

Identification of the allergenic components of kiwi fruit and evaluation of their cross-reactivity with timothy and birch pollens

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Background: Only a few food allergens have as yet been identified, mainly because of the difficulty of obtaining a sufficient number of patients who are clinically sensitized to a given food. This is more feasible in the case of the oral allergy syndrome (OAS), a common form of food allergy, which is especially prevalent in patients with pollinosis.

Objective: We designed a study to identify the allergens of kiwi fruit (*Actinidia chinensis*) by analyzing the sera of patients with OAS for kiwi and to examine the cross-reactivity of these allergens with timothy and birch pollen allergens.

Methods: Twenty-seven patients with OAS for kiwi, a positive skin prick test response and serum IgE antibody to kiwi, and a positive open kiwi challenge test result and three patients who had OAS with severe systemic symptoms, which excluded a challenge test, were included in this study. The different polypeptide components of an extract of fresh kiwi were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis and analyzed by IgE immunoblotting with sera from these patients. Cross-reactivity with the two pollen extracts was assessed by inhibition of the immunoblots with pooled and individual patients' sera.

Results: Twelve IgE-binding components with molecular weights ranging from 12 to 64 kd were identified in the kiwi extract, but only a 30 kd component acted as major allergen, being recognized by sera of 100% of these patients. Inhibition of kiwi immunoblots with timothy and birch pollen extracts demonstrated strong cross-reactivity with some of the kiwi allergens, suggesting complete identity between certain food and pollen allergens; whereas others, particularly the 30 kd allergen, were only partially inhibited, suggesting much weaker cross-reactivity.

Conclusions: Kiwi fruit contains a large number of allergens widely cross-reacting with allergens in grass and birch pollen extracts. Nevertheless, the major allergen at 30 kd appears to be specific for kiwi. (*J Allergy Clin Immunol* 1996;98:601-10.)

Key words: Kiwi, oral allergy syndrome, food allergy, cross-allergenicity, immunoblotting, timothy, birch

Identification and characterization of allergens is of major importance because the information adds to our basic knowledge of allergy and leads to more reliable extracts. In contrast to the present

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Received for publication Oct. 19, 1995; revised Jan. 18, 1996; accepted for publication Jan. 30, 1996.

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0091-6749/96 \$5.00 + 0 1/1/72532

Abbreviations used

MW:	Molecular weight
OAS:	oral allergy syndrome
OD:	Optical density
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SPT:	Skin prick test

situation with numerous aeroallergens, for which many allergenic components have been identified, there are relatively few published data on food allergens.¹ This is due mainly to the difficulty of

recruiting a large number of patients sensitized to any given food item. The oral allergy syndrome (OAS), a very common clinical form of food allergy caused by fresh fruits and vegetables, appears to be a more promising source of subjects for such investigations. OAS is an immediate hypersensitivity reaction that begins within a few minutes after a specific sensitizing food comes in contact with the orolabial mucosa,² and it is frequently associated with certain pollen allergies.³ Thus it has been possible to identify the major allergens of some fruits and vegetables and to assess their cross-reactivity with pollen allergens (e.g., apple and birch pollen⁴; celery and birch and mugwort pollens).⁵⁻⁷

Kiwi, the fruit of the kiwi tree *Actinidia chinensis*, was brought onto the commercial market from New Zealand about 20 years ago and has become increasingly popular throughout the world. There have been reports of allergy to kiwi since the early 1980s,⁸⁻¹⁰ and kiwi allergy has been associated with birch pollen allergy.^{11, 12} We recently reported that kiwi often causes OAS in patients with allergic rhinitis caused by grass and/or birch pollen.¹³ This study was designed to identify the allergenic components of kiwi and to characterize cross-reactions between kiwi allergens and allergens from timothy pollen and birch pollen by using serum from patients with kiwi-related OAS.

METHODS

Subjects

Individuals who claimed to have the symptoms of OAS when they ate kiwi fruit were recruited for this study from among patients with OAS who were seen in consultation by an allergist in either the Third Division of General Medicine of the University of Milan or the Bizzozzero Division of the Niguarda Ca' Granda Hospital of Milan. To avoid the risks associated with an oral food challenge, patients who had experienced a severe systemic reaction on eating kiwi were not challenged, but they were nevertheless included as a source of serum. All the patients gave their informed consent to the study.

Potential subjects underwent skin prick testing with fresh kiwi according to the procedure described by Dreborg and Foucard,¹⁴ and their serum was assayed for IgE antibodies specific for kiwi, timothy (*Phleum pratense*) pollen, and birch (*Betula verrucosa*) pollen in the ImmunoCAP System (Pharmacia, Uppsala, Sweden). Patients with a positive skin prick test (SPT) response to kiwi (wheal diameter ≥ 3 mm) and a positive kiwi ImmunoCAP result (≥ 0.35 kU/L) underwent an open oral food challenge with fresh kiwi. The sera of patients with a positive challenge result were used in the in vitro studies described below.

Skin test

For the kiwi SPT, the fruit was cut in half and pricked with the 1 mm tip of an Allergy Pricker (DHS, Slough, U.K.), and the skin of the volar surface of the patient's forearm was then pricked with the same lancet. SPTs were also done with DHS timothy, ryegrass, Bermuda grass, and birch pollen extracts (1:20 wt/vol) by using an Allergy Pricker.

