

Quantitative IgE inhibition experiments with purified recombinant allergens indicate pollen-derived allergens as the sensitizing agents responsible for many forms of plant food allergy

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Background: Type I allergic symptoms in the oropharyngeal mucosa upon contact with plant-derived food in patients with pollen allergies have been termed oral allergy syndrome (OAS). IgE cross-reactivity between pollen and food allergens represents the molecular basis for this phenomenon. The sensitizing allergen source (pollen or plant food) in OAS is a controversial issue.

Objective: We sought to determine the primary sensitizing molecules in patients with OAS.

Methods: We used recombinant birch pollen (rBet v 1 and rBet v 2) and plant food allergens (apple, rMal d 1; celery, rApi g 1; and carrot, rDau c 1), as well as natural pollen (birch and timothy grass) and plant food (apple, peach, kiwi, hazelnut, celery, and carrot) allergens, to identify cross-reactive allergens by using qualitative immunoblot inhibitions. In addition, we determined the percentage of plant food-specific IgE that can be preadsorbed with recombinant and natural pollen allergens by quantitative RAST inhibitions by using sera from 71 patients with OAS.

Results: Preincubation of sera with recombinant and natural pollen allergens led to an almost complete inhibition of IgE binding to plant food allergens in Western blots, as well as in RAST inhibition experiments. In contrast, recombinant plant food allergens poorly inhibited IgE binding to Bet v 1.

Conclusion: Most IgE epitopes in plant food recognized by patients with OAS are resembled by pollen allergens. Thus pollen allergens may be responsible for the elicitation and maintenance of OAS. (*J Allergy Clin Immunol* 1999;116:25.)

Key words: Food allergy, pollen allergy, cross-reactivity, recombinant allergens

The estimations for the prevalence of IgE-mediated food allergy vary between 0.5% and 10% of the population.^{1,2} Depending on the allergen-containing sources, 2 forms of food allergy may be discriminated. Certain patients have allergy to foods (eg, peanuts, soja, milk, fish, hen's egg, and meat) without necessarily exhibiting a concomitant pollen allergy, whereas others who are sensitized to certain fruits, vegetables, and spices (eg, hazelnut, celery, apple, carrot, and fennel) frequently have respiratory pollen allergies as well.^{3,4} The frequent association of pollen allergy with allergic reactions to fruits and vegetables was defined as oral allergy syndrome (OAS).^{5,6} Patients with OAS exhibit a broad variety of symptoms on direct contact of the oral mucosa with plant food, including oral itching, lip swelling, labial angioedema, glottis edema, and occasionally systemic reactions.⁶ Evidence for the importance of sensitization to pollen allergens in patients with OAS came from RAST studies indicating the presence of cross-reactive IgE epitopes in pollen and plant-derived food.⁷⁻¹² Furthermore, patients exhibit more severe symptoms of food allergy during and after the pollen season.¹³ By using specific antibody probes, IgE immunoblot inhibition experiments, and molecular biology techniques, it could be demonstrated that plant-derived food contains allergens that share epitopes with the major birch pollen allergen, Bet v 1, and profilin, a highly cross-reactive plant panallergen.¹⁴⁻¹⁸ In addition, a high molecular weight allergen complex of approximately 60 kD present in pollens from grasses and weeds was found to share IgE epitopes with allergens in fruits and vegetables.¹⁹ These findings suggested that patients with OAS contain IgE antibodies that cross-react with allergens or epitopes present in pollen, as well as plant-derived food. Recently, the complementary (c)DNAs coding for the Bet v 1-homologous allergens from apple (Mal d 1),²⁰ celery (Api g 1),²¹ and carrot (Dau c 1)²² were isolated and shown to share an average sequence identity of approximately 45% with Bet v 1. Moreover, it could be demonstrated that T cells and T-cell clones with specificity for

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Supported by a grant of the Austrian Ministry of Science and Transport, by grant Y078GEN of the Austrian Science Fund, and by the Interdisciplinary Cooperative Project program of the Austrian Ministry of Science and Transport.

Received for publication July 2, 1999; revised Sep 7, 1999; accepted for publication Sep 7, 1999.

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0091-6749/2000 \$12.00 + 0 1/1/102872

Abbreviation used

OAS: Oral allergy syndrome

food allergens and rMal d 1, respectively, cross-reacted with the major birch pollen allergen Bet v 1.^{23,24} The demonstration that immunization of rhesus monkeys with recombinant birch pollen allergens, rBet v 1 and rBet v 2, induced immediate-type skin reactions to food containing Bet v 1- and Bet v 2-related allergens finally raised the question of whether pollen or food allergens are the disease-eliciting agent in patients with OAS.²⁵

