

Recombinant birch pollen allergens (rBet v 1 and rBet v 2) contain most of the IgE epitopes present in birch, alder, hornbeam, hazel, and oak pollen: A quantitative IgE inhibition study with sera from different populations

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Background: Pollen from trees of the order Fagales are important allergen sources in most parts of the world. Clinical, immunochemical, and molecular biology studies indicate that they contain cross-reactive allergens. The major birch pollen allergen, Bet v 1, and birch profilin, Bet v 2, a highly cross-reactive allergen, have been cloned and expressed in *Escherichia coli*.

Objective: The purpose of this study was to demonstrate the presence of allergens in Fagales pollens that share IgE epitopes with recombinant Bet v 1 and Bet v 2 and to determine the percentage of birch, alder, hornbeam, hazel, and oak pollen-specific IgE that can be preabsorbed with rBet v 1 and rBet v 2 from 102 sera of different populations of subjects allergic to Fagales tree pollen.

Methods: The presence of rBet v 1- and rBet v 2-homologous allergens in tree pollen extracts was investigated by IgE immunoblot inhibition experiments, and the percentage of tree (birch, alder, hornbeam, hazel, and oak) pollen-specific IgE that was bound by a mixture of rBet v 1 and rBet v 2 was determined by RAST-based quantitative IgE inhibition experiments. The clinical significance of IgE antibody cross-reactivity was studied by skin prick testing with rBet v 1, rBet v 2, and Fagales pollen extracts.

Results: Natural birch, alder, hornbeam, hazel, and oak pollen contain allergens that share IgE epitopes with rBet v 1 and rBet v 2. A combination of rBet v 1 and rBet v 2 accounted for

82% of tree pollen-specific IgE on average. Most of the tree pollen-specific IgE was directed against rBet v 1.

Conclusion: rBet v 1 and rBet v 2 contain most of the Fagales pollen-specific IgE epitopes and may therefore substitute natural tree pollen extracts not only for diagnosis but also for patient-tailored immunotherapy of tree pollen allergy. (*J Allergy Clin Immunol* 1998;102:579-91.)

Key words: Recombinant birch pollen allergens, Bet v 1, profilin (Bet v 2), cross-reactivity, immunoabsorption, skin prick testing, tree pollen allergy

Pollen of trees belonging to the order Fagales (birch, alder, hazel, hornbeam, and oak) are important elicitors of allergic symptoms in the northern parts of Europe, North America, East Asia, Northwest Africa, and certain parts of Australia.¹⁻³ The prevalence of allergy to pollens of trees belonging to the Fagales order has been estimated to be 20% of the allergic individuals in Europe.^{4,5} Birch was identified as the most relevant allergen source among the Fagales trees for 2 reasons: (1) population studies indicated that most of the patients allergic to tree pollen are sensitized primarily to birch pollen and less to other Fagales pollens,^{4,6} and (2) birch pollen contained most of the IgE epitopes present in pollen of other Fagales species.⁶

The immunochemical and molecular characterization of the major tree pollen allergens has therefore led to the definition, purification, and partial amino acid sequence characterization of major allergens of birch and related trees.⁷⁻¹¹ Bet v 1, a 17-kd protein, has been identified as the major birch pollen allergen that shares epitopes with the major pollen allergens of trees belonging to the Fagales order and plant-derived food.⁸⁻¹⁸ Bet v 1 represents a target for IgE antibodies of more than 95% of patients allergic to birch pollen, and almost 60% of them are exclusively sensitized to Bet v 1.¹¹ Another well-characterized birch pollen allergen, Bet v 2 (birch profilin), is recognized by 10% to 20% of patients allergic to tree pollen and represents an important cross-reactive plant allergen that is present in tree, weed, and grass pollens, as well as plant-derived food.¹⁹⁻²⁴ The cDNAs coding for Bet v 1 and birch profilin (Bet v 2) have been iso-

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Abbreviations used

kUA: Kilounits of antigen

SPT: Skin prick test

lated recently.^{19,25} Recombinant Bet v 1 and Bet v 2 have been characterized extensively in vitro and in vivo. Both recombinant birch pollen allergens bind IgE antibodies from patients allergic to tree pollen,²⁶ elicit specific and dose-dependent histamine release from patients' basophils,²⁷ and stimulate Bet v 1-specific human T cells.²⁸ By means of qualitative IgE inhibition experiments, it was demonstrated that recombinant Bet v 1 and Bet v 2 share IgE epitopes with allergens from various pollens and plant-derived foods and thus can be responsible for clinically relevant IgE-mediated cross-reactions.^{18,20,21} Moreover, it could be demonstrated that rBet v 1 and rBet v 2 induced IgE responses in experimental animal models^{29,30} (mice and rhesus monkeys) and elicited immediate-type skin reactions,^{31,32} as well as nasal/bronchial hyperreactivity³³ in human subjects comparable with the those induced by the natural allergen preparations.

Because of the extensive in vitro and in vivo characterization of recombinant Bet v 1 and Bet v 2, both molecules are proposed to be used for diagnostic and therapeutic purposes.^{34,35} The concept of using only a few cross-reactive extracts/allergens for immunotherapy gained support from a previous study demonstrating that birch pollen extract is equally effective in treatment of allergy to Fagales pollens as a combination of alder, birch, and hazel.³⁶⁻³⁸ Prerequisites for the attempt to use rBet v 1 and rBet v 2 for treatment of tree pollen allergy are (1) that Bet v 1- and Bet v 2-homologous allergens are present in pollens of trees belonging to the Fagales order, (2) that rBet v 1 and rBet v 2 share a large proportion of the IgE epitopes with the homologous Fagales allergens, and (3) that patients from different countries display a similar IgE cross-reactivity to recombinant birch pollen allergens and their homologues.

In this study we investigate the presence of rBet v 1- and rBet v 2-homologous allergens in extracts of alder, hornbeam, hazel, and oak pollen by qualitative IgE-immunoblot inhibition experiments with sera from patients with well-defined IgE reactivity profiles to birch pollen allergens. In addition, we determine the proportion of IgE to birch, alder, hornbeam, hazel, and oak pollen that can be preabsorbed with rBet v 1 and rBet v 2 from sera of 102 patients allergic to tree pollen collected from different parts of Europe (Austria, France, Sweden, and Switzerland) by quantitative RAST inhibition experiments. The clinical relevance of IgE antibodies that cross-react with Bet v 1- and Bet v 2-homologous allergens was documented by skin prick tests (SPTs) of 20 patients with recombinant birch pollen allergens (rBet v 1 and rBet v 2) and birch, alder, hornbeam, hazel, and oak pollen extracts.