A panel of foods was also used to identify the negative control to be used in the food challenge. These foods were: apple, pear, lemon, tomato, zucchini, peanut, carrot, fennel, melon, and pea.

Open food challenges

Open food challenges were done throughout the year except in patients with a positive SPT response to birch and grass pollens, in which case the patients were tested out of the birch pollen season (February to April) or the grass pollen season (May to July). Any drug that might affect the result of the challenge test was discontinued for at least 1 week before the scheduled test. The challenge was carried out by an allergist not involved in this study. In brief, the challenge was initiated by having the patient chew a small piece of fresh kiwi (weighing about 2 gm) for about 30 seconds and then spitting it out. If no symptoms occurred within a period of 15 minutes, the test was repeated with double the dose of kiwi. Then, until intense symptoms or signs of an OAS occurred, the challenge was repeated at 15-minute intervals with doubling doses to a maximum dose of 64 gm. If the 32 gm dose did not elicit a reaction, the patient was asked to swallow this dose, and the same procedure was followed with the final dose of kiwi. The challenge test result was considered positive only if both subjective and objective evidence of an OAS occurred. Patients with a positive test result were considered to be symptomatic. If the patient did not react to the challenge or reported only itching and tingling of the lips and/or mouth but no other symptoms and the allergist observed no objective evidence of an allergic reaction, then the challenge was repeated twice on separate days before the test result was classified as negative. If, however, there was subjective evidence of an allergic reaction on both the second and third challenges, the test result was classified as positive. Patients with a negative challenge test result were considered to be free of symptoms.

All the patients also underwent a negative control challenge with a fresh fruit selected on the basis of a negative clinical history and a negative SPT response to that fresh fruit. Patients with either a doubtful kiwi food challenge result or a positive response to the control fruit challenge were not included in the subsequent part of the study.

In vitro methods

Kiwi extract. The pulp of the fresh fruit was homogenized in 1:2 wt/vol 0.1 mol/L potassium phosphate buffer (pH 7) and then shaken gently for 2 hours at 8° C. The

resulting homogenate was centrifuged at 40,000 g for 30 minutes, and the supernatant was then dialyzed for 48 hours at 4° C against 0.1 mol/L phosphate buffer. At the end of this period, the extract was centrifuged again at 10,000 g for 5 minutes to remove any precipitate. It was then stored at -20° C.

The protein content of this kiwi extract, determined by using the folin reagent according to the method of Lowry et al.,¹⁵ was 2.1 mg/ml.

Pollen extracts. Lyophilized timothy and birch pollen extracts were obtained from Pharmacia, and the protein content was measured by the Bradford method. The lyophilized extracts were reconstituted with distilled water to obtain a protein concentration of 2 mg/ml each.

Sodium dodecylsulfate-polyacrylamide gel electrophoresis

The allergen extracts were separated in a discontinuous buffer system by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a 6% stacking gel and a 7.5% to 20% separation gradient gel at 6 mA for 16 hours in a Bio-Rad Protein Ixi vertical electrophoresis slab cell (Bio-Rad Laboratories, Richmond, Calif.), essentially as described by Neville.¹⁶

Before being introduced into the gel, the extracts were diluted 1:2 in sample buffer, containing Tris (hydroxymethyl-aminomethane) adjusted to pH 6.1 with concentrated sulfuric acid, 2% SDS, 3% 2-mercaptoethanol, 5% glycerol, and 0.001% bromphenol blue. The diluted extracts and the low molecular weight (MW) markers (Pharmacia) were denatured by heating at 100° C for 5 minutes and then centrifuged at 10,000 rpm for 5 minutes to remove carbohydrate or lipid precipitates that might affect the electrophoresis. The low MW markers used were: rabbit muscle phosphorylase B, 94 kd; bovine serum albumin, 67 kd; ovalbumin, 43 kd; bovine erythrocyte carbonic anhydrase, 30 kd; soybean trypsin inhibitor, 20.1 kd; and bovine milk α -lactalbumin, 14.4 kd.

For kiwi, we applied 100 μ l per centimeter of gel at a final concentration of 1 mg/ml.

After separation, a part of the gel was fixed in a solution of 30% methanol and 7% acetic acid in distilled water for 2 hours on a horizontal shaker, then immersed in a staining solution with Coomassie Brilliant Blue R-250 (Pharmacia) for 1 hour, and after that in methanol-acetic acid solution for destaining.