The fact that most patients with OAS have concomitant pollen allergy⁴ and that even those patients who reportedly have allergy to Rosaceae fruits without related pollinosis exhibited IgE reactivity to pollen extracts²⁶ indicated that pollen allergens may predominate as sensitizing agents. Furthermore, most of the cross-reactive allergens are more abundantly expressed in pollen than in somatic plant tissue.²⁷ The latter observations gave rise to the hypothesis that OAS is caused by a primary respiratory sensitization to pollen allergens.²⁷ Here we present an experimental in vitro system that aims to investigate the nature of the primary sensitizing molecules in patients with OAS. This experimental in vitro system is based on the assumption that the primary sensitizing molecules will carry most, if not all, of the relevant IgE epitopes, whereas the secondary cross-reactive allergen will contain less IgE-reactive epitopes. We performed IgE inhibition experiments with sera from 71 patients with OAS by using recombinant birch pollen (rBet v 1 and rBet v 2) and plant food allergens (apple, rMal d 1; carrot, rDau c 1; and celery, rApi g 1) and natural allergens from birch and timothy grass pollen, as well as from apple, carrot, celery, hazelnut, kiwi, and peach. By using IgE immunoblot inhibition experiments, we identified the cross-reactive moieties and determined, by means of quantitative RAST inhibitions, the percentage of plant food-specific IgE that is directed against recombinant and natural pollen allergens. Our finding that a combination of rBet v 1, rBet v 2, and timothy grass pollen allergens resembles most of the IgE epitopes present in apple, carrot, celery, hazelnut, kiwi, and peach, whereas recombinant plant food allergens poorly inhibit IgE binding to birch pollen allergens, provides evidence for a primary respiratory sensitization in patients with OAS.

METHODS

Patients

Sera from 71 patients (46 Austrian and 25 French patients; 29 male and 42 female patients; mean age, 34 years) with OAS were analyzed. All patients had clinically relevant sensitization to at least one of the following plant-derived foods: apple, carrot, celery, hazelnut, kiwi, and peach. Food allergy was diagnosed according to the recommendations of the Royal College of Physicians and Pathologists (London, UK)²⁸ and according to the guidelines of the European Academy of Allergology and Clinical Immunology.¹ The diagnosis of associated pollen allergy was based on a case history indicative of seasonal sensitization, skin prick test results, and positive serology performed as described.^{29,30} The identification of

allergens in apple, carrot, celery, hazelnut, kiwi, and peach extracts that cross-react with the recombinant birch pollen allergens rBet v 1 and rBet v 2 and natural timothy grass pollen allergens was performed by using IgE immunoblot inhibition experiments with sera from 21 representative patients, where sufficient serum was available. The latter group consisted of 12 women and 9 men aged between 22 and 54 years (mean age, 35 years). Demographic, clinical, and serologic data of these 21 patients are displayed in Table I. For control purposes, serum from a nonatopic individual was examined. The capacity of rBet v 1-homologous proteins from apple (rMal d 1), carrot (rDau c 1), and celery (rApi g 1) to inhibit IgE binding to natural Bet v 1 was investigated by using 5 sera from patients with associated birch pollen allergy (Table I, patients 2, 6, 17, 19, and 21) and from a nonatopic individual.

Quantitative CAP-RAST IgE inhibition experiments were performed with sera from 71 patients with clinically relevant food allergies (apple, n = 52; carrot, n = 14; celery, n = 9; hazelnut, n = 26; and peach, n = 4).

Natural plant food and pollen extracts

Pollen from birch (*Betula verrucosa*) and timothy grass (*Phleum pratense*) was purchased from Allergon (Välinge, Sweden). Pollen allergen extracts were prepared by aqueous extraction, as previously described.³¹

Fresh fruits (apple, kiwi, and peach), vegetables (carrot and celery), and nuts (hazelnut) were purchased at a local market. Plant food extracts were prepared by homogenizing 8.4 g of fresh material in 20 mL of SDS-PAGE sample buffer³² by using an Ultraturrax (IKA, Heidelberg, Germany). The homogenates were extracted by boiling for 10 minutes, and insoluble particles were removed by centrifugation (10,000g for 10 minutes at 20°C). Cleared supernatants of each extract were placed in aliquots and stored at -20°C until use. Protein contents and quality of the extracts were analyzed by SDS-PAGE and Coomassie Brilliant Blue staining (BioRad, Richmond, Calif).^{33,34}

Production and purification of recombinant allergens

Recombinant, bacterially expressed birch pollen allergens (rBet v 1; rBet v 2 = birch profilin) were obtained from BIOMAY (Linz, Austria). rBet v 1 and rBet v 2 represent the isoforms encoded by the cDNA originally isolated by IgE immunoscreening.^{35,36} rMal d 1, rApi g 1, and rDau c 1 were expressed in *Escherichia coli* and purified, as described previously.²⁰⁻²² The specific IgE-binding capacity of all recombinant proteins was determined by immunoblotting before use for IgE competition experiments.

IgE immunoblot inhibition experiments

Plant food and birch pollen extracts (approximately 200 µg of protein per centimeter of gel) were separated by 12.5% preparative SDS-PAGE³³ and blotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany).³⁷ Sera from allergic patients and, for control purposes, from a nonatopic individual were diluted 1:5 in buffer A (50 mmol/L Na phosphate [pH 7.5], 0.5% wt/vol BSA, 0.5% vol/vol Tween 20, and 0.05% wt/vol NaN₃) and preincubated either with recombinant allergens (5 µg of each recombinant allergen per 1 mL of serum dilution), with natural timothy grass pollen allergens (100 µg of natural timothy grass pollen extract per 1 mL of serum dilution), or, for control purposes, with BSA (5 µg per 1 mL of serum dilution) overnight at 4°C. Preadsorbed sera were then incubated overnight at 4°C with nitrocellulose-blotted allergen extracts, and bound IgE antibodies were detected as described.^{18,19}

TABLE I. Demographic, clinical, and serologic characterization of individuals tested in Fig 1

