

Comparison of the allergenicity of *Actinidia deliciosa* (kiwi fruit) and *Actinidia chinensis* (gold kiwi)

Lucas JSA, Lewis SA, Trewin JB, Grimshaw KEC, Warner JO, Hourihane JO'B. Comparison of the allergenicity of *Actinidia deliciosa* (kiwi fruit) and *Actinidia chinensis* (gold kiwi).

Pediatr Allergy Immunol 2005; 16: 647–654. © 2005 Blackwell Munksgaard

Actinidia chinensis (gold kiwi) is a newly available fruit which has been shown to have *in vitro* immunoglobulin E (IgE) cross-reactivity with green kiwi. This is the first study to investigate clinical reactivity of gold kiwi. Five patients clinically allergic to green kiwi were investigated by skin test and double-blind placebo controlled food challenge (DBPCFC) with gold kiwi fruit. IgE-binding patterns of individual sera from the five challenged patients and a pool of sera from a further nine patients with kiwi allergy were compared in the two fruits by Western blotting. Cross reactivity of proteins in the two fruits was assessed by inhibition of immunoblots and by IgE enzyme-linked immunosorbent assay (ELISA) inhibition. Four of the five patients had a positive DBPCFC to gold kiwi. Western blotting showed marked differences in the allergen patterns of green and gold kiwi. However, inhibition of the immunoblots and ELISA assay reveals extensive inhibition of IgE binding to proteins in each fruit by the alternative species. Gold kiwi fruit is allergenic and patients allergic to green kiwi are at risk of reacting to the gold kiwi fruit. Despite having different protein profiles and IgE-binding patterns, the two species have proteins that extensively cross-inhibit the binding to IgE.

Jane S. A. Lucas¹, Stella A. Lewis¹, Jennifer B. Trewin², Kate E. C. Grimshaw^{1,2}, John O. Warner² and Jonathan O'B. Hourihane^{1,2}

¹Allergy and Inflammation Sciences, University of Southampton, ²Wellcome Trust Clinical Research Facility, Southampton University NHS Trust, Southampton, UK

Key words: *Actinidia*; kiwi fruit; allergens; double blind food challenge; immunoglobulin E; skin tests; sodium dodecylsulphate-polyacrylamide gel electrophoresis

Jane Lucas, University Child Health (MP 803), Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK
Tel.: +44 238 0796 867
Fax: +44 238 0796 378
E-mail: jlucas1@soton.ac.uk

Accepted 29 June 2005

The genus *Actinidia* contains about 60 species (1), but until recently only one has been eaten regularly in the Western world. Green kiwi fruit, *Actinidia deliciosa*, originated in China, but has been cultivated in New Zealand since 1904 (1). It has become a common food throughout the world over the last 30 yr, and is now grown in many countries. *Actinidia chinensis* is a species very similar to *A. deliciosa* and until 20 yr ago they were classified in the one species. However, there are distinct differences between the two. *Actinidia chinensis* fruit is almost hairless and the flesh ranges from a lime green colour to bright yellow. The fruit are generally sweeter than *A. deliciosa*. *Actinidia chinensis* seeds were taken from China to New Zealand in 1977 for commercial development. Fruit from a mother plant

with yellow flesh was considered particularly suitable for commercial development, and is now grown and marketed under the name ZespriTM Gold (Zespri, Bay of Plenty, New Zealand). Exports were first made to USA and Europe in 2000, and this novel food is increasingly available in many parts of the world. Net sales of New Zealand ZespriTM Gold have rapidly increased from NZ 95.7 million in 2001–02, to NZ 175.6 in 2003–04 (Zespri Group Limited Annual Report 2003–04).

Allergenicity can vary considerably between different varieties of the same species. For example, differences in allergenicity have been confirmed between varieties of apple (2), olive pollen (3), sesame seed (4) and dates (5). The allergic potential of each novel food species or

variety therefore needs assessment. This would appear particularly important in the case of different species, which are likely to present different allergens.

Allergy to kiwi fruit (*A. deliciosa*) is well recognized. Although symptoms may be mild, there is a spectrum of reactivity [reviewed in Lucas et al. (6)] and severe anaphylactic reactions are not uncommon, particularly in infants and young children (7, 8). A recent study using sera from patients with reported kiwi allergy has confirmed *in vitro* immunoglobulin E (IgE) cross-reactivity of proteins in green and gold fruit (9), but to date there have been no studies confirming clinical reactivity to gold kiwi.