Immunoblotting and autoradiography

Allergens separated on the part of the SDS-PAGE gel that was not stained were then electroblotted to nitrocellulose paper (pore size 0.45 μ m; Amersham, Buckinghamshire, U.K.) by using a Trans-Blot Cell from Bio-Rad at 0.45 Å, 100 V, for 4 hours at 4° C. The blot solution was composed of 25 mmol/L Tris, 192 mmol/L glycine, and 20% methanol. The paper was first blocked by immersion in a blocking solution (phosphate-buffered

saline, pH 7.4, with 0.5% Tween-20) for 30 minutes at room temperature and then cut into strips, which were incubated overnight in the test sera diluted 1:4 in blocking solution. To reveal IgE bound to allergen, each strip was washed three times in a washing solution (normal saline solution with 0.5% Tween-20) for 10 minutes on a horizontal shaker and then incubated for 6 hours with iodine 125-labeled anti-human IgE antiserum (Pharmacia) diluted 1:4 in blocking solution, followed by washing and exposure on x-ray film for 5 days.¹⁷

Immunoblotting inhibition

To examine the inhibition by timothy and birch allergens of IgE binding to kiwi allergen components, 600 μ l of timothy and birch pollen extracts, used in doubling dilutions from 2 to 0.00195 mg/ml, was added to 600 μ l of the pooled serum or to individual sera from kiwi-sensitive patients plus 1200 μ l of phosphate-buffered saline and incubated for 1 hour. Six hundred microliters of distilled water was substituted for allergen in the mixture to provide a diluent control. Nitrocellulose strips with kiwi extract were then incubated in each of these mixtures, and IgE binding was detected as described previously. To control for nonspecific allergen binding, this procedure was also carried out with pooled sera from nonallergic donors in place of the allergen-specific sera.

The same procedure at the same dosages was performed to examine the inhibition of our kiwi blot by our kiwi extract.

The serum pool was made by mixing 2.5 ml of sera from all the patients with positive responses to timothy and birch pollen (i.e., patients 1 to 20), and in addition, sera from patients 21 and 26 for timothy and patients 27 and 29 for birch, because they had very high specific IgE levels.

The immunoblots were scanned in an Image Master Desktop Densitometer (Pharmacia) and analyzed for absorption at 633 nm by using Image Master Software (Pharmacia). For each band, the peak optical density (OD) was obtained after subtracting the background OD (OD of the film). The percent inhibition of IgE binding to each band was calculated as follows:

$$100 - 100 \times \frac{\text{Peak OD of band incubated with allergen extract}}{\text{Peak OD of band incubated with diluent control}}$$

RESULTS

Among all the patients tested for a clinically suspected kiwi allergy, the 27 who had a positive SPT response, a positive kiwi ImmunoCap result, and a positive oral challenge test result were included in the in vitro part of the study. In

KIWI IgE IMMUNOBLOTTING IN 30 PTS

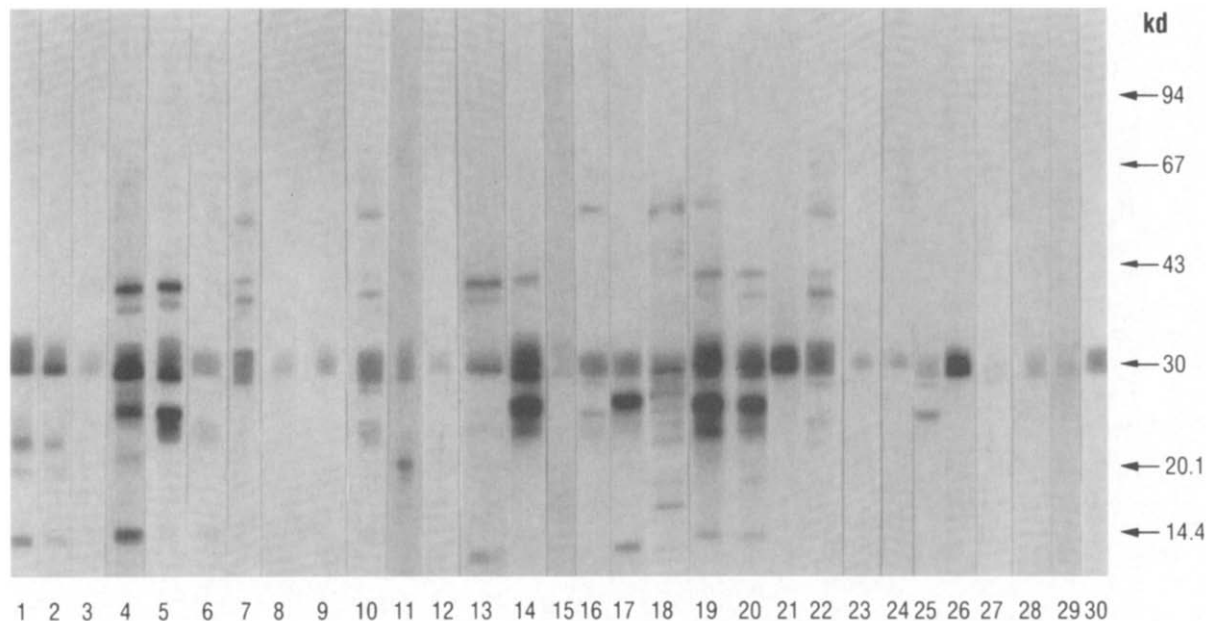


FIG. 1. IgE immunoblots of kiwi extract with sera from 30 kiwi-sensitive patients. MWs of markers are reported at right. *PTS*, Patients.

addition, three patients with a positive SPT response and a positive ImmunoCAP result who were not challenged because of reported severe allergic reactions while eating kiwi were also included. Of these 30 patients (13 men and 17 women; mean age, 35.8 years; range, 16 to 69 years), 26 (86.6%) had timothy pollen-specific IgE (patients 1 to 26), and 22 (73.3%) had birch pollen-specific IgE (patients 1 to 20, 27, and 28).