METHODS**Characterization of patients and sera**

For the immunologic characterization of natural tree pollen extracts by IgE-immunoblot inhibition, 11 sera from patients allergic to birch pollen were selected to comprise possible IgE reactivity profiles to birch pollen allergens.¹¹ Serum from a nonallergic person (serum 1) was included for control purposes. The 12 individuals were characterized by case history, SPTs, serology, and IgE immunoblotting with birch pollen extract (Table I). In addition, the sera were tested for reactivity to rBet v 1, rBet v 2, and rBet v 4.³⁹ Sera from patients with exclusive (sera 4 and 6) or almost exclusive (sera 11 and 12; additional weak IgE binding to components of 25 to 30 kD) reactivity to the major birch pollen allergen, Bet v 1, represent the most common reactivity profile to birch pollen.¹¹ In more rare cases patients are sensitized to other allergens (sera 3, 5, and 9) in addition to Bet v 1. An exclusive reactivity to allergens other than Bet v 1, as exemplified by sera 2, 8, and 10, can be observed only occasionally.¹¹

For the quantitative IgE inhibition experiments, sera from 102 patients allergic to tree pollen from Austria ($n = 38$), France ($n = 27$), Sweden ($n = 19$), and Switzerland ($n = 18$) were collected randomly. Inclusion criteria were a positive case history indicative for tree pollen allergy (at least 1 of the following symptoms: rhinitis, conjunctivitis, or asthma during spring) and a positive SPT response to at least birch pollen. SPTs with rBet v 1, rBet v 2, and natural birch, alder, hornbeam, hazel, and oak pollen extracts were performed in 20 of the Austrian patients (Tables II and III, a1-a20). Sera were characterized by assessment of total IgE and specific IgE antibodies to birch pollen extract and recombinant pollen allergens (rBet v 1 and rBet v 2) by means of the CAP system (Pharmacia) (Tables I through IV). The cut-off levels for a positive IgE reactivity to recombinant birch pollen allergens and natural allergen extracts in the CAP have been set to greater than 0.35 kilounits of antigen (kUA)/L by the manufacturer. The IgE reactivity profile of the 102 sera to nitrocellulose-blotted birch pollen extract was determined by immunoblotting (Tables I, II, IV).

Preparation and analysis of natural tree pollen extracts

Pollens from *Betula verrucosa*/birch, *Alnus glutinosa*/alder, *Carpinus betulus*/hornbeam, *Corylus avellana*/hazel, and *Quercus alba*/oak were purchased from Allergon (Välinge, Sweden). Pollens were pure as determined by light microscopy. Protein extracts were prepared by homogenization of 0.5 g pollen in 50 mL distilled water containing 5 mmol/L phenylmethylsulfonyl fluoride by using an ultraturax (Ika, Heidelberg, Germany). Proteins were extracted by shaking at 4° C for 3 hours. Extracts were then centrifuged at 16,000 g for 15 minutes to remove insoluble particles. The supernatants were frozen as 1-mL aliquots in liquid nitrogen, lyophilized, and stored at -20° C until use.⁴⁰ Lyophilized extracts were analyzed by SDS-PAGE⁴¹ and Coomassie Brilliant Blue staining (BioRad, Richmond, Calif)⁴² to estimate quality and quantity of proteins. Skin test solutions (birch, alder, hornbeam, hazel, oak pollen extracts, sodium chloride, and histamine) were purchased from ALK (Horsholm, Denmark).

Recombinant allergens

Recombinant birch pollen allergens (rBet v 1 and rBet v 2 [birch profilin]), which had been expressed in *Escherichia coli* and purified as described,^{43,44} 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.^{19,25} Recombinant birch pollen allergens used for SPTs were dissolved in sterile 0.9% sodium chloride at a concentration of 20 μ g/mL and 5 μ g/mL.

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

Patient	Sex	Demographic and clinical data				Total IgE (kU/L)	Birch (kUA/L)	rBet v 1 (kUA/L)	rBet v 2 (kUA/L)	IgE immunoblot (birch)				
		Age	Allergies	Symptoms	Treatment					<10 kd	14 kd	17 kd	25-30 kd	>45 kd
1	M	34				9.7	<0.35	<0.35	<0.35					
2	F	30	t,g,mi	r	ah	130	2.0	<0.35	<0.35					+
3	M	30	t,g,f,a	r,c	hyp,ah	553	49.6	51.8	<0.35	+		++		+++
4	F	29	t,g,a	r,c,as	ah	74.9	15.2	14.0	<0.35			+		
5	F	29	t,g,a	r,ad	ah	1371	96.8	91.7	1.4	±		+++		±
6	F	45	t,f	r,c	is	370	27.8	34.8	<0.35			++		
7	F	31	t,g,w,mi,a	r,c,as	hyp	242	10.5	2.8	9.8		++	±		+
8	M	35	t,g	r,c	ah	105	4.9	<0.35	3.3			±		+
9	M	38	t,g,mi,mi,a	r,c,as	hyp,ah	473	46.8	37.9	4.1	+	++	+++	+	++
10	M	49	t,g,w,f,a	r,c	ah	439	12.4	<0.35	15.4		+++			
11	M	32	t,g,f,mi,a	c,a	ah	193	44.3	68.2	<0.35			+++	±	
12	F	50	t,g	r,c	ah	196	19.3	24.4	<0.35			++	+	

Individuals (nonallergic person: 1; patients allergic to tree pollen: 2 to 12) are numbered as in Fig 1.

Allergies: t, Tree pollen; g, grass pollen; mi, mites; f, food; a, animals; w, weed pollen; mo, molds. Symptoms: r, Rhinitis; c, conjunctivitis; as, asthma; ad, atopic dermatitis. Treatment: ah, Antihistamines; hyp, tree pollen-specific hyposensitization; is, immunosuppression. Immunoblot: +++, Very strong; ++, strong; +, average; ±, weak binding.