The aims of this study were to identify and compare the allergenicity and immunogenicity of *A. deliciosa* and *A. chinensis*. This is the first report of allergy to gold kiwi confirmed by double-blind placebo controlled food challenge (DBPCFC).

Method

The Southampton and South West Hants Joint Research Ethics Committee approved the study.

Clinical investigations

Five patients with allergy to green kiwi fruit confirmed by DBPCFC (8) were investigated by skin test and DBPCFC to gold kiwi fruit. They included adults and children with systemic and isolated oral symptoms (Table 1). Peeled, pureed pulp of gold kiwi fruit, including crushed seeds, was masked in yoghurt containing a mix of orange pulp, tea leaves and liquid food colouring. Three placebo doses were randomly interspersed with four active incremental doses (2.5, 10, 20 and 60 g) and cumulative doses (2.5, 12.5, 32.5 and 92.5 g) at intervals of 30 min. An average green or gold kiwi fruit weigh approximately 60 g. Challenge doses were freshly prepared. Prior to performing the challenges on patients, a panel of non-allergic patients confirmed that the active and placebo mixtures were indistinguishable in all ways, including texture, taste, smell and appearance. A challenge was considered positive if the patient developed objective signs, or if they had symptoms that corresponded appropriately to all active and placebo doses. All DBPCFC took place in the Wellcome Trust Clinical Research Facility, which is fully equipped for physiological monitoring and resuscitation. All patients were well on the day of the challenge.

Table 1. Characteristics and clinical investigations results of the patients who underwent double-blind placebo controlled food challenges

Volunteer	Age (yr)	Reported reactions to green	Other allergies (symptoms and SPT)	Green SPT (mm)	Gold SPT (mm)	Specific immunoglobulin E Green	Green challenge	Gold challenge	Binding bands to green (kDa)	Binding bands to gold (kDa)
1	10	Pharyngeal oedema, urticaria, angioedema	BP/GP/cat HDM	7	3	3.6	Lip swelling, urticaria	Oral tingling and soreness	30, 38	40, 42, 60
2	10	GI, facial and oral swelling	BP/peanut/HDM	8	8	2.38	Lip swelling, abdominal pain	Lip swelling, abdominal pain	24, 30, 38, 42	40, 42, 45, 60
3	9	Rash, GI	Peanut/walnut	7	0	1.9	Rash and abdominal pain	Negative	38	8, 20, 34
4	52	OAS	Nil	13	12.5	6.8	Intense oral itching	Intensity of itching milder	30, 38	8, 20, 34
5	44	OAS	Nil	6	2	0.5	Intense oral itching	Intensity of itching milder	38	8, 20, 34

Symptoms were as reported by the patient during a reaction in the community. All allergies to allergens other than fruit were confirmed by SPT; patients who reported seasonal rhinitis were skin tested to a mix of grass pollens and birch pollen.

BP, birch pollen; TP, tree pollen; HDM, house dust mite; GI, abdominal pain plus vomiting and/or diarrhoea; OAS, oral allergy syndrome; SPT, skin prick test.

Prick-to-prick skin testing was performed using fresh pulp of kiwi on the volar aspect of the distal forearm. The fruit was cut, and the lancet was pricked into the pulp and then immediately afterwards into the skin at 90°. Patients were also skin tested with a panel of common allergens (birch pollen, latex, house dust mite, egg, milk – all ALK-Abellö), with fresh foods known to cross-react with kiwi fruit (avocado, apple and banana) and with any allergen of clinical relevance to the individual patient. A negative control of saline 0.9% and a positive control of histamine 10 mg/ml were used. The reaction was regarded as positive if the mean wheal diameter was at least 3 mm in the presence of appropriate reactions to the positive and negative controls. Serum was tested for specific IgE to kiwi fruit (f84) using the (Fluorescent enzyme immunoassay) FEIA-CAP system (Pharmacia, Uppsala, Sweden) in the hospital immunology laboratory. A pool of sera for Western blotting and immunoblots and enzyme-linked immunosorbent assay (ELISA) inhibition studies, was produced from nine patients with a history supportive of IgE mediated allergy to green kiwi fruit and measurable specific IgE to kiwi (Table 2). These patients had declined to have a food challenge or had been unsuitable to challenge because of severity of symptoms or distance from the research centre. They included adults and children, and their symptoms ranged from anaphylaxis to oral allergy syndrome.