Patient No.	Sex	Age (y)	Allergies			Symptoms			Therapy	Birch (kUA/L)	Timothy grass	Mugwort
			Pollen	Food	Other	Pollen	Food	(kUA/L)			(kUA/L)	
1	F	42	t	H, K, P	None	r, c	e: lips	ah	7.50	<0.35	<0.35	
2	M	32	t	A, H, P	None	r, c, as	e: lips	ah, c	12.0	<0.35	<0.35	
3	F	32	t, g, w	A, S	a	r, c, as	u	ah, it	9.74	7.83	16.0	
4	F	32	t	A, H	None	r, c	e: lips, tongue	ah	3.97	<0.35	<0.35	
5	F	29	g	H, W, Al	None	r, c	e: lips	ah	<0.35	1.42	<0.35	
6	F	30	t, g	A, H, P, C, Ca	a	r, ad	e: lips	ah	67.8	>100	2.55	
7	F	36	t, g, w	A, Ce, Ch, T, S, Ci	a, mi	r, as	e, m, p, f	it, ah, ed	4.14	17.0	<0.35	
8	F	22	t, g, w	A	a	c, r, d	e: lips, tongue	ah	2.14	78.1	1.74	
9	M	42	g, t	A, H, Ca	None	r, c	e, ear itching	it	6.75	6.87	<0.35	
10	M	32	t, g	A, P, B	a, mi	r, as	e: lips	ah	13.4	8.63	2.65	
11	M	50	t, g	A	a	r, c, as	n, e	ah	6.17	45.1	3.41	
12	M	39	t, g, w	A, C, Ce, s	a, mi, mo	r	e, D	ah, c	19.2	44.6	1.36	
13	F	25	t, g	H, P	None	r, c	e	ah	22.1	37.7	1.13	
14	M	35	t, g	A, Ca	None	r, c	e	ah	14.3	6.82	0.68	
15	M	54	t, g	H	a, mo	r, c	e: lips	None	19.9	12.1	<0.35	
16	F	30	g, w	C	None	r, c	a	ah	<0.35	29.6	0.81	
17	F	41	t	Ca, C, P, K, S, H, W, A, Pe, Po	None	r, c	e: lips, tongue	ah	58.1	0.36	<0.35	
18	M	35	t, g	A, H	None	r, c	e: lips	ah	11.2	19.9	0.45	
19	F	34	t	A, Ca, K, B, PA, W	mi	r, c, as	e: lips, tongue	ah, c	14.4	<0.35	<0.35	
20	F	24	t, g	A, Ca	mi, a	r, c, as	u, p	ah	54.5	37.0	1.13	
21	M	36	t, g	H, A, P, Pe	a	r, c	p	it	14.5	25.9	<0.35	

Pollen (allergies): t, trees; g, grass; w, weed; *Others (allergies):* mi, mites; mo, molds; a, animals; *Food (allergies):* A, apple; Ca, carrot; C, celery; H, hazelnut; K, kiwi; P, peach; Pe, pear; S, strawberry; B, banana; PA, pineapple; W, walnut; Al, almonds; Ce, cereals; s, sea food; Po, potatoes; Ch, cheese; T, tomatoes; Ci, citrus fruits; *Pollen (symptoms):* r, rhinitis; c, conjunctivitis; as, asthma; *Food (symptoms):* u, urticaria; p, pruritus; a, anaphylaxis; ad, atopic dermatitis; d, dyspnea; n, nausea; e, edema; m, migraine; f, flush; D, diarrhea; *Therapy:* ah, antihistamines; it, immunotherapy; ed, elimination diet; c, corticosteroids.

Quantitative measurement of allergen extract-specific IgE antibodies and quantitative IgE inhibition experiments

Specific IgE antibody levels to pollen and fruit extracts were determined by using the Pharmacia CAP system (Pharmacia & Upjohn AB, Uppsala, Sweden).

Quantitative IgE inhibition experiments were performed by using the Pharmacia CAP-RAST System. Fifty-microliter serum aliquots were preadsorbed with 25 μ L of PBS containing either a mixture of rBet v 1 and rBet v 2 (3 μ g of each protein), 25 μ L of PBS containing a mixture of rBet v 1 and rBet v 2 (3 μ g of each protein) and 50 μ g of timothy grass pollen extract, or, for control purposes, with 25 μ L of PBS containing 50 μ g of BSA overnight at 4°C. The levels of IgE specific for CAP-bound food extracts (apple, carrot, celery, hazelnut, and peach) were determined as kilounits of allergen per liter. The percentage inhibition after preincubation with allergens was calculated as follows: percentage inhibition = $100 - \text{kUA/L (serum + allergen)} \times 100 / \text{kUA/L (serum + BSA)}$.

RESULTS

Characterization of patients with OAS

For the identification of cross-reactive allergens in pollen and plant food (apple, carrot, celery, hazelnut, kiwi, and peach), we used sera from 21 allergic individ-

uals who exhibited a clinically relevant sensitization to at least one plant food and to at least one source of pollen allergens (Table I and Fig 1, A-F). We, as other investigators before us,³⁸ noted that the presence of IgE antibodies against certain plant food allergens does not necessarily predict that the patient will exhibit clinical symptoms after contact with the very same allergen source (Table I and Fig 1, A-F).

Each of the patients examined in our study contained IgE antibodies against the plant food responsible for their clinical symptoms.