IgE immunoblots and IgE immunoblot inhibition experiments

Approximately 200 µg/cm gel of each of the 5 tree pollen extracts were separated by preparative SDS-PAGE and blotted on nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany).⁴⁵ Nitrocellulose-blotted allergen extracts were probed with sera diluted 1:10 in buffer A (buffer A: 50 mmol/L Naphosphate [pH 7.5], 0.5% wt/vol BSA, 0.5% vol/vol Tween 20, and 0.05% wt/vol NaN₃), and bound IgE antibodies were detected with iodine 125 (¹²⁵I)-labeled anti-human IgE antibodies (RAST; Pharmacia, Uppsala, Sweden) and visualized by autoradiography with KODAK X-OMAT films and intensifying screens (Kodak, Heidelberg, Germany) at -70° C. The presence of rBet v 1- and rBet v 2-homologous allergens in alder, hornbeam, hazel, and oak pollen extracts was studied by IgE immunoblot inhibition experiments. One milliliter of 1:10 diluted serum was preincubated with a mixture of rBet v 1 and rBet v 2 (10 µg each) or, for control purposes, with 20 µg BSA before exposure to nitrocellulose-blotted tree pollen extracts. Bound IgE was detected as described above. Because hazel, hornbeam, and oak pollen possessed a much weaker IgE-binding capacity compared with birch and alder pollen, different exposure times (birch and alder, 16 hours; hazel and hornbeam, 40 hours; and oak, 64 hours) were chosen (Fig 1).

Quantitative IgE inhibition experiments

Quantitative IgE inhibition experiments were performed in a manner similar to that for RAST inhibition experiments. Approximately 50 µg of nondenatured PBS-dissolved pollen extract (birch, alder, hornbeam, hazel, and oak) was dotted on nitrocellulose strips of exactly 6 × 30 mm in size. To ensure that the absorption experiment is performed at conditions of antigen excess, appropriate serum dilutions and amounts of recombinant allergens were determined in pilot experiments. One milliliter aliquots of sera diluted 1:10 in buffer A were preincubated with a mixture of rBet v 1 and rBet v 2 (10 µg each) or, for control purposes, with 20 µg BSA before exposure to nitrocellulose-dotted pollen extracts. All determinations were performed as duplicates. Bound IgE was detected with ¹²⁵I-labeled anti-human IgE antibodies and quantified by gamma counting (Wizzard, Automatic Gamma Counter; Wallac, Uppsala, Sweden). The percentage inhibition of IgE binding to natural extracts after preabsorption with rBet v 1 and rBet v 2 was calculated as follows: %inhibition = 100 - cpm (rBet v 1 + rBet v 2) × 100/cpm(BSA).

SPT experiments

SPTs with natural allergen extracts, rBet v 1, and rBet v 2 were performed in 20 patients allergic to tree pollen as described.^{31,32} The skin was pricked with prick lancets (ALK), and reactions were recorded 20 minutes thereafter by photography and by transferring the ballpoint pen-surrounded wheal area with a scotch tape to paper. The mean wheal diameter (MD) was calculated by measuring the maximal longitudinal diameter (D) and the maximal transversal diameter (d) according to the following formula: [D + d]/2 = MD.

RESULTS

Sera with defined reactivity profile to birch pollen allergens as tools to compare the allergen-profile of birch with that of alder, hornbeam, hazel, and oak pollen

To study the allergen profile in alder, hornbeam, hazel, and oak pollen extracts, we used sera from patients with a well-characterized IgE reactivity profile to birch pollen allergens. The group of patients allergic to birch pollen consisted of 6 women and 5 men with a mean age of 36 years. Demographic and clinical data of the 11 patients and the nonallergic control person are summarized in Table I. The most common IgE reactivity profile to birch pollen allergens (ie, exclusive or almost exclusive IgE reactivity to the major birch pollen allergen, Bet v 1, at 17 kd) is represented by sera 4, 6, 11, and 12 (Table I; Fig 1, A). These sera reacted with the Bet v 1-homologous allergens, Aln g 1^{10,46} (alder), Car b 1^{10,47} (hornbeam), Cor a 1^{10,48} (hazel), and Que a 1¹⁰ (oak) at approximately 17 kd (Fig 1, B through E, lanes -). Sera (eg, 7, 9, and 10) with reactivity to birch profilin (Bet v 2) at approximately 12 to 14 kd (Fig 1, A, lanes -) bound to profilin-related allergens in all Fagales pollen extracts tested (Fig 1, B through E, lanes -). Bet v 4, a very recently characterized 2 EF-hand calcium-binding allergen of approximately 8 to 9 kd,^{39,49} was detected by sera 3, 5, and 9 in

birch pollen (Fig 1, A, lanes –). The Bet v 4–reactive sera bound to a Bet v 4–related allergen of 8 to 9 kd in alder, hornbeam, and oak pollen extracts (Fig. 1, B, C, and E). The high molecular weight IgE-reactive bands (45 to 100 kd) detected in birch pollen by sera 2, 3, 5, 7, 8, and 9 were found in all Fagales pollen extracts (Fig 1, B through E). In addition to Bet v 5, a pectinesterase-like allergen,⁵⁰ they may contain other cross-reactive moieties. In summary, we found that alder, hornbeam, hazel, and oak pollen extracts contain an allergen profile that is almost identical to that of birch pollen. Although certain sera contained IgE antibodies with specificities directed to molecules other than Bet v 1– and Bet v 2–related allergens (eg, Bet v 4; high molecular weight bands), the quantitative IgE measurements (Table I) indicated that only sera from Bet v 1– and Bet v 2–reactive patients contained high levels of birch pollen–specific IgE. Serum 2, which did not react with Bet v 1 or Bet v 2, had a very low (2.0 kUA/L) birch pollen–specific IgE level (Table I).

Alder, hornbeam, hazel, and oak pollen contain rBet v 1 and rBet v 2 cross-reactive allergens

The qualitative analysis of birch, alder, hornbeam, hazel, and oak pollen extracts with selected sera (Table I; Fig 1, A through E) indicated the presence of Bet v 1 and Bet v 2 cross-reactive allergens. To demonstrate that the Bet v 1– and Bet v 2–related Fagales allergens share IgE epitopes with rBet v 1 and rBet v 2, IgE immunoblot inhibition experiments were performed. Sera from the patients allergic to tree pollen described in Table I were preabsorbed with a mixture of rBet v 1 and rBet v 2 and, for control purposes, with a comparable amount of BSA before exposure to nitrocellulose-blotted pollen extracts. Preabsorption of Bet v 1–reactive sera (sera 3, 4, 5, 6, 9, 11, and 12) with the allergen combination resulted in a great reduction or loss of IgE binding to the rBet v 1–homologous allergens present in birch, alder, hornbeam, hazel, and oak pollen extracts (Fig 1 A through E, lanes +). The IgE reactivity of sera 7, 9, and 10 to Bet v 2 and Bet v 2–related Fagales allergens likewise was greatly reduced after preincubation with rBet v 2 (Fig 1 A through E, lanes +). The results of the IgE immunoblot inhibition thus identify rBet v 1 and rBet v 2 cross-reactive allergens in pollens of alder, hornbeam, hazel, and oak.