Components of kiwi extract that bind kiwi-specific IgE

To identify the IgE-binding components of kiwi extract and to determine their relative importance as allergens, we examined immunoblots made with sera from the 30 patients. The blots are shown in Fig. 1. All sera reacted with a band of about 30 kd, and therefore we identify it as the major kiwi allergen. Eleven other components also bound IgE, but none of them could be considered a major allergen, that is, recognized by sera from at least half of these patients. Components of 41, 38, 32, 28, 24, and 22 kd were considered to be allergens of intermediate importance because they were recognized by 30% to 50% of the sera; whereas other components of 64, 20, 17, 14, and 12 kd were considered to be minor allergens because they were recognized by less than 30% of the sera.

Components of timothy pollen extract that bind timothy pollen-specific IgE

The major IgE-binding polypeptide components of timothy pollen were identified in three MW ranges by analysis of the sera of the 23 patients with kiwi sensitivity and grass pollen allergy (there was not enough sera from patients 19, 20, and 21 after collection for use in the serum pool). As shown in Fig. 2, Phl p 4 is represented by bands in the range of 60 to 65 kd, Phl p 1 by bands in the range of 30 to 37 kd, Phl p 5 by bands in the range of 25 to 32 kd, and Phl p 6 by the band at 11 kd.

Components of birch pollen extract that bind birch pollen-specific IgE

As shown in Fig. 3, the major IgE-binding polypeptide components in this extract, as identified by using the sera of 20 patients with kiwi sensitivity and birch pollen allergy (there was not enough serum from patients 19 and 20 after collection for use in the serum pool), were in bands at 20, 17, 14, and 13.5 kd. Intermediate or minor allergens were identified in bands at 70, 37, and 33 kd.

Inhibition of binding of kiwi-specific IgE to kiwi allergens

Inhibition of the binding of kiwi-specific IgE to kiwi allergens by kiwi, timothy pollen, and birch

TIMOTHY IgE IMMUNOBLOTTING IN 23 PTS

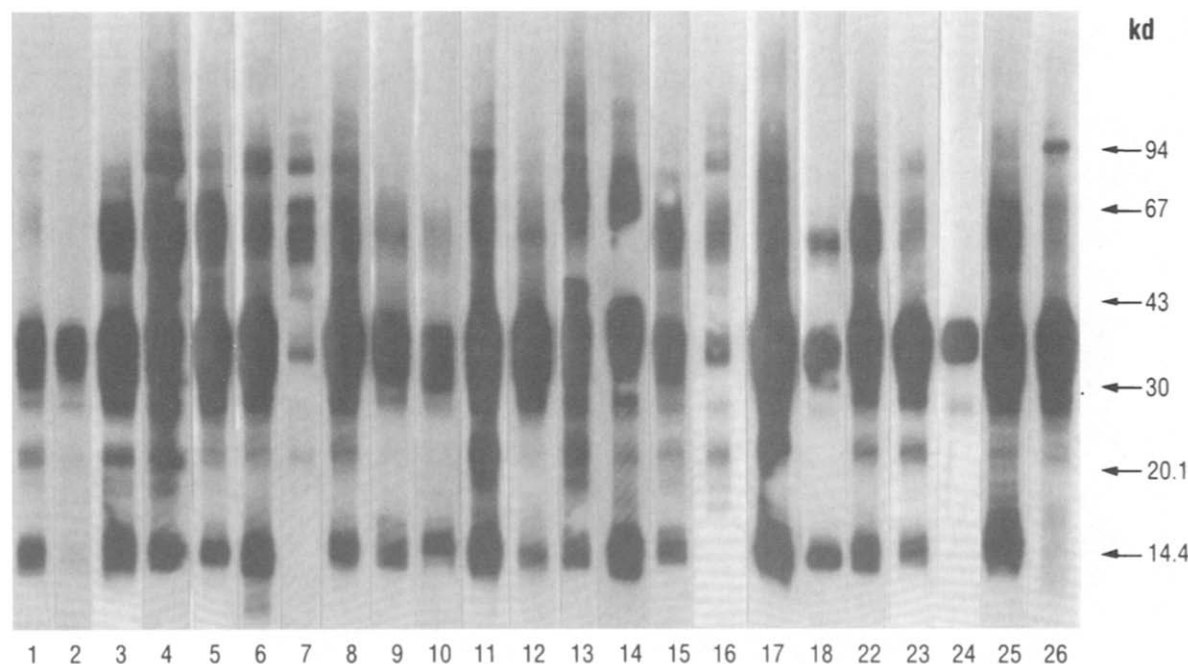


FIG. 2. IgE immunoblots of timothy pollen extract with sera from 23 patients with symptoms of allergy to kiwi fruit who also have specific IgE to timothy pollen. MWs of markers are reported at right. *PTS*, Patients.

pollen allergens was investigated by mixing increasing dilutions of each of the extracts with the pooled sera from kiwi-sensitive patients and then reacting the mixtures with immunoblotted kiwi extract (prepared with 0.1 mg of protein per centimeter of gel). Experiments were also performed with individual sera of six kiwi-sensitive patients mixed with kiwi extract at a dilution of 1 mg protein/ml.