Identification of allergens in apple, hazelnut, kiwi, peach, carrot, and celery that cross-react with pollen allergens as demonstrated by IgE immunoblot inhibition

We used sera from 21 patients with clinically relevant allergy to plant food (Table I) to identify plant food allergens that share IgE epitopes with pollen allergens. Sera that were not preadsorbed with pollen allergens (Fig 1, A-F, lanes A) displayed IgE reactivity to components of 10 to 14 kd, 17 to 20 kd, and 30 to 100 kd in plant food extracts (Fig 1, A-F). In kiwi extracts IgE-

A

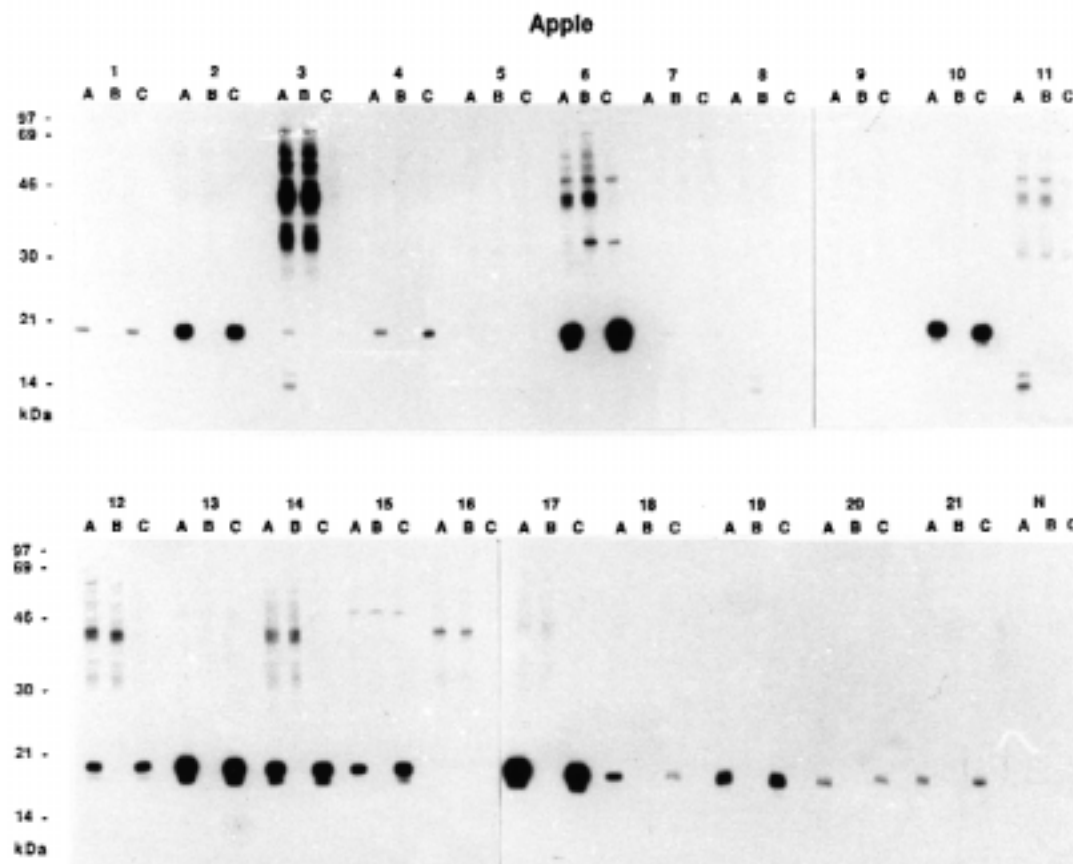


FIG 1. Inhibition of serum IgE reactivity to nitrocellulose-blotted plant food extracts by preadsorption of sera with recombinant or natural pollen allergens. Nitrocellulose-blotted extracts from apple (**A**), carrot (**B**), celery (**C**), hazelnut (**D**), kiwi (**E**), and peach (**F**) were probed with sera from 21 patients with OAS (*panels 1-21*) and with serum from a nonallergic individual (*panel N*). Sera were preadsorbed with an unrelated control protein (*lanes A*, BSA), with a combination of rBet v 1 and rBet v 2 (*lanes B*), or with natural timothy grass pollen allergens (*lanes C*). Bound IgE antibodies were detected with iodine 125-labeled anti-human IgE antibodies and visualized by autoradiography. Molecular weights are displayed in kilodaltons on the left margins. For *panels 10 to 11* and *19 to N* (hazelnut) and *9 to 11* and *18 to N* (peach), molecular weights are displayed on the right side.

reactive antigens of 25 and 30 kD were frequently detected (Fig 1, *E*, sera 1, 3, 6, 8, 11, 12, and 15). These kiwi-derived bands most likely represent the major kiwi allergen actinidin.³⁹ Preincubation of sera with a combination of rBet v 1 and rBet v 2 almost completely abolished IgE reactivity to the 10- to 14- and 17- to 21-kD allergens (Fig 1, *A-F*, lanes B). The 10- to 14-kD components detected by sera 3, 6, 8, 11, 13, and 20 and the 17- to 20-kD moieties recognized by sera 1, 2, 4, 6, 9, 10, 12 to 15, and 17 to 21 thus represent allergens that cross-react with birch profilin (Bet v 2) and Bet v 1, respectively. IgE binding to the higher molecular weight bands (30-100 kD) detected by sera 3, 6, 8 to 14, and 16 to 18 was completely abolished or strongly reduced after preincubation of sera with timothy grass pollen extract containing the high molecular weight cross-reactive allergen described by Heiss et al¹⁹ (Fig 1, *A-F*, lanes C). Timothy grass pollen extract contains profilin (Phl p 12) and thus inhibited IgE binding to the 10- to 14-kD profilin band. However, because of the lack of a Bet v 1-homologous allergen, timothy grass

pollen extract failed to influence IgE binding to the 17- to 21-kD Bet v 1 homologue. Serum from a nonatopic individual (Fig 1, *A-F*, panels N) displayed no IgE reactivity to any of the extracts.