Recombinant birch pollen allergens (rBet v 1 and rBet v 2) and nitrocellulose-blotted birch pollen extract define the IgE reactivity profile (allergogram)

Before immunoabsorption experiments, the IgE reactivity profile to birch pollen extract, rBet v 1, and rBet v 2 was determined by quantitative CAP (RAST) measurements for the 102 European sera (Sweden, $n = 19$; France, $n = 27$; Austria, $n = 38$; and Switzerland, $n = 18$), and immunoblotting experiments (birch pollen extract) were performed. One hundred of 102 sera (99%) contained IgE antibodies to rBet v 1, and 14.7% displayed IgE reactivity to rBet v 2. In all but 4 sera (Tables II and

IV: *a13*, *f25*, *swe3*, and *swe12*) rBet v 1 accounted for most of the birch pollen–specific IgE. In many cases (eg, Tables II and IV: *a5*, *a19*, *f2*, *f9*, *f10*, *f27*, *swe5*, *swe7*, *swe8*, *swe16*, *swi3*, *swi6*, *swi9*, *swi10*, and *swi13*) rBet v 1 bound even more IgE than total birch pollen extract, indicating a better coupling efficacy of the purified rBet v 1 compared with its natural counterpart being part of the total birch pollen extract. Although certain sera displayed IgE reactivity to allergens other than Bet v 1 and Bet v 2 in the immunoblotting experiments (Tables II and IV: components of <10 kd, 20 kd, 25 to 30 kd, and >46 kd), rBet v 1 represented by far the most frequently (99% of positive sera) detected allergen and bound most of the birch pollen–specific IgE.

Preferential sensitization to birch pollen in different parts of Europe

Our study supports early observations obtained for Sweden,^{4,6} which indicated that birch may represent the most potent sensitizing allergen source among trees belonging to the Fagales order for the following reasons. (1) Sera from all 102 patients allergic to tree pollen contained IgE antibodies to birch extract or to natural/recombinant Bet v 1. (2) Sera from patients allergic to tree pollen failed to display IgE reactivity to Fagales pollen extracts other than birch, depending on the distance of the taxonomic relationship to birch. Twenty-six sera failed to react with oak, 13 with hornbeam, 11 with hazel, and 6 with alder pollen extract (Tables II and IV). (3) The IgE binding (average of the 102 sera) to the pollen extracts as represented by the cpm values of bound ¹²⁵I-labeled anti-human IgE antibodies were as follows: birch, 2215 cpm; alder, 1540 cpm; hornbeam, 652 cpm; hazel, 723 cpm; and oak, 289 cpm. In summary, birch pollen appeared to be by far the most prominent source of allergens/IgE epitopes.

A combination of rBet v 1 and rBet v 2 contains most of the IgE epitopes present in Fagales pollens

The quantitative CAP measurements indicated that a combination of rBet v 1 and rBet v 2 accounts for a high percentage of birch pollen–specific IgE. To estimate the amount of IgE to Fagales pollen extracts that can be preabsorbed with a mixture of rBet v 1 and rBet v 2, RAST-based IgE inhibition experiments were performed. Sera from 102 patients allergic to European tree pollen were preabsorbed with rBet v 1 and rBet v 2 before exposure to birch, alder, hornbeam, hazel, and oak pollen extract. Tables II and IV detail the percentage inhibition of IgE binding to the 5 Fagales pollen extracts for each serum. The results indicate that a mixture of rBet v 1 and rBet v 2 contains most of the IgE epitopes present in Fagales pollen. On an average, 82% of Fagales-specific IgE could be preabsorbed with the 2 recombinant allergens (Table V). Some relatively minor variations in the degree of inhibition of IgE binding were found, which were dependent on patient populations and botanical relationships of tree species. Regarding the popu-

TABLE II. Serologic characterization of sera from Austria and Switzerland

Patient	Total IgE (kU/L)	Birch (kUA/L)	Bet v 1 (kUA/L)	Bet v 2 (kUA/L)	IgE immunoblot (birch)						%inhibition of IgE binding				
					<10 kd	14 kd	17 kd	20 kd	25-30 kd	>46 kd	Bet v	Aln g	Car b	Cor a	Que a
a1	359	43.1	47.8	<0.35			+++				92	85	66	71	44
a2	55.8	1.53	1.61	<0.35			+				93	96	93	87	—
a3	39.7	2.43	2.34	<0.35			+				91	76	70	86	74
a4	589	33.0	21.2	9.97		++	+++				94	93	82	87	87
a5	363	12.1	18.1	1.62		+	+++				94	96	87	84	89
a6	476	6.14	3.43	5.06	±	±	+				90	88	82	88	87
a7	553	49.6	51.8	<0.35	+		++			+++	75	77	61	27	31
a8	196	58.3	48.4	<0.35			+++		±		82	94	37	57	37
a9	483	3.66	4.47	<0.35	±	±	+			+	49	50	0	3	18
a10	56.8	6.38	6.23	<0.35			++				78	50	36	32	36
a11	317	82.3	62.9	6.81	+	++	+++				96	93	86	62	76
a12	344	95.3	84.7	<0.35		+	+++		+		90	80	76	81	81
a13	169	6.09	<0.35	5.48		+					77	91	65	73	88
a14	81.5	8.22	9.92	<0.35			++				97	89	70	62	26
a15	>2000	93.2	92.1	<0.35			+++				81	89	35	74	48
a16	200	18.9	15.2	6.70		+	+++		+		89	95	77	73	89
a17	151	12.7	12.4	<0.35		±	++				98	98	89	91	74
a18	248	16.1	16.9	<0.35			++				91	81	60	80	29
a19	45.1	24.6	25.5	<0.35			+++				96	100	96	95	99
a20	518	49.0	29.1	4.24	+	++	+++	+	+	+	31	35	60	16	28
a21	167	14.5	14.1	<0.35			++				90	89	—	71	25
a22	49.0	3.69	3.69	<0.35			+				98	77	41	70	0
a23	26.2	4.41	5.44	<0.35			+				99	100	96	99	22
a24	ND	ND	ND	ND			+				97	100	86	97	97
a25	>2000	25.3	24.9	<0.35			++			+	100	99	79	65	—
a26	27.6	19.0	15.5	<0.35			+		+		95	74	—	78	100
a27	48.8	21.7	20.1	<0.35			+				100	99	88	24	—
a28	84.0	61.1	54.3	<0.35			+++		+		100	100	93	96	100
a29	1020	34.1	30.9	<0.35			++			+	96	96	71	68	37
a30	89.8	13.9	13.3	<0.35			++				100	100	82	—	—
a31	65.0	23.8	22.9	<0.35			++				97	98	89	97	83
a32	15.2	3.53	3.69	<0.35			+				100	100	89	100	100
a33	152	41.6	38.3	<0.35			+++			±	96	97	84	68	75
a34	45.3	31.5	30.3	<0.35			+++			+	82	89	85	72	93
a35	105	8.31	8.99	<0.35			+			±	93	98	67	60	30
a36	44.9	13.5	13.4	<0.35			++				100	100	87	89	96
a37	160	47.0	51.5	<0.35			+++				99	97	70	74	95
a38	36.9	3.88	4.08	<0.35			+				97	81	79	—	39
swi1	115	13	12.8	<0.35			++				84	45	72	69	100
swi2	385	3.6	3.4	<0.35			+				99	100	96	100	—
swi3	230	26.4	32.2	<0.35			++				100	100	97	97	100
swi4	186	7.53	5.29	<0.35			+				100	100	91	85	100
swi5	337	47.7	45.9	1.01			++				100	100	91	86	73
swi6	275	6.61	8.4	<0.35			+			+	67	100	82	74	21
swi7	1320	>100	97.5	<0.35			+++				89	100	97	99	97
swi8	193	4.95	6.19	<0.35			+				100	100	80	—	—
swi9	314	25.1	31.0	<0.35			++				100	100	99	98	—
swi10	291	51.1	64.7	<0.35			+++		+	+	99	99	93	83	98
swi11	29.1	6.41	9.16	<0.35			+				100	100	85	—	—
swi12	186	26.8	14.4	<0.35		+	+				90	86	97	96	91
swi13	332	59.1	71.3	<0.35			+++				100	100	96	87	98
swi14	393	28.6	29.5	<0.35			++				98	99	87	14	49
swi15	286	82.5	76.4	<0.35			++				100	99	93	74	100
swi16	87.2	14.9	16.3	<0.35			++				100	98	91	98	—
swi17	91.3	6.64	9.20	<0.35			+				100	100	84	89	—
swi18	394	28.5	27.0	<0.35			++				98	100	78	87	—