Preparation of kiwi fruit extracts

Three published methods for extraction of kiwi fruit proteins (10–12) were compared. The extraction methods varied very little in terms of protein yield, but Pastorello's method (12) was found to provide the most comprehensive pattern of *A. deliciosa* proteins, and was therefore adapted as the extraction method for this study.

Extracts of kiwi fruit protein were made from fresh green and gold kiwi fruit. Fresh fruit was peeled and pureed during which some of the seeds were crushed. The pulp was homogenized in 1:2 (w/v) in phosphate-buffered saline (PBS) pH 7.4, and stored on ice whilst vortexing every 15 min for 2 h. The resulting homogenate was centrifuged (4000 g, 30 min, 4°C), and the supernatant dialysed at 4°C. The specimen was frozen at –80°C and then lyophilized. The resulting extract was stored at –80°C until required.

SDS-PAGE

Size fractionation of the kiwi proteins was performed by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, to determine the protein profiles of the two extracts. Extract containing 25 µg of protein was loaded in each well of a NuPAGE™ (Invitrogen, Paisley, UK) 12% Bis-Tris Gel. Reference markers with known molecular weights (Magic Marker; Invitrogen, Paisley, UK) were run on the same gel in a XCell Surelock™ Mini-Cell (Invitrogen) for 45 min at 200 V constant using MOPs running buffer. The gel was stained with Coomassie stain (EZBlue; Sigma, Gillingham, UK).

Protein analysis

For 2-D gel electrophoresis, green and gold extracts were cleaned using 2-D Clean-Up Kit (Amersham Biosciences, Chalfont St. Giles, UK), and were resuspended in rehydration buffer (8 M urea, 2% CHAPS, 0.5% (v/v) ZOOM® Carrier Ampholytes (Invitrogen, Paisley, UK), 0.002% bromophenol blue, DTT 0.5 M). A 400 µg (for MS analysis) or 200 µg (for staining) of the protein extracts were incubated with a 3–10 pH gradient strip (ZOOM® Strip; Invitrogen) for 16 h at room temperature. Isoelectric focusing was performed using a ZOOM®IPG-

Table 2. Characteristics of subjects in the kiwi sera pool

Age (yr)	Kiwi allergy symptoms	Other allergies	Specific immunoglobulin E kiwi
12	Throat swelling, difficulty breathing, tingling mouth	Peanut, tree nut, tree pollen, grass pollen, house dust mite	0.6
12	Wheeze, tight throat, swelling of mouth, angioedema, rash	Nil	0.7
20	Anaphylaxis	Banana, latex, grass pollen, dog	4.6
35	Wheeze, rash, angioedema, tight throat	Peanut, tree nuts, egg, shell-fish, tree pollen, grass pollen	3.9
58	Tingling mouth, throat tightening, vomiting	Nil	0.6
63	Tingling sore throat, difficulty swallowing	House dust mite, latex	2.4
68	Tingling mouth, throat tightening	Nil	2.4
69	Tight throat, throat burning, vomiting	Nil	2.1
71	Tingling mouth	Nil	2.6

Runner™ (Invitrogen, Paisley, UK) in a Mini Cell using a step voltage protocol (150 V for 20 min, 300 V for 15 min, 600 V for 15 min, 2000 V for 7 h). The strip was incubated for 15 min with NuPAGE LDS sample buffer and reducing agent followed by 15-min incubation with alkylating solution. The 2-D electrophoresis was performed at 200 V for 40 min using a NuPAGE Novex Bis-Tris ZOOM (Invitrogen, Paisley, UK) Gel (4–12%). Proteins selected for mass spectrometry (MS) analysis were excised from the 2D gel. MS analysis was performed at the Centre for Proteomic Research, University of Southampton. Matrix-assisted laser desorption ionization time of flight MS was used to measure the masses of the peptides derived from the enzymatic digestion (trypsin) of the gel-separated proteins. Peptide mass fingerprinting was used in an attempt to identify proteins, but no positive matches were identified. Electrospray ionization (ESI) NanoLC tandem MS was then used to obtain fragmentation data from each peptide and *de novo* sequence information obtained. Sequence homology between proteins in the two fruits was sought.