Our IgE inhibition experiments thus identified a Bet v 1-homologous allergen, a high molecular weight allergen complex that shares IgE epitopes with timothy grass pollen allergens, and profilin in our plant food extracts.

Recombinant plant food allergens poorly inhibit IgE binding to the major birch pollen allergen Bet v 1

Next we investigated whether preadsorption of sera with recombinant plant food allergens in patients with OAS can inhibit IgE binding to Bet v 1. For this purpose, we preadsorbed sera from 5 representative patients with birch pollen allergy and clinically relevant sensitization to at least one plant-derived food (apple, carrot, and celery) either with rBet v 1, the major birch pollen allergen,

B

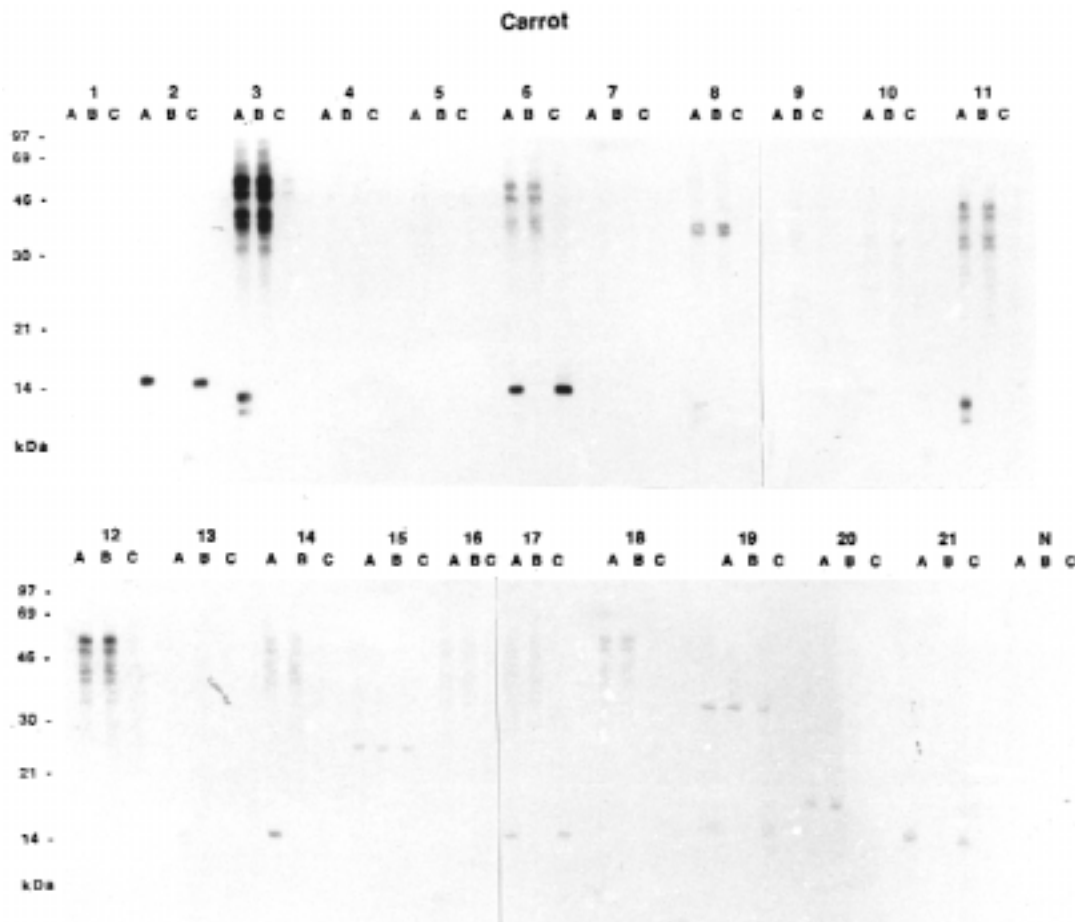


FIG 1. CONT

or with the Bet v 1-related major allergens from apple (rMal d 1), carrot (rDau c 1), or celery (rApi g 1). PreadSORption of the sera with rBet v 1 completely inhibited IgE binding to nitrocellulose-blotted natural Bet v 1 (Fig 2, lanes A), whereas preincubation with recombinant food allergens weakly (Fig 2, serum 4) or not at all (Fig 2, sera 1-3 and 5) reduced IgE binding to natural Bet v 1. Serum from a nonatopic individual showed no IgE reactivity to nitrocellulose-blotted birch pollen extract (Fig 2, serum 6).

Recombinant birch pollen allergens (rBet v 1 and rBet v 2) and natural timothy grass pollen allergens contain most IgE epitopes present in apple, carrot, celery, hazelnut, and peach