Total IgE, birch pollen-specific IgE, and IgE to rBet v 1 and rBet v 2 were determined by CAP measurement. The IgE reactivity to components of certain molecular weights (<10 kd, 14 kd, 17 kd, 20 kd, 25 to 30 kd, and >46 kd) in nitrocellulose-blotted birch pollen extract is displayed for each serum (+++, Very intensive; ++, intensive; +, distinct; ±, weak IgE binding). The percentage inhibition of IgE binding to birch (*Bet v*), alder (*Aln g*), hornbeam (*Car b*), hazel (*Cor a*), and oak (*Que a*) measured after preabsorption of each serum with a combination of rBet v 1 and rBet v 2 is also shown. Dashes indicate lack of IgE reactivity to the given extract. a, Austrian; swi, Switzerland; ND, not done.

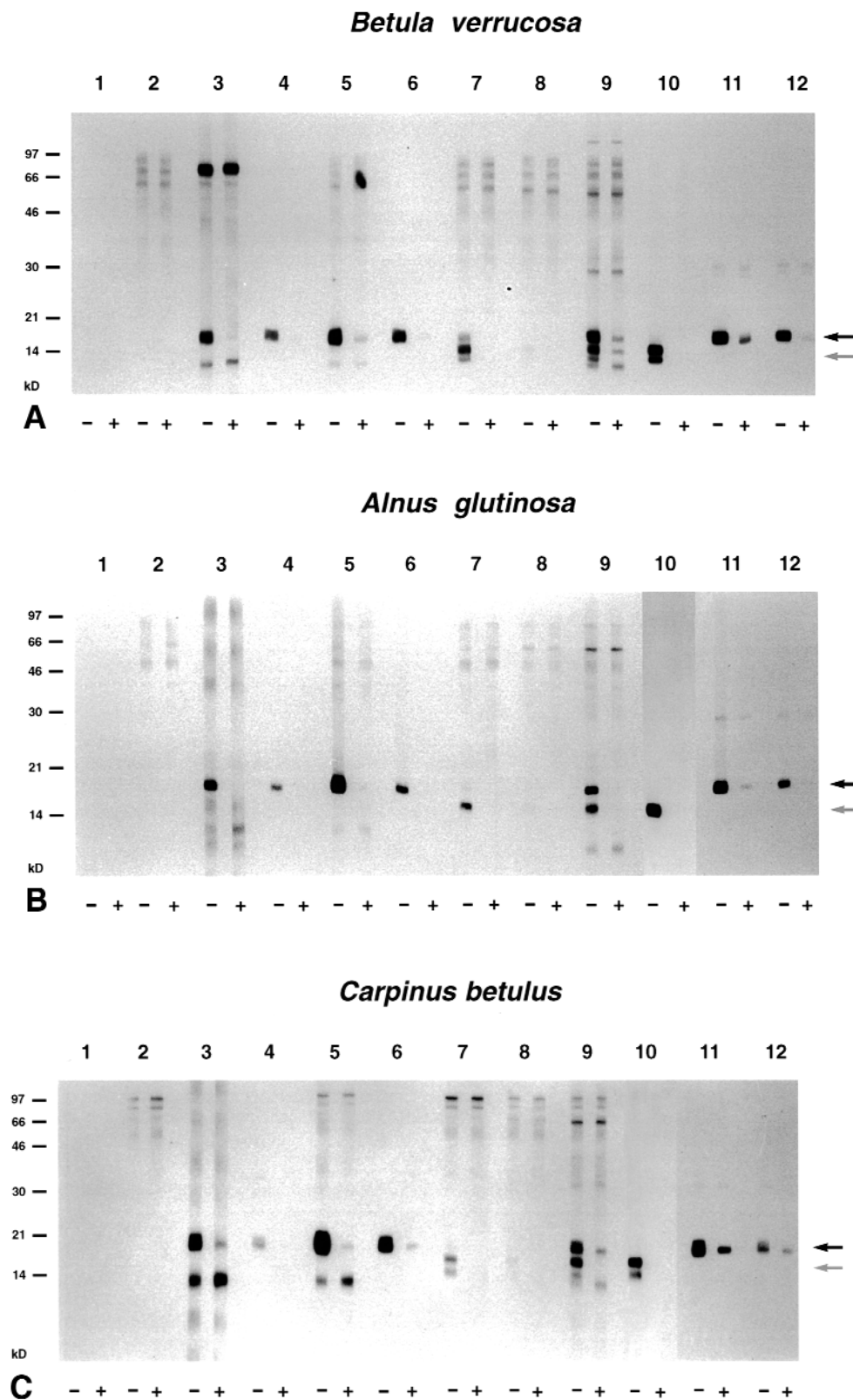


FIG 1. Inhibition of IgE binding to nitrocellulose-blotted natural tree pollen extracts (**A**, *Betula verrucosa*/birch; **B**, *Alnus glutinosa*/alder; and **C**, *Carpinus betulus*/hornbeam) with a combination of rBet v 1 and rBet v 2.