Western blotting

Numerous blotting optimization studies were conducted in an attempt to eliminate the non-specific binding at approximately 20 kDa in both green and gold extracts, and a band at approximately 38 kDa in the gold extract. These bands were present when the protein strips were incubated with sera from non-atopic patients or without sera. We studied seven different secondary antibodies over a range of concentrations, and many blocking conditions and incubation times. These non-specific binding sites were detected during all optimization studies where sensitivity to detect actinidin and other kiwi allergens was maintained. Corresponding bands in kiwi allergic patients were therefore not considered significant.

Our final protocol was as follows: 10 µg per lane of kiwi proteins were separated with pre-stained molecular markers (SeeBlue Plus2; Invitrogen) by 1D SDS-PAGE under reducing conditions using the above protocol. The extracts were transferred onto polyvinylidene fluoride (PVDF) membranes (0.2 µm pore; Invitrogen) at 30 V for 1 h using XCell II blotting apparatus (Invitrogen). Membranes were blocked in 3% bovine serum albumin (BSA) in Tris-buffered saline (TBS) with 0.1% Tween-20 for 1 h at room temperature. IgE binding to kiwi fruit proteins was determined by incubating strips of the separ-

ated extracts in a pool of sera from nine patients with green kiwi fruit allergy and individual sera from a further five patients who had undergone DBPCFC to green and gold fruit. Sera were diluted 1 in 10, in a diluent of 0.3% BSA in TBS with 0.1% Tween-20, and the strips were incubated at room temperature overnight. Membranes were then incubated in 1:500 biotin-labelled goat anti-human IgE (Vector BA 3040, Peterborough, UK) followed by 1:120,000 AP-linked extravidin (Sigma) each for 45 min. Following each incubation, membranes were washed twice with TBS-containing 0.05% Tween-20 and twice in TBS alone. IgE-binding sites were identified by developing with BCIP/NBT for 15 min.

Immunoblotting inhibition

Green kiwi proteins were blotted onto a PVDF membrane, and blocked as described above. 900 µl of doubling dilutions (0.1–0.00313 mg/ml) of gold kiwi extract were incubated with 100 µl of the pooled sera for 1 h. A volume of 100 µl of TBS without additives, and 100 µl of 0.1 mg/ml BSA in TBS were incubated with the sera as controls. Strips of the PVDF were then incubated with each of the mixtures overnight. Immunodetection of IgE binding was performed as previously described. The procedure was repeated by incubating the sera with extract of green kiwi fruit.

Gold kiwi extract proteins were similarly separated, and immunoblotting performed using the pooled sera incubated with green kiwi extract and gold kiwi extract. The competitive inhibition of gold kiwi was only at a 'maximal blocking dose' of 0.1 mg/ml of extract, because of limited sera. Individual sera (100 µl) from the 5 patients who had had oral challenges was incubated with 900 µl of 0.1 mg/ml of the green and the gold extracts, before incubating with PVDF strips of green and gold extracts.

ELISA inhibition

A Corning half area 96-well plate was coated with green kiwi extract 100 µg/ml in carbonate-bicarbonate buffer (50 µl/well), and incubated overnight at 4°C. A second plate was coated with BSA (100 µg/ml; 50 µl/well) as a background control. Plates were washed with PBS and blocked with BSA 3% in PBS (150 µl/well) for 2 h at room temperature. After washing with PBS, 50 µl of patient (green kiwi allergic pool) and control sera diluted with 1% BSA in T-PBS were added to the wells. For the standard curves sera were serially diluted (1 in 5; 1 in 10; 1 in 20; 1

in 40; 1 in 80; 1 in 160). Inhibition experiments were conducted by pre-incubation of pool sera (1 in 10) with serial dilutions of the inhibitor in 1% BSA and TPBS (inhibitor concentrations: 0.2, 0.1, 0.025, 0.00625, 0.00156 and 0.0004 mg/ml). The inhibition mixtures (including sera with no inhibitor as a positive control) and standard curves were dispensed in the wells (50 µl/well) and incubated at room temperature for 2 h. The plates were then incubated with anti-human IgE (Vector BA3040) 1:500; 50 µl/well, followed by horseradish peroxidase (HRP)-labelled Neutra-vidin (Pierce, Rockford, IL, USA) 1:5000; 50 µl/well. IgE binding was detected by TMB (BD UK Ltd, Cowley, UK) 50 µl/well, and the reaction stopped after 30 min with the addition of 1 M sulphuric acid 100 µl/well. The absorbance (OD) in each well was measured at 450 nm. The absorbance in wells of the control plate coated with BSA were subtracted from the absorbance in the kiwi coated wells to account for non-specific binding. Assays were performed in duplicate. Percentage inhibition was calculated using the formula:

$$\% \text{inhibition} = 100$$

$$\times \frac{(\text{OD}_{\text{of serum with no inhibitor}} - \text{OD}_{\text{of serum with inhibitor}})}{(\text{OD}_{\text{of serum with no inhibitor}})}.$$

Results

Clinical investigations

All five patients reacted adversely to green kiwi (entry requirement to study), and four of the five patients allergic to green fruit reacted adversely to gold kiwi during a DBPCFC (Table 1). A total of 2.5 mg was the lowest dose for green kiwi and gold kiwi DBPCFCs. The first dose of each challenge elicited symptoms in some patients.

Patients 4 and 5 had oral pruritus corresponding to active but not placebo doses during DBPCFC with green and gold fruit. These two patients expressed the subjective feeling that the intensity of itching was less throughout the gold kiwi challenge than the DBPCFC using green fruit (the gold kiwi challenge occurred some months after the green kiwi challenge). The green challenge of patient 1 was stopped at dose 1 (2.5 g kiwi fruit) because she developed lip swelling and facial urticaria. She completed all doses of the gold challenge (cumulative dose 92.5 g kiwi fruit) but complained of oral tingling and soreness that occurred during active, but not placebo doses. The reactions for patient 2 were identical during the two DBPCFCs. On the first

dose she developed marked lip swelling, complained of abdominal pain and became withdrawn. Patient 3 developed abdominal pain and an urticarial rash with green kiwi but completed the whole gold challenge without symptoms.

Prick-to-prick skin testing with gold kiwi correctly predicted the patient who had a negative food challenge. Two of the patients were birch pollen allergic, but no patients had latex allergy. Two of the patients had mono-allergy to kiwi.

SDS-PAGE electrophoresis

The SDS-PAGE separation of proteins provided distinctly different protein profiles in the two fruit (Fig. 1). Both extracts showed an abundant band just below 30 kDa. In the green fruit this corresponds visually on gel analysis to the protein reported as actinidin by previous studies (13). There were more bands and greater intensity of bands with a molecular mass > 30 kDa in the gold kiwi extract compared with the green kiwi extract. There were three clearly resolved protein bands in the green kiwi extract with molecular weights in the range approximately 14–21 kDa, but a poorly resolved band in the corresponding range in the gold kiwi extract.

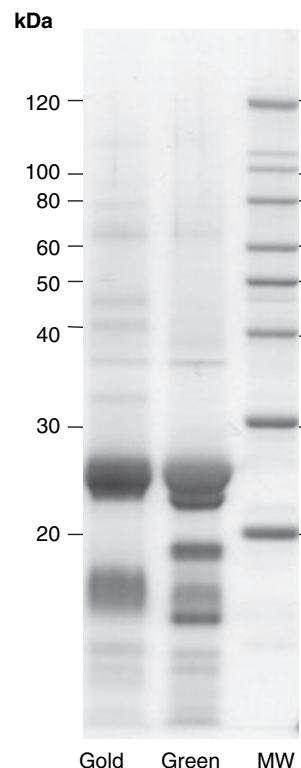


Fig. 1. Green kiwi and Zespri Gold protein extracts separated by 1D sodium dodecylsulphate-polyacrylamide gel electrophoresis on a 12% gel with MOPS running buffer. Molecular standards are in right lane.

An abundant protein in green kiwi fruit with molecular weight approximately 30 kDa and pI approximately 3 was confirmed to be the major allergen actinidin using ESI-MS and a BLAST search of the NCBnr database. There was no corresponding 30 kDa/pI 3 protein in the gold fruit. However, a less abundant protein with similar molecular weight but basic pI in gold kiwi fruit had significant homology with Act c 1 and is likely to be a basic form of actinidin.