To determine the percentage of IgE antibodies specific for plant food allergens that can be preadsorbed with rBet v 1, rBet v 2, and natural timothy grass pollen extract, quantitative RAST inhibition studies were performed with sera from 71 patients with OAS (Table II). PreadSORption of 52 sera from patients with clinically relevant sensitization to apples with a combination of rBet

v 1 and rBet v 2 inhibited IgE binding to apple extract in an average of 91.7% of sera. Additional preadsorption with natural timothy grass pollen allergens slightly increased the degree of inhibition of IgE binding to 96.1% of sera (Table II). A combination of rBet v 1 and rBet v 2 inhibited the average IgE reactivity to celery (n = 9 sera) by 79%, to carrot (n = 14 sera) by 64%, to peach (n = 4 sera) by 60%, and to hazelnut (n = 26 sera) by 83% (Table II). When natural timothy grass pollen allergens were included as additional inhibitors, the mean percentage of inhibition to celery increased to 89%, carrot to 93%, peach to 100%, and hazelnut to 94% (Table II). The results of the quantitative IgE inhibition studies thus demonstrate that rBet v 1 and rBet v 2 account for most of the IgE antibodies directed to apple, carrot, celery, hazelnut, and peach extract. Timothy grass pollen extract contains additional cross-reactive allergens that account for approximately 10% of the IgE-binding capacity of the plant food extracts investigated. After preincubation of sera with a combination of 2 recombinant birch pollen allergens, rBet v 1 and rBet v 2, and natural timothy grass pollen extract, a close to 100% inhibition of IgE binding to apple, carrot, celery, hazelnut, and peach extracts was achieved (Table II).

C

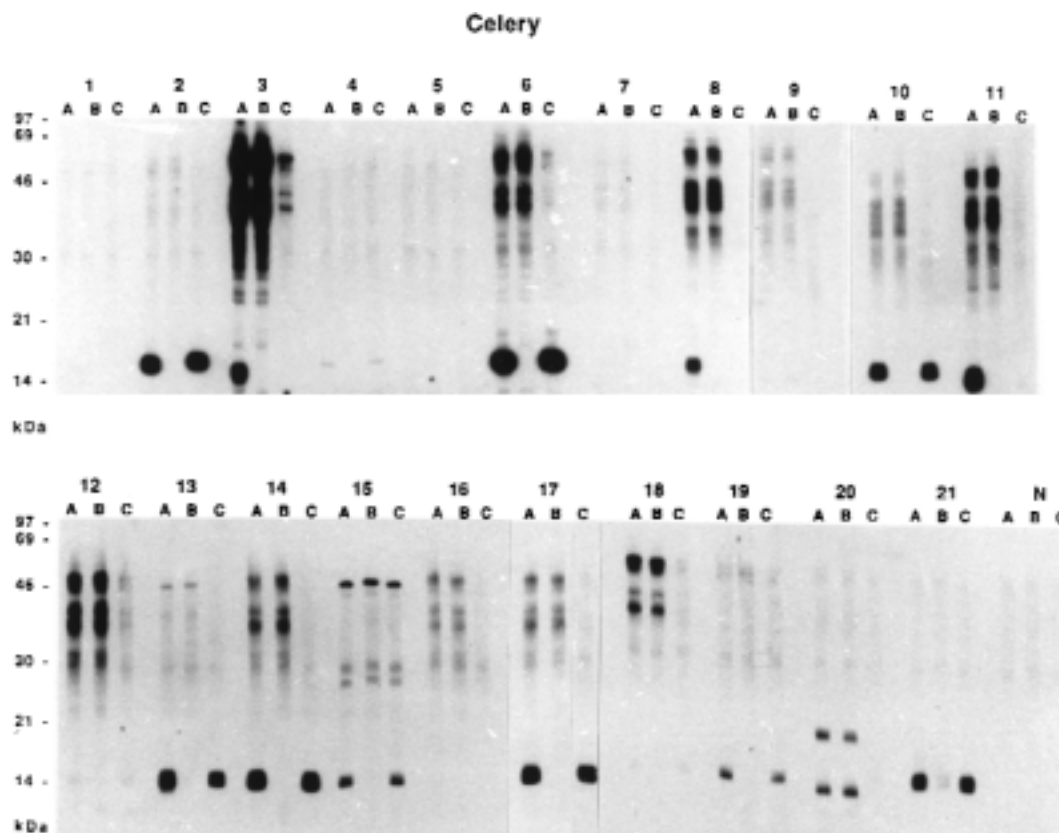


FIG 1. CONT

TABLE II. Percentage of IgE inhibition (mean and range) to plant food extracts after preincubation of sera with recombinant birch pollen allergens (rBet v 1 and rBet v 2) and natural timothy grass pollen extract