Inhibition by kiwi extract. Complete inhibition with the homologous allergen required concentrations of less than 1 mg/ml; some bands were inhibited even at much lower concentrations (Fig. 4).

Inhibition by timothy pollen extract. IgE binding to kiwi polypeptides with MWs of 41, 38, and 22 kd was completely inhibited by timothy pollen extract in concentrations as low as 0.00195 mg/ml (Fig. 5). The maximum inhibition of bands at 30, 28, and 24 kd was 66% (with 0.0039 mg protein/ml), 66% (with 0.016 mg protein/ml), and 93% (with 0.031 mg protein/ml), respectively.

Inhibition by birch pollen extract. IgE binding to kiwi polypeptides with MWs of 41, 38, 24, 22, and 14 kd was completely inhibited by birch pollen extract in concentrations as low as 0.06 mg/ml (Fig.

6). The maximum inhibition of bands at 32, 30, and 27 kd was 77%, 70%, and 69%, respectively, with the birch pollen extract at a concentration of 0.06 mg protein/ml. Higher concentrations were unable to inhibit binding to these polypeptide bands.

Inhibition of binding with individual sera. In experiments with six individual sera, selected because they reacted with nearly all the kiwi allergens, IgE binding was completely inhibited by kiwi extract at 1 mg/ml. Both timothy and birch pollen extracts, also at 1 mg/ml, completely inhibited binding to kiwi allergens of the IgE in the sera of four of these individuals; inhibition was not observed, however, with the other two sera.

DISCUSSION

OAS caused by fresh fruits and vegetables is a form of food allergy that occurs frequently in patients with pollinosis.³ The clinical association between these two kinds of allergy has led some investigators to study its immunologic basis, and they found that it could be explained by the presence of similar allergens in certain pollens and vegetable foods. As examples, a 17.5 kd polypeptide in birch pollen and apple,⁴ 14 kd and 18 kd polypeptides in hazel tree pollen and hazelnuts,¹⁸

BIRCH IgE IMMUNOBLOTTING IN 20 PTS

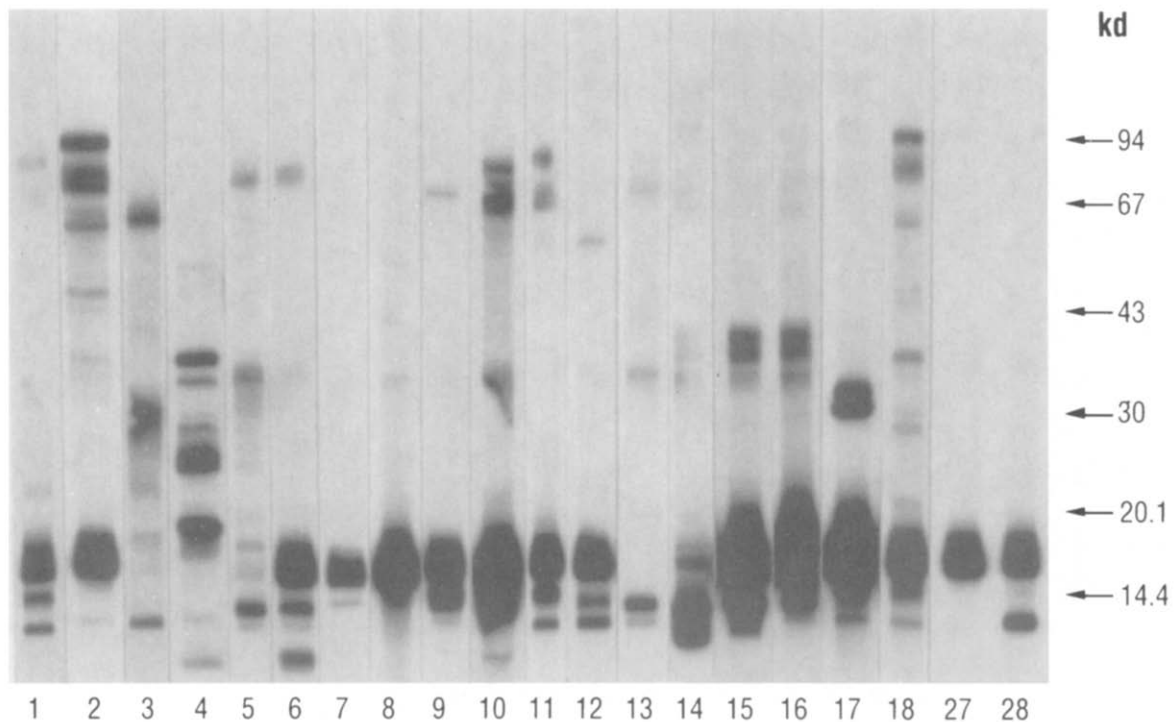


FIG. 3. IgE immunoblot of birch pollen extract with sera from 20 patients with symptoms of allergy to kiwi fruit who also have specific IgE to birch pollen. MWs of markers are reported at right. *PTS*, Patients.