Western blotting

The IgE-binding patterns to green and gold kiwi extracts by a pool of sera from patients with allergy to green kiwi fruit, based on clinical history and specific IgE are shown in Fig. 2, lanes KA. IgE-binding bands recognized by individual sera from patients who had DBPCFC to green and gold kiwi are shown in Fig. 2 lanes 1–5, and described in Table 1. The most common green kiwi bands recognized by the five patients were at approximately 38 and 30 kDa, and these bands were also present on the kiwi sera pool blots. Two patients had IgE-binding gold kiwi proteins of 40, 42 and 60 kDa, and three patients, including patient 3 who did not react to the gold DBPCFC, reacted to proteins of 8, 20 and 34 kDa.

Immunoblotting inhibition

Complete inhibition of IgE binding to green kiwi extract occurred when the kiwi pool was pre-incubated with gold extract at 0.05 mg/ml and

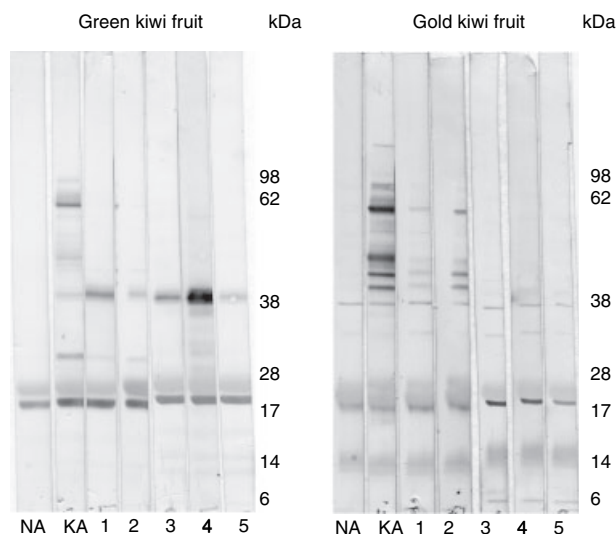


Fig. 2. Western blots using green (left) and gold kiwi fruit extract. Lanes were incubated with pooled or individual sera (NA, non-atopic pool; KA, kiwi allergic pool; volunteers, lanes 1–5 corresponding to volunteer numbers in Table 1).

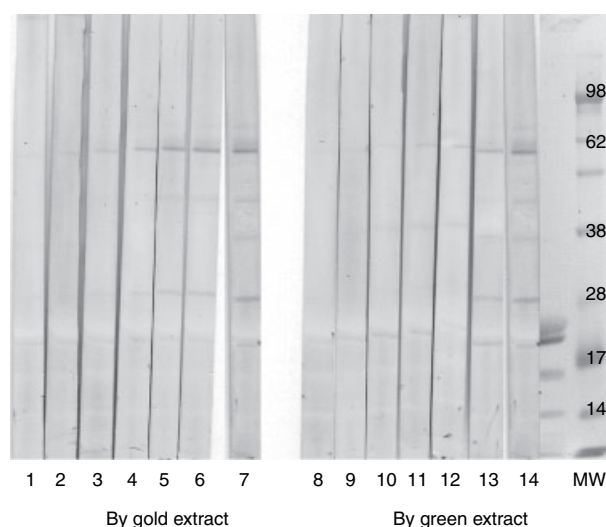


Fig. 3. Inhibition of green kiwi extract immunoblots using doubling dilutions of gold kiwi extract and green kiwi extract using pooled kiwi sera. Dilutions of inhibitors: lanes 7 and 14, no inhibitor; lanes 1 and 8, 0.1 mg/ml; lanes 2 and 9, 0.05 mg/ml; lanes 3 and 10, 0.025 mg/ml; lanes 4 and 11, 0.0125 mg/ml; lanes 5 and 12, 0.00625 mg/ml; lanes 6 and 13, 0.003125 mg/ml.

some bands were inhibited at concentrations as low as 0.00625 mg/ml (Fig. 3). There was no inhibition of IgE binding when the sera was incubated with 0.1 mg/ml of BSA, thus excluding non-specific protein binding of the IgE. IgE binding to gold kiwi extract was likewise inhibited by green extracts using pooled sera.

The IgE binding to green and gold kiwi extracts by the individuals who had had food challenges was also inhibited by green and gold kiwi extracts. However patient 4 had only partial inhibition of binding to a protein of approximately 38 kDa in green kiwi extract to which he had developed a particularly strong band on Western blot.