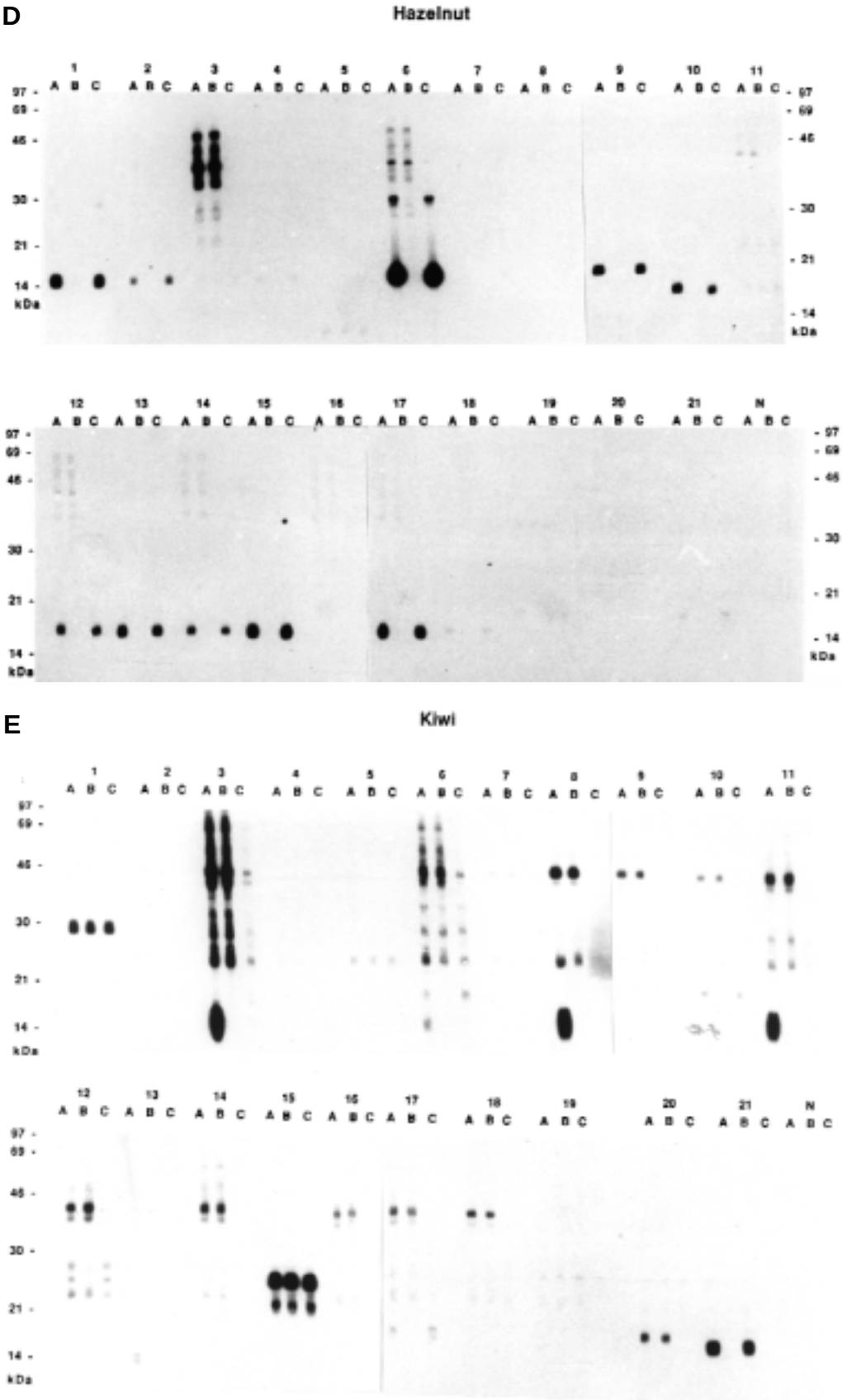
	No. of sera	B1+B2 mean (min-max)	B1+B2+P mean (min-max)
Apple	52	91.7% (4.7%-100%)	96.1% (23.2%-100%)
Peach	4	60% (17.9%-100%)	100% (100%-100%)
Carrot	14	63.8% (0%-100%)	93.2% (29.2%-100%)
Celery	9	78.9% (2%-100%)	89.3% (4.1%-100%)
Hazelnut	26	82.7% (3.6%-100%)	93.6% (16.8%-100%)

B1+B2, Percentage inhibition achieved by preincubation of sera with rBet v 1 and rBet v 2; B1+B2+P, percentage inhibition achieved by preincubation of sera with rBet v 1, rBet v 2, and natural timothy grass pollen extract.

DISCUSSION

The co-occurrence of allergic symptoms to certain plant-derived foods (fruits, vegetables, and spices) and pollen allergy has been designated as OAS.⁶ In this study we used quantitative IgE inhibition studies performed with purified recombinant allergens to investigate whether the majority of IgE epitopes of plant food extracts are present on recombinant pollen allergens. We found that a combination of recombinant and natural pollen allergens almost completely inhibited IgE binding to plant food extracts, whereas IgE reactivity to pollen allergens was poorly inhibited by recombinant plant food allergens. The recombinant food allergens used in our study were soluble up to concentrations of 1 mg/mL, bound IgE antibodies of patients with food allergy, and

inhibited IgE binding to the corresponding natural food allergens.²⁰⁻²² We therefore believe that the poor inhibition of IgE binding to pollen allergens by recombinant plant food allergens is not caused by lack of native-like structure of the recombinant food allergens. It rather seems that pollen allergens contain the majority of IgE epitopes, indicating that most patients with OAS were sensitized primarily against pollen allergens. This assumption is further supported by our observation that each of the 71 patients who were included in this study, primarily on the basis of a clinically relevant food allergy, had allergy to at least one pollen species. In addition, it has been described by other investigators that symptoms of plant food allergy tend to exacerbate during and after pollen seasons.¹³ In this context it is noteworthy that the respiratory mucosa has been proposed as a site