and a 15 kd component in birch pollen and celery⁷ were found to be cross-reacting allergens. Of course, vegetable foods also have specific allergens not shared with pollens. For example, we have identified a 13 kd allergen in peach and other fruits of the *Prunoideae* subfamily, which does not cross-react with birch and grass pollen allergens.²

The aim of this study was to identify the allergens of kiwi fruit, to which allergic reactions are being reported with increasing frequency.⁸⁻¹⁰ Allergic reactions to kiwi have been reported in association with birch¹² and grass¹³ pollen allergies. To obtain reliable clinical data to correlate with our immunologic findings, we assessed our patients' clinical reactivity by means of an open food challenge with kiwi fruit. From its results or from a documented history of an anaphylactic reaction, 30 patients with a positive SPT response and CAP/RAST result for kiwi were included in our study. We analyzed the frequency of IgE binding to the components of kiwi in sera from these 30 patients. Polypeptides with MWs ranging from 64 to 12 kd were identified as allergenic components. Sera from all these patients recognized a 30 kd component, which appears to be the major kiwi allergen,

and should therefore be designated Act c 1 (according to current allergen nomenclature) from the taxonomic name of *Actinidia chinensis*. Polypeptides at 41, 38, 32, 28, 24, and 22 kd were identified as other important (although not as major) allergens. These findings confirm recent observations of Vocks et al.¹⁹ concerning three kiwi-sensitive patients whose sera reacted to a kiwi polypeptide of about 30 kd and, in one case, also to components of about 22 and 24 to 43 kd. Only few sera reacted with the 24 kd polypeptide, which is probably actinidin, one of the major components of kiwi. Actinidin, a thiol protease with a MW of about 23.5 kd, has been fully characterized and sequenced,²⁰ and has a structure very similar to Der p 1, the major allergen of *Dermatophagoides pteronyssinus*.²¹

We then analyzed the cross-reactivity of kiwi fruit with two common allergen sources, timothy and birch pollens. Almost all of the patients were allergic to timothy grass pollen, and immunoblots with their sera showed reactivity against the well-known major *Phleum pratense* allergens, the 34 kd Phl p 1 and the 29 to 31 kd Phl p 5. On immunoblots, IgE binding kiwi allergens of 41, 38, 24, 22,

INHIBITION OF KIWI BLOT BY KIWI

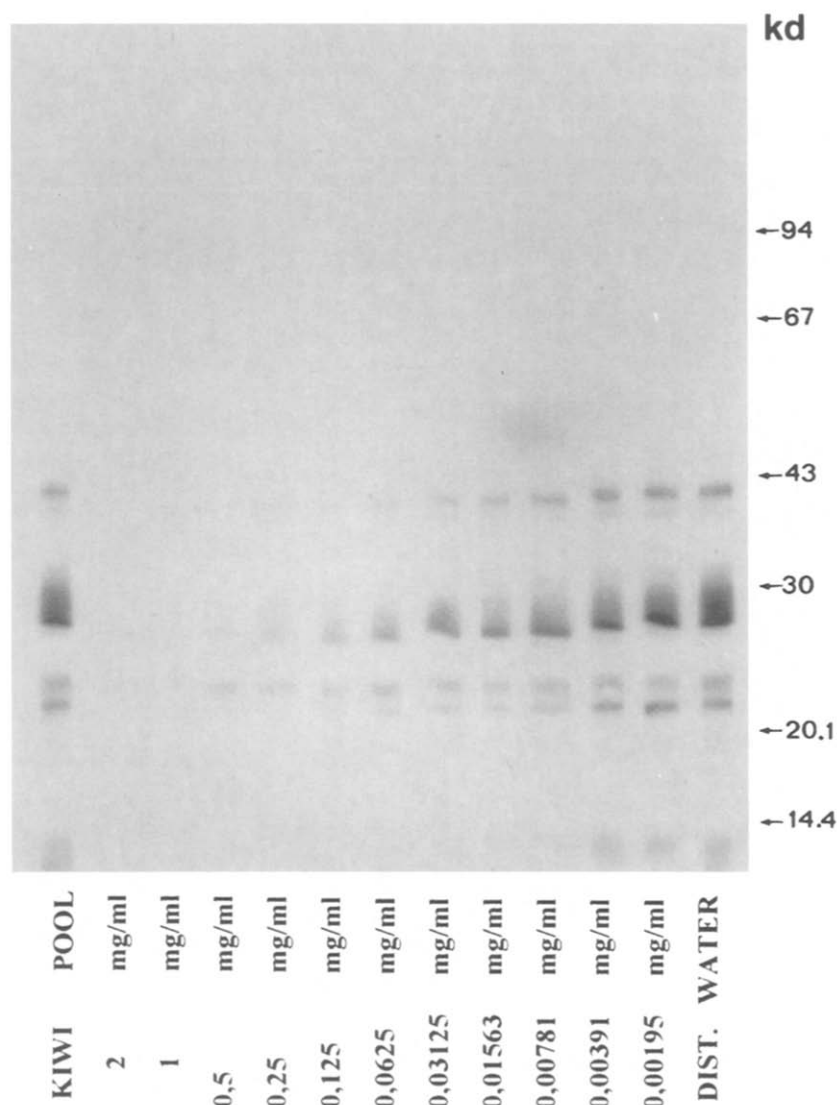


FIG. 4. Inhibition of kiwi immunoblots by kiwi extract at different concentrations (reported on lower part of figure). MWs of markers are reported at right.