ELISA inhibition

The ELISA inhibition with green kiwi extract as a positive control showed strong inhibition which was maintained even at low dilutions. Inhibition with gold extract showed inhibition equivalent to green kiwi at higher dilutions of inhibitor, but the inhibition was not maintained with dilution (Fig. 4). This confirms significant cross-reactivity.

Discussion

This is the first study to confirm that *A. chinensis* is an allergen source, four of our five patients reacting during a DBPCFC. Green kiwi fruit, *A. deliciosa*, has been widely available throughout

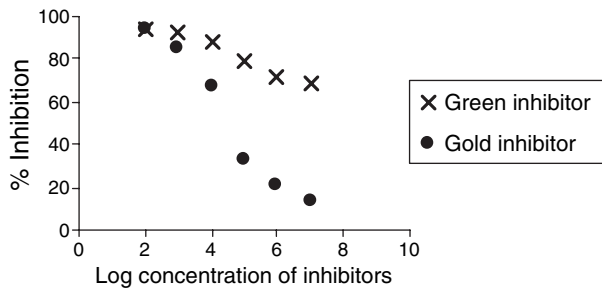


Fig. 4. Inhibition of immunoglobulin E binding to green kiwi extract in enzyme-linked immunosorbent assay by gold extract and green extract (control) in logarithmic concentrations. A pool of kiwi allergic sera was used.

the world for several decades, and allergic reactions, first reported in 1981 (14), appear to be increasingly common (8, 15). *Actinidia chinensis* marketed as gold kiwi, has only been exported from New Zealand since 1998, and imported to Europe since 2000. We have demonstrated that patients allergic to green kiwi fruit are at high risk of allergy to gold kiwi. A recent paper has demonstrated the *in vitro* immunogenicity of gold kiwi (9), but did not examine the clinical relevance. Serological cross-reactivity does not necessarily correlate with clinical allergy. Serum from patient 3 who tolerated gold kiwi showed inhibition in immunoblotting experiments. This highlights the need to confirm the results of *in vitro* work with clinical investigations.

One child reacted as severely to gold kiwi fruit as to green kiwi, but three patients had milder symptoms with the gold kiwi fruit and one patient showed no allergic symptoms. The relatively mild symptoms to gold kiwi in some patients may reflect a lower concentration of actinidin, the major allergen in green kiwi. The absence of actinidin in gold kiwi fruit has been previously described (9, 16). Selecting proteins from a 2D gel, we have used ESI NanoLC tandem MS to obtain fragmentation data for peptides and obtained *de novo* sequence information. An abundant protein in the green fruit with molecular weight approximately 30 kDa and *pI* approximately 3 was confirmed to be actinidin using a BLAST search of the NCBI database. There was no corresponding 30 kDa/*pI* 3 protein in the gold fruit. However, a much less abundant protein, with similar molecular weight but basic *pI* in gold fruit had significant sequence alignment with actinidin, and may be the same protein with post-translational modification. It has been suggested that allergen containing foods can be made safer by reducing or eliminating the responsible allergen. The fact that one patient had a systemic reaction to a low

dose of gold kiwi highlights the fact that such modifications may not render the food safe for all consumers.

Our SDS-PAGE has confirmed distinct differences between green and gold extracts as described by Bublin et al. (9), particularly the higher intensity of higher molecular weight proteins in gold extract. The Western blots suggest that IgE in sera from patients with allergy to green kiwi fruit recognize differing proteins in the two fruit, but the inhibition studies indicate that these proteins have extensive cross-reactivity. The DBPCFC results support the conclusion that the *in vitro* cross reactivity has clinical relevance.

Act c 1, actinidin, has been reported as the major allergen in green kiwi fruit (13). Act c 1 is an unglycosylated thiol protease with a mean isoelectric point of 3.5 (13). Actinidin, the most abundant protein in kiwi fruit, has a molecular weight of 30 kDa, but on the SDS-PAGE the apparent molecular weight is lower than this (Fig. 1). This finding is observed in kiwi protein SDS-PAGE by other groups (9, 11). This may reflect some fragmentation of the protein, or physical properties effecting passage through the gel.