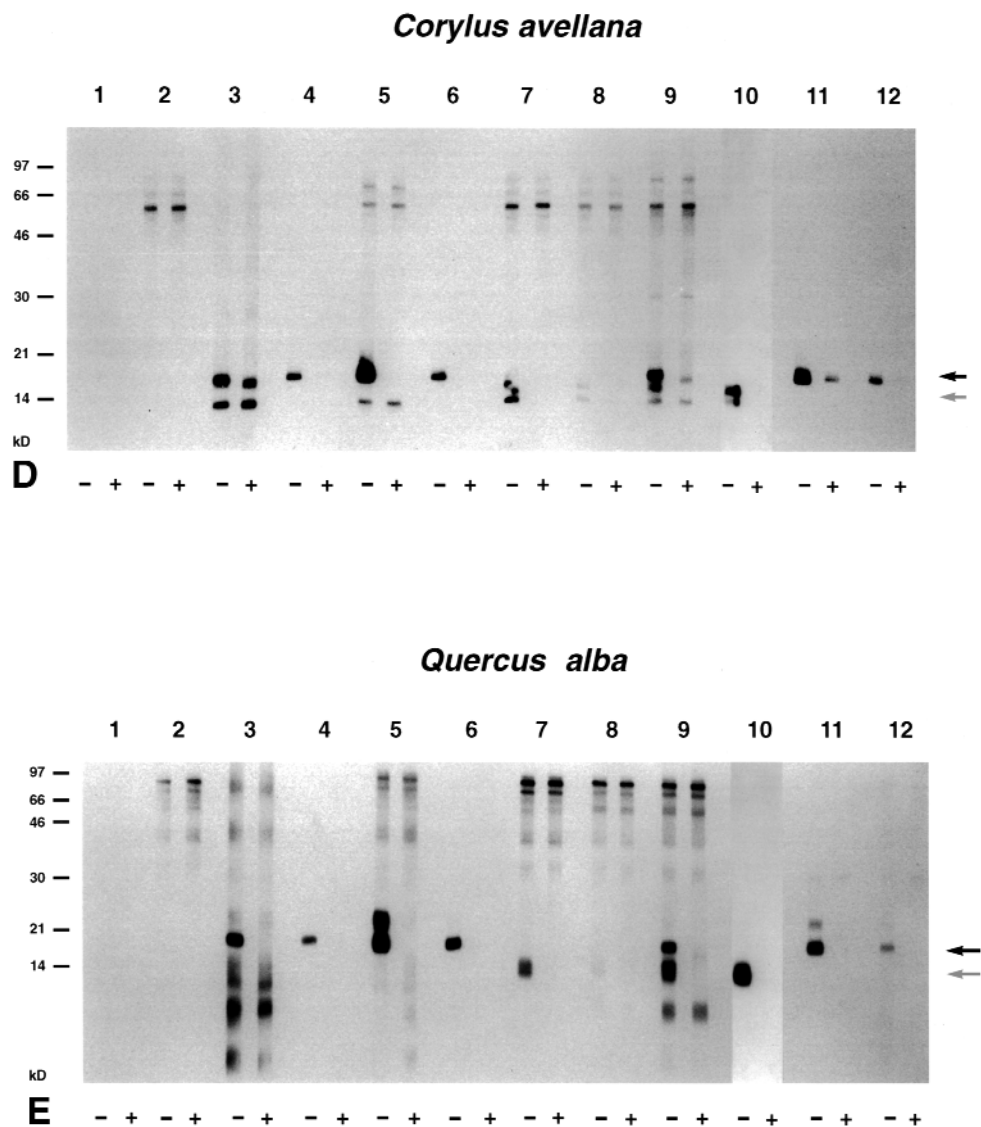


FIG 1. Cont'd. Nitrocellulose-blotted tree pollen extracts (**D**, *Corylus avellana*/hazel; and **E**, *Quercus alba*/oak) were probed with sera from 11 representative patients allergic to tree pollen (*panels 2 to 12*) and, for control purposes, with serum from a nonallergic individual (*panel 1*). Sera were preabsorbed with a mixture of rBet v 1 and rBet v 2 (*lanes +*) or, for control purposes, with BSA (*lanes -*). Molecular weights are displayed at left. *Black arrows* indicate position of Bet v 1 and Bet v 1-homologous allergens, and *grey arrows* indicate those of Bet v 2 and Bet v 2-homologous allergens.

TABLE III. RAST results and skin reactivity to rBet v 1, rBet v 2, and natural tree pollen extracts

Patient	rBet v 1			rBet v 2			Birch	
	RAST (kUA/L)	SPT (5 mg/mL) MD	SPT (20 mg/mL) MD	RAST (kUA/L)	SPT (5 mg/mL) MD	SPT (20 mg/mL) MD	RAST (kUA/L)	SPT (MD)
a1	47.8	5	5	<0.35	—	—	43.1	9
a2	1.61	3	5	<0.35	—	—	1.53	4.5
a3	2.34	4	5.5	<0.35	—	—	2.43	4.5
a4	21.2	10	9.5	9.97	4.5	10.5	33.0	8.5
a5	18.1	6.5	10.5	1.62	3	5.5	12.1	7
a6	3.43	3.5	7	5.06	3	7.5	6.14	6
a7	51.8	8	19	<0.35	—	—	49.6	10.5
a8	48.4	10.5	10.5	<0.35	—	—	58.3	11
a9	4.47	5.5	7.5	<0.35	—	—	3.66	6
a10	6.23	6	8	<0.35	—	—	6.38	8
a11	62.9	8.5	9.5	6.81	6.5	7	82.3	7.5
a12	84.7	4.5	8.5	<0.35	—	—	95.3	8
a13	<0.35	—	—	5.48	—	4	6.09	2.5
a14	9.92	5.5	12.5	<0.35	—	—	8.22	8.5
a15	92.1	6.5	9	<0.35	—	—	93.2	7.5
a16	15.2	3.5	6.5	6.70	—	4.5	18.9	6
a17	12.4	3	11	<0.35	—	—	12.7	7
a18	16.9	2	2.5	<0.35	—	—	16.1	3
a19	25.5	—	6	<0.35	—	—	24.6	5
a20	29.1	5	11	4.25	4	5	49.0	13
N1	<0.35	—	—	<0.35	—	—	<0.35	—
N2	<0.35	—	—	<0.35	—	—	<0.35	—

MD, Mean diameter of the wheal reaction in millimeters; N, nonatopic individual; —, no reaction.