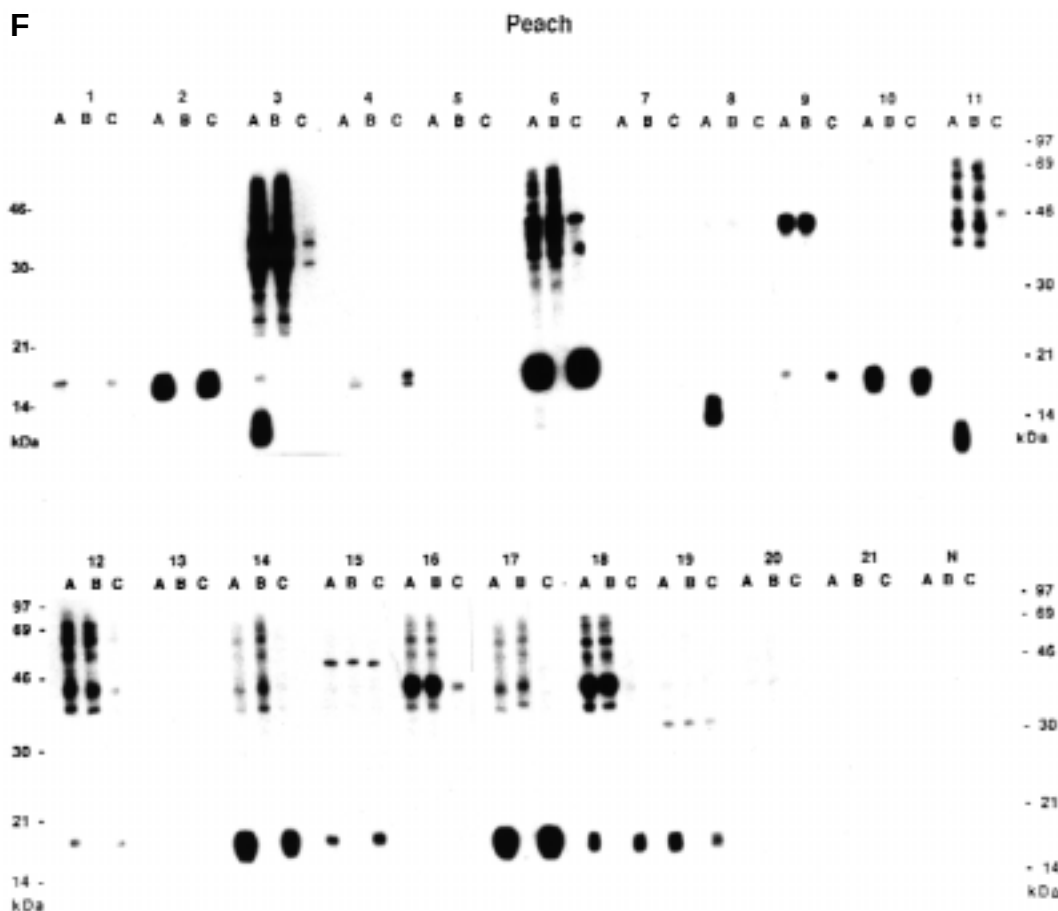


FIG 1. CONT

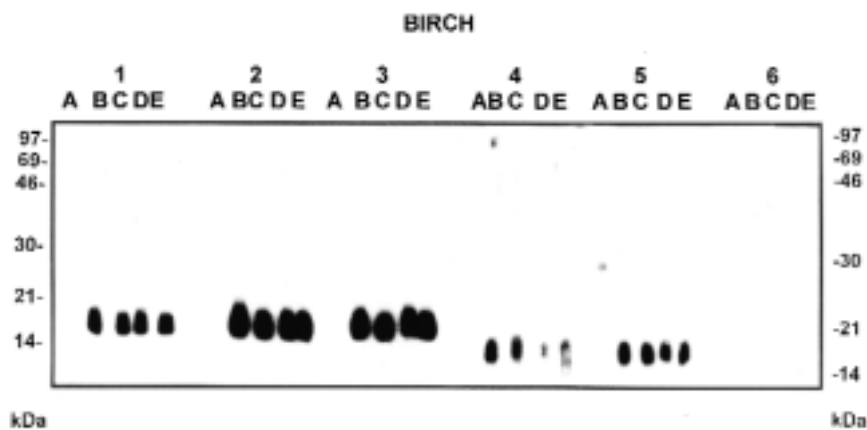


FIG 2. Inhibition of serum IgE reactivity to nitrocellulose-blotted natural birch pollen extract by preadsorption of sera with recombinant Bet v 1 and recombinant plant food allergens. Nitrocellulose-blotted birch pollen extract was probed with sera from 5 patients with OAS (*panels 1-5*) and with serum from a nonallergic individual (*panel 6*). Serum samples were preadsorbed with rBet v 1 (*lanes A*), BSA as a control protein (*lanes B*), rApi g 1 (*lanes C*), rMal d 1 (*lanes D*), or rDau c 1 (*lanes E*). Bound IgE antibodies were detected with iodine 125-labeled anti-human IgE antibodies and visualized by autoradiography. Molecular weights are displayed in kilodaltons on the left margins. For *panels 4 to 6*, molecular weights are displayed on the right side.

for local IgE production.⁴⁰ On the basis of all these findings, it is likely that contact with pollen allergens through the respiratory system could induce, maintain, and boost the production of the cross-reactive IgE antibodies that

are responsible for plant food allergy in patients with OAS.

The demonstration that a combination of 2 recombinant birch pollen allergens, rBet v 1 and rBet v 2, con-

tained most of the IgE epitopes of plant food extracts may also be relevant for the diagnosis of plant food allergy. rBet v 1 and rBet v 2, together with the 60-kd cross-reactive allergen complex produced as purified recombinant proteins, may be used for standardized in vivo provocation testing (eg, double-blind, placebo-controlled food challenge⁴¹ and colonoscopic allergen provocation⁴²).

By using the serologic competition experiments described in our study, it is possible to identify patients with plant food allergy whose IgE reactivity is mainly directed against epitopes on pollen allergens. In such patients performance of pollen immunotherapy may be considered, as well as immunotherapy to plant food allergy, by using a few cross-reactive recombinant pollen allergens.

In conclusion, we provide evidence that a few pollen allergens represent the primary sensitizing agents for the majority of patients with OAS caused by certain plant foods. This finding may lead to the development of novel forms of diagnosis and treatment that are based on the use of recombinant pollen allergens bearing the relevant epitopes of the cross-reactive food allergens.

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