and 14 kd was completely inhibited by timothy grass pollen extract, but binding to the main kiwi allergenic components at 32 and 30 kd was only partially inhibited (about 60%). It is thus conceivable that although all the other components are completely cross-reactive, there is only similarity and not identity between the 32 and 30 kd components of kiwi and timothy grass pollen. This is supported by the fact that sera from patients 27 to 30, who are not allergic to timothy grass pollen, reacted only to the 30 kd kiwi component, an allergen that is apparently specific to kiwi.

Immunoblots with birch pollen extract identified its major allergens, Bet v 1 and Bet v 2, in bands at

17 and 14 kd, respectively. In the experiment with birch pollen as inhibiting allergen, as in the experiments with timothy pollen, we observed partial inhibition of binding to 32 and 30 kd kiwi polypeptides, although equivalent polypeptides in birch are not major allergens. It is possible that the epitopes of these allergens are hidden but that they can nevertheless act as allergens in particular circumstances. A significant cross-reactivity was also observed between the 14 kd allergens of birch (Bet v 2) and kiwi. The latter is likely to belong to profilins, ubiquitous proteins involved in plant fertilization, which have been identified in a number of vegetable sources.²² However, profilin does

INHIBITION OF KIWI BLOT BY GRASS

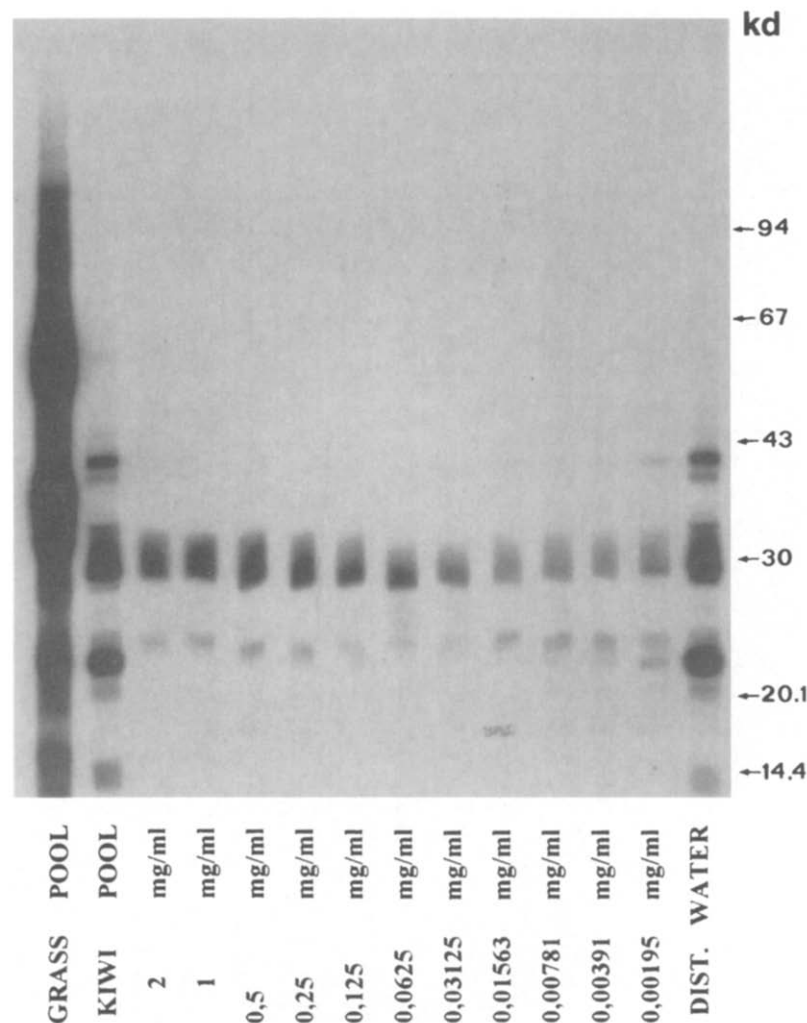


FIG. 5. Inhibition of kiwi immunoblots by timothy pollen extract at different concentrations (reported on lower part of figure). The sera used were collected from patients with positive kiwi challenge results who also have specific IgE to timothy pollen. MWs of markers are reported at right.

not seem to be an important allergen of kiwi, being recognized by specific IgE from only 26% of our patients allergic to kiwi.