Serum IgE levels and skin reactions to rBet v 1, rBet v 2, birch, alder, hornbeam, hazel, and oak pollen extracts and histamine are displayed for 20 patients allergic to Austrian tree pollen (a1-a20; see also Table II) and 2 nonatopic individuals (N1, N2).

lations, we found that most Fagales-specific IgE was depleted from the Swiss sera (90%) and less from the Austrian and French sera (80% and 83%, respectively) (Table V). The lowest degree of inhibition was obtained for the Swedish sera (75%), which, according to the immunoblotting experiments, frequently reacted with molecules other than Bet v 1 and Bet v 2 (Tables II, IV, and V).

The combination of rBet v 1 and rBet v 2 inhibited IgE binding to pollen extracts derived from trees taxonomically more closely related to birch (alder, Betulaceae; hornbeam and hazel, Corylaceae) better than to the more distantly related species (oak, Fagaceae) (Table V). The average inhibition of IgE binding to alder pollen extract was 88%, to hornbeam 77%, to hazel 80%, and to oak 72% (Table V).

IgE cross-reactivity to Bet v 1- and Bet v 2-homologous Fagales pollen allergens: Immediate-type skin reactions to multiple extracts

Twenty patients experiencing clinical symptoms during spring (ie, the flowering period of trees of the Fagales order) and, for control purposes, 2 nonatopic individuals underwent SPTs with rBet v 1, rBet v 2, birch, alder,

hazel, hornbeam, and oak pollen extracts. Results obtained showed that with the exception of only 3 patients who failed to mount immediate-type skin reactions to oak pollen extract (Tables II and III: a2, a9, and a13), all 20 patients displayed immediate-type skin reactions to all of the 5 tree pollen extracts, as well as to rBet v 1 or rBet v 2 (Table III). Fig 2 shows the photodocumentation of the SPT results obtained for representative patients a1, a2, a3, a4, and a5 (Tables II and III). Serum samples obtained from these patients were analyzed for the presence of specific IgE antibodies, and the percentage of tree pollen-specific IgE that could be preabsorbed with rBet v 1 and rBet v 2 was determined (Table II). Preabsorption of the sera from these 20 patients with recombinant birch pollen allergens yielded a similarly high inhibition of IgE binding to natural pollen extracts as observed for sera of the other populations (Table II).

DISCUSSION

Pollen of trees belonging to the order Fagales (birch, alder, hornbeam, hazel, and oak) belong to the most potent elicitors of Type I allergy in early spring.¹⁻³ In this study we demonstrate that pollen from alder, hornbeam, hazel, and oak contain allergens that share IgE epitopes

Alder		Hornbeam		Hazel		Oak		Histamine
RAST (kUA/L)	SPT (MD)	RAST (kUA/L)	SPT (MD)	RAST (kUA/L)	SPT (MD)	RAST (kUA/L)	SPT (MD)	SPT (MD)
32.9	11	13.7	11.5	30.3	8	11.0	7.5	6.5
1.37	5.5	0.70	5	1.50	4	<0.35	—	5.5
1.87	5.5	1.71	5.5	1.92	4	1.56	2	4
25.6	7.5	19.7	12.5	20.1	8	19.2	4.5	5
9.98	5	7.19	7	5.06	5.5	4.34	3.5	3
5.15	5	3.94	3.5	2.90	4	3.52	6.5	4.5
33.6	14	36.7	16	31.2	10.5	34.9	5	7.5
46.9	7.5	23.2	8	35.7	9.5	10.8	4.5	5.5
3.91	5.5	2.29	2.5	1.30	2.5	1.66	—	7
4.69	8.5	2.75	10.5	4.41	6.5	2.87	5.5	6
47.4	6.5	36.1	12.5	26.0	7.5	32.5	9.5	3.5
74.4	8	43.8	5	51.0	7.5	25.4	6.5	4
6.29	3	5.98	3	4.67	2	4.87	—	4.5
5.48	6	2.18	6	5.62	6	2.40	3	3.5
69.5	7	63.0	9	81.4	10	65.2	5.5	8.5
18.5	10.5	14.2	5	16.3	4.5	9.92	2.5	4
9.95	4.5	4.73	4.5	5.74	6	3.75	2.5	6
13.6	2.5	8.98	2	15.2	2.5	6.73	2	2
18.9	6	18.1	4	19.8	3	12.3	6.5	2
36.8	11	39.7	7	14.8	9	35.5	5	4
<0.35	—	<0.35	—	<0.35	—	<0.35	—	3.5
<0.35	—	<0.35	—	<0.35	—	<0.35	—	4.5

with recombinant birch pollen allergens (rBet v 1),¹⁹ the major birch pollen allergen, and rBet v 2 (birch profilin).²⁵ By means of quantitative IgE inhibition studies performed with 102 sera from different European populations (Austria, France, Sweden, and Switzerland) we show that a combination of rBet v 1 and rBet v 2 contains most (82%) of the IgE epitopes present in Fagales pollens. Although previous immunochemical, protein-chemical, and molecular biologic investigations have already indicated that Fagales pollens contain structurally related allergens that share IgE epitopes,^{10,12,46-48,51} it was unexpected to find that a combination of only 2 recombinant birch pollen allergens, rBet v 1 and rBet v 2, accounts for such high levels (average 82%) of Fagales pollen-specific IgE. This result is also interesting in light of reports that major tree pollen allergens are composed of several isoforms (ie, variants with minor sequence variations that can, however, lead to dramatically altered antibody binding and T-cell stimulating capacity of the individual isoforms).^{47,48,52,53} Most of the reported isoforms with altered antibody-binding capacity were, however, obtained by PCR-based technologies, and only for a few of them could the existence of corresponding natural isoforms be shown by extensive protein-chemical work.⁵² The reason for the high IgE-binding capacity of rBet v 1

and rBet v 2 used in our study may be that both recombinant birch allergens are derived from cDNA clones that were isolated by immunoscreening with serum IgE from allergic patients and therefore most closely correspond to their natural counterparts.^{19,25} We believe that the definition of 2 recombinant birch pollen allergens (rBet v 1 and rBet v 2) that share most of the IgE epitopes with natural Fagales pollen extracts may have implications for diagnosis, as well as specific immunotherapy.