Non-specific bands on Western blots was a problem shared by some other groups investigating kiwi allergy. One group has reported clear kiwi blots using non-atopic sera under extreme blotting conditions, blocking with 15% fetal calf sera (7), but 39% of their patients with positive DBPCFC had negative blots. Some researchers have not reported the results of control sera to exclude non-specific binding (12). Other research teams have produced clear blots but we were unable to replicate their results (11, 17). Non-specific bands were present on the Western blots of a recent publication reporting on kiwi allergy (9). Our non-specific bands were present when the blots were incubated in diluent with no sera (0.3% BSA, 0.1% Tween in TBS) excluding non-specific IgG binding as a cause. The bands were absent if no secondary antibody was incubated with the extract. Therefore non-specific bands were not caused by direct activation of the developer by peroxidase or phosphatase activity. In the optimization studies, the concentration of AP- or HRP-extravidin had little influence on the intensity of the non-specific binding. Altering the concentration of the secondary antibodies did affect the intensity of binding, but we could not find a concentration at which non-specific binding was eliminated but sensitivity for actinidin and other kiwi allergens was maintained. This suggests that the non-specific binding was due to a direct binding of the secondary antibodies to the binding sites. For the purposes of this study

we therefore ignored patient sera binding to bands that also produced non-specific binding.

To date there have not been any confirmed reports of allergy to Zespri™ Gold in the community, perhaps because individuals with kiwi allergy are successfully avoiding this fruit. It is important for consumers to be aware that the fruit is marketed as Zespri™ Gold and labels do not refer to kiwi. If the popularity of this novel food increases in a similar fashion to the traditional kiwi fruit, it is likely that reports of allergic reactions to *A. chinensis* will increase accordingly.

Acknowledgements

We are grateful for support from The Food Standards Agency (UK) who funded this study (Study Code TO7038). We would like to thank Snita Bansal and Lesley-Anne Gudgeon for co-ordination of study patients and administration of the study. We are grateful to Karen Collins and the staff of the Wellcome Trust Clinical Research Facility for assisting with clinical investigations.

Funding

This work was funded by Food Standards Agency (UK) Grant Code TO7038.

References

1. FERGUSON AR. New temperate fruits: *Actinidia chinensis* and *Actinidia deliciosa*. In: JANICK J, ed. Perspectives on New Crops and New Uses. Alexandria, VA: ASHS Press 1999: 342–7.
2. VIETHS S, JANKIEWICZ A, SCHONING B, AULEPP H. Apple allergy: the IgE-binding potency of apple strains is related to the occurrence of the 18-kDa allergen. *Allergy* 1994; 49: 262–71.
3. CARNES J, ISPANO M, SCHEINER O, FLORIDO LOPEZ JF, BONGRAND P, FERNANDEZ CALDAS E. Allergenicity and immunochemical characterization of six varieties of *Olea europaea*. *Allergy* 2002; 57: 313–8.
4. FREMONT S, ZITOUNI N, KANNY G et al. Allergenicity of some isoforms of white sesame proteins. *Clin Exp Allergy* 2002; 32: 1211–5.
5. KWAASI AA, HARFI HA, PARHAR RS, COLLISON KS, AL SEDAIRY ST, AL MOHANNA FA. Cultivar-specific IgE-epitopes in date (*Phoenix dactylifera* L.) fruit allergy. Correlation of skin test reactivity and ige-binding properties in selecting date cultivars for allergen standardization. *Int Arch Allergy Immunol* 2000; 123: 137–44.
6. LUCAS JS, LEWIS SA, HOURIHANE JO. Kiwi fruit allergy: a review. *Pediatr Allergy Immunol* 2003; 14: 420–8.
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. 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.
10. 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.
11. MOLLER M, PASCHKE D, VIELUF D, KAYMA M, VIETHS S, STEINHART H. Characterisation of allergens in kiwi fruit and detection of cross reactivities with allergens of birch pollen and related fruit allergens. *Food and Agricultural Immunology* 1997; 9: 107–21.
12. 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.
13. 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.
14. FINE AJ. Hypersensitivity reaction to kiwi fruit (Chinese gooseberry, *Actinidia chinensis*). *J Allergy Clin Immunol* 1981; 68: 235–7.
15. 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.
16. NISHIYAMA I, OOTA T. Varietal differences in actinidin concentration and protease activity in kiwifruit juice. *J Jpn Soc Food Sci Technol* 2002; 49: 401–8.
17. 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.