by IgE or by IgG4 from any tested serum from kiwifruit-allergic patients (Bublin et al., 2011), although a prevalence of 72% was reported when the sera of subjects with a convincing history of allergy to kiwifruit were tested by IgE immunoblotting (<http://www.allergen.org>). A member of pectin methylesterase inhibitor family has also been described as the major plane tree (*Platanus acerifolia*) pollen allergen, Pla a 1 (Asturias, Ibarrola, Eraso, Arilla, & Martinez, 2003).

3.7. Act d 7 (pectin methylesterase)

Act d 7 causes sensitization of 22% of patients with kiwifruit allergy (Bublin et al., 2011). All patients' sera positive to Act d 7 were also positive to Api g 5, a glycoallergen from celery, and Ana c 2, bromelain from pineapple, which are known as allergens containing cross-reactive carbohydrate determinants (Altmann, 2007). Members of the pectin methylesterase family have also been described as allergens in pollen from Russian thistle (*Salsola kali*) and olive (*Olea europea*).

3.8. Act d 8 (pathogenesis-related protein PR-10)

Act d 8 belongs to group 10 of the pathogenesis-related proteins (PR-10). Primary sensitization to Act d 8 predominantly occurs via Bet v 1, the major birch pollen allergen, resulting in cross-reactivity of IgE to Act d 8 affecting about 40% of kiwifruit-allergic patients. The Bet v 1 homologue from gold kiwifruit is termed as Act c 8. The amino acid sequence identity between Act d 8 and Act c 8 is 70%. Act d 8 and Act c 8 showed 53% and 54% identity with Bet v 1 (Oberhuber et al., 2008). Act d 8 is highly labile to denaturation and to proteolytic degradation by endogenous enzymes contributing to the low diagnostic sensitivity of the kiwifruit protein extracts (Bublin et al., 2010).

3.9. Act d 9 (profilin)

Profilins are ubiquitous proteins with molecular mass between 13 and 15 kDa responsible for allergic cross-reactivity among a wide variety of unrelated plants (Radauer et al., 2006). The kiwifruit profilin, Act d 9, is involved in pollen-related kiwifruit allergy with a sensitization rate of 18–20% (Bublin et al., 2011, 2010).

3.10. Act d 10 (nonspecific lipid-transfer protein)

The nonspecific lipid-transfer proteins (nsLTPs), Act d 10 and Act c 10, have been identified in both green and gold kiwifruit. IgE reactivity to the two proteins has been observed, but no clinical relevance for a subset of kiwifruit-allergic patients tested in the study could be assured (Bernardi et al., 2011). Nonspecific LTPs are ubiquitous proteins of 8–10 kDa and represent important allergens in fruits and vegetables mainly in the Mediterranean population. In addition to being allergens in plant-derived foods such as stone-fruits, apples, or nuts, LTP have been shown to constitute important allergens in pollen. However, at present, there is no evidence to suggest a

clinical association between sensitization to plant food and pollen nsLTPs (Egger, Hauser, Mari, Ferreira, & Gadermaier, 2011).

3.11. Act d 11 (major latex protein)

Act d 11 is a member of the major latex protein/ripening-related protein family, which belongs to the Bet v 1-superfamily (Radauer & Breiteneder, 2007). The 17-kDa allergen is a minor allergen, recognized by IgE of 22% of kiwifruit-allergic patients. Act d 11 may have a clinical relevance for subjects sensitized to major birch pollen allergen, Bet v 1, and Bet v 1-related allergens in other pollen and fruits (D'Avino et al., 2011). The amount of Act d 11 in protein extracts of *A. deliciosa* is influenced by natural ripening and post-harvest treatments, including the exposure to the plant hormone ethylene (Ciardiello et al., 2009).



4. ALLERGENICITY OF DIFFERENT KIWIFRUIT CULTIVARS

There is only limited information about the differences of kiwifruit varieties with respect to allergen composition. A difference in protein and allergen pattern has been demonstrated between green-fleshed kiwifruit cultivar and gold kiwifruit. The most striking differences between the two cultivars were the lack of actinidin in gold kiwifruit and the higher amounts of protein with molecular weights above 30 kDa extracted from this cultivar (Bublin et al., 2004). The distinctive protein composition of green and gold kiwifruits was reproduced by the results of IgE immunoblots that showed allergens common for both cultivars and species-specific IgE-binding proteins. The distinct allergen profiles of the two cultivars give rise to regionally divergent IgE recognition patterns. Whereas sera of patients from Central Europe mainly recognized green kiwifruit and actinidin (Act d 1), almost all sera from a Mediterranean array showed IgE binding to both kiwifruit cultivars, but only half of them contained Act d 1-specific IgE. This difference in IgE reactivity may originate from regionally distinct pollen sensitization patterns of the patients. Birch and grass pollen are the major pollen species in Central Europe, while cypress, olive, and *Parietaria* pollen are the most important causes of pollinosis in South Europe (Bublin et al., 2004).

However, despite having different protein and allergen profiles, the two species were shown to be highly cross-reactive (Bublin et al., 2004; Lucas et al., 2005). *In vivo* reactivity of gold kiwifruit (*A. chinensis* cv. 'Hort 16A')

in five patients showed that patients allergic to green kiwifruit are also at risk of reacting to gold kiwifruit (Lucas et al., 2005).

Some kiwifruit-allergic individuals may suffer allergic reactions if they consume raw hardy kiwifruit (*Actinidia arguta*), but heat processing of the kiwifruit significantly reduced *in vitro* IgE binding (Chen et al., 2006).

Differences in allergenicity were found by *in vivo* testing with six kiwifruit cultivars from three different species (*A. deliciosa* cv ‘Hayward’ and cv ‘Summer 3373’, *A. chinensis* cv Zespri Gold™ ‘Hort 16A’ and cv ‘Jintao’, and *Actinida. eriantha* cv ‘Eriantha 96’ and cv ‘Eriantha 114’). The cultivars could be classified into groups with low (Zespri Gold™), moderate (Summer, Jintao), and high allergenicity (Eriantha 96/114 and Hayward), with a significant difference between the high and low allergenicity cultivars. Differences in allergenicity between Zespri Gold™ and Summer versus Hayward were confirmed by a food challenge test (Le et al., 2011). Nevertheless, differences in allergenicity between kiwifruit cultivars must be interpreted with some caution, because reactivity to cultivars can differ among subjects.



5. CONCLUSION

Further characterization of kiwifruit allergens at a molecular level, together with an increased understanding of immune mechanisms underlying the allergenic response to kiwifruit, should lead to improved diagnostic and therapeutic approaches to kiwifruit allergy.

Most kiwifruit-allergic patients avoid eating the fruit, abstaining themselves from a food source of high nutritive and health value. Therefore, allergy sufferers may benefit from the selection and breeding of low-allergenic kiwifruit cultivars.

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