Regarding diagnosis, our data indicate that it is possible to use only a few recombinant birch pollen allergens (eg, rBet v 1 and rBet v 2) for in vitro and probably in vivo diagnosis of not only birch but also alder, hornbeam, hazel, and oak pollen allergy. A positive reaction to each of these molecules together with clinical symptoms in response to exposure to birch, alder, hornbeam, hazel, or oak pollen will define a patient as being allergic to tree pollen. This assumption was supported by our finding that patients containing IgE antibodies that cross-reacted with Bet v 1- or Bet v 2-homologous allergens present in pollen extracts of trees of the order Fagales (birch, alder, hornbeam, hazel, and oak) displayed immediate-type skin reactions to the latter extracts.

As a logical consequence, recombinant birch pollen allergens (eg, rBet v 1 and rBet v 2) may substitute crude

TABLE IV. Serologic characterization of sera from Sweden and France

Patient	Total IgE (kU/L)	Birch) (kUA/L)	Bet v 1 (kUA/L)	Bet v 2 (kUA/L)	IgE immunoblot (birch)						% inhibition of IgE binding				
					<10 kd	14 kd	17 kd	20 kd	25-30 kd	>46 kd	Bet v	Aln g	Car b	Cor a	Que a
swe1	211	>100	>100	<0.35			+++				100	100	100	77	—
swe2	262	22.3	27.4	<0.35			++		+		93	65	—	100	89
swe3	348	13.5	3.74	7.4	+	++	++			+	67	83	54	54	56
swe4	50	8.9	8.5	1.3		+	+				87	89	93	96	92
swe5	140	72.4	88.4	<0.35			+++				99	100	96	98	69
swe6	73	13.3	10.0	<0.35			+		+		82	70	51	55	44
swe7	145	32.6	41.8	<0.35			++	+			95	56	25	70	5
swe8	120	44.7	52.0	<0.35			+++				100	100	100	97	99
swe9	789	10.3	8.4	<0.35			+				95	—	56	—	—
swe10	380	62.6	82.4	<0.35	±		+++	±	++		94	97	71	98	100
swe11	315	14.0	15.9	<0.35			+			+	95	97	19	80	—
swe12	592	80.1	40.6	0.6	+	+	+	+	+++	+	90	56	14	4	28
swe13	121	4.0	4.1	<0.35			+				87	16	—	79	—
swe14	161	29.5	32.6	<0.35			++	+	+		89	87	61	67	0
swe15	356	8.4	10.0	<0.35			+				95	—	—	—	—
swe16	277	41.3	52.7	<0.35			++				100	100	97	—	81
swe17	396	12.5	14.1	<0.35			+				97	100	—	—	—
swe18	126	4.24	3.8	<0.35	±		+		+		81	59	100	79	—
swe19	280	10.5	11.5	<0.35			+				100	—	—	—	—
f1	121	16.0	20.9	ND			+				59	92	—	97	100
f2	299	71.9	81.5	<0.35		+	++			+	81	80	41	63	75
f3	389	32.1	44.3	ND			++				97	94	76	97	87
f4	1216	>100	>100	4.4	+	+	+++	+	+	+	87	88	68	79	55
f5	>2000	>100	>100	1.6		±	+++				99	97	94	92	81
f6	360	>100	>100	<0.35			+++				100	99	94	100	99
f7	158	31.0	36.7	2.6			++				99	100	89	99	100
f8	30	8.1	10.0	ND			+				100	100	—	96	—
f9	201	82.4	>100	<0.35			++				100	98	91	100	100
f10	168	48.1	80.9	<0.35			++				99	99	92	97	97
f11	38	7.9	10.8	<0.35			+				93	—	—	—	—
f12	184	21.0	25.5	<0.35			+				99	99	91	87	86
f13	990	9.7	11.4	<0.35			+			+	42	70	49	34	0
f14	301	>100	>100	ND			++				99	100	78	100	—
f15	254	93.8	>100	<0.35			+++	+			93	95	84	59	66
f16	590	>100	>100	<0.35			+++			+	90	86	73	66	90
f17	425	96.7	83.9	<0.35			++				100	100	98	100	100
f18	106	18.3	22.8	<0.35			+				98	99	89	93	—
f19	107	7.7	9.4	<0.35			+				100	99	81	73	—
f20	294	22.9	9.7	<0.35	++		+				65	0	—	35	4
f21	70	20.5	18.5	<0.35			++				88	91	65	74	89
f22	113	4.8	5.4	<0.35			+				100	—	100	100	—
f23	61	30.4	38.1	<0.35			++				100	100	71	82	—
f24	1096	>100	>100	<0.35			+++				97	87	—	97	93
f25	604	4.0	<0.35	1.8	±	±	±			+	63	65	—	59	69
f26	117	1.6	1.9	<0.35			+				79	—	25	—	35
f27	136	44.5	60.4	<0.35			+++				99	95	89	87	96

Total IgE, birch pollen-specific IgE, and IgE to rBet v 1 and rBet v 2 were determined by CAP measurement. The IgE reactivity to components of certain molecular weights (<10 kd, 14 kd, 17 kd, 20 kd, 25 to 30 kd, and >46 kd) in nitrocellulose-blotted birch pollen extract is displayed for each serum (+++, Very intensive; ++, intensive; +, distinct; ±, weak IgE binding). The percentage inhibition of IgE binding to birch (*Bet v*), alder (*Aln g*), hornbeam (*Car b*), hazel (*Cor a*) and oak (*Que a*), measured after preabsorption of each serum with a combination of rBet v 1 and rBet v 2 is also shown. Dashes indicate lack of IgE reactivity to the given extract. swe, Sweden; f, France; ND, not done.

Fagales pollen extracts for in vitro and in vivo allergy diagnosis in most cases. The advantage of using recombinant birch pollen allergens for diagnosis of Fagales pollen allergy would be the possibility of determining

exactly which components the patient is sensitized against and measuring the levels of IgE antibodies against the individual molecules. In this context it is relevant to note that rBet v 1 and rBet v 2 not only cross-