With both timothy and birch pollens we observed less inhibition at the higher concentrations of the inhibiting extracts. This might be a technical artifact, surely not determined by an inappropriate blocking solution because this would affect the whole strip and not only some bands. A possible explanation is provided by nonspecific binding of the inhibitor to the blot, or by the inhibitory pollen allergens binding to both inhibitor and the kiwi fruit allergen. Because a complete structural identity between the IgE-binding components of kiwi and pollens is unlikely, the phenomenon may occur

only at higher concentrations when specificity related to structural identity is less important for IgE binding. Another explanation may depend on the fact that very high concentrations of complex allergen extracts may contain aggregates of the polypeptide components of the extracts, resulting in inadequate antibody binding with inhibitor. In consideration of this, we then used the most convenient concentration, 1 mg/ml, to test the sera of individual patients, and we found complete inhibition of binding to all the kiwi allergens by timothy and birch extracts in four of six cases. This indicates that IgE antibodies specific for 32 and 30 kd polypeptides also recognize polypeptides of the same MW in timothy and birch pollen extracts.

INHIBITION OF KIWI BLOT BY BIRCH

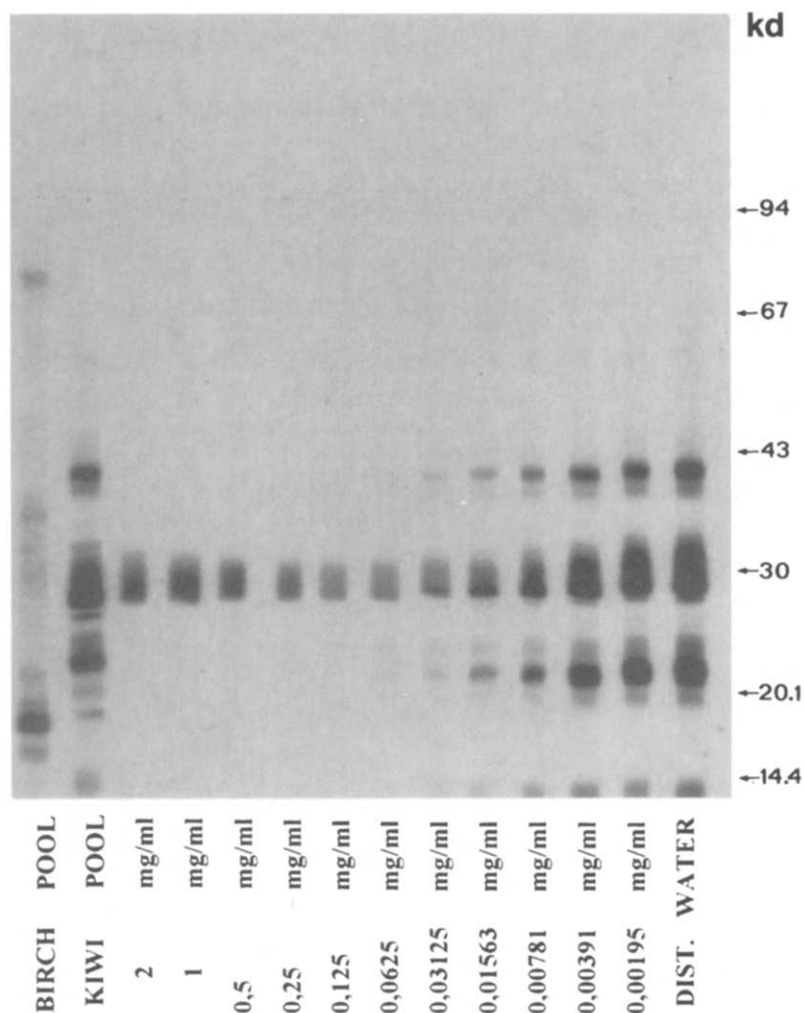


FIG. 6. Inhibition of kiwi immunoblots by birch pollen extract at different concentrations (reported on lower part of figure). The sera used were collected from patients with positive kiwi challenge results who also have specific IgE to birch pollen. MWs of markers are reported at right.

Taken together, these results further suggest that the 32 and 30 kD allergens from these three plant sources share some but not all of their antigenic determinants.

It would also be interesting to investigate the possibility of cross-reactivity of IgE antibodies to the 30 kD kiwi allergen with allergens of other vegetable foods or other allergenic sources. In fact, a 30 kD IgE-binding component has been identified in fruits such as peach, banana, mandarin orange, guava, and strawberry by Wade²³; in cherries by us²; and in natural rubber latex by Alenius et al.²⁴ By focusing attention on allergens that are widespread in nature, recent reports have suggested the existence of "panallergens" in several botanical and animal sources, such as the above-mentioned

profilin in vegetable foods and plants,²² cytochrome *c* in grasses and Compositae,^{25, 26} and carbohydrate allergens in foods and Hymenoptera venoms.²⁷ The 30 kD kiwi polypeptide might be such a "panallergen." At least with respect to kiwi and cherry, we have found a high degree of homology, as suggested by complete reciprocal inhibition (unpublished observation). Recently, Lavaud et al.²⁸ identified, as a 30 kD component, the allergen cross-reacting between banana, avocado, and latex; the latter is known to cross-react, including clinically, with kiwi.

In conclusion, immunoblots with specific IgE-containing sera from patients with OAS who are clinically sensitive to kiwi have allowed us to identify 11 allergens in this fruit. Most of these

polypeptides, including the 30 kd major allergen, were shown to cross-react with allergens present in timothy and birch pollen extracts.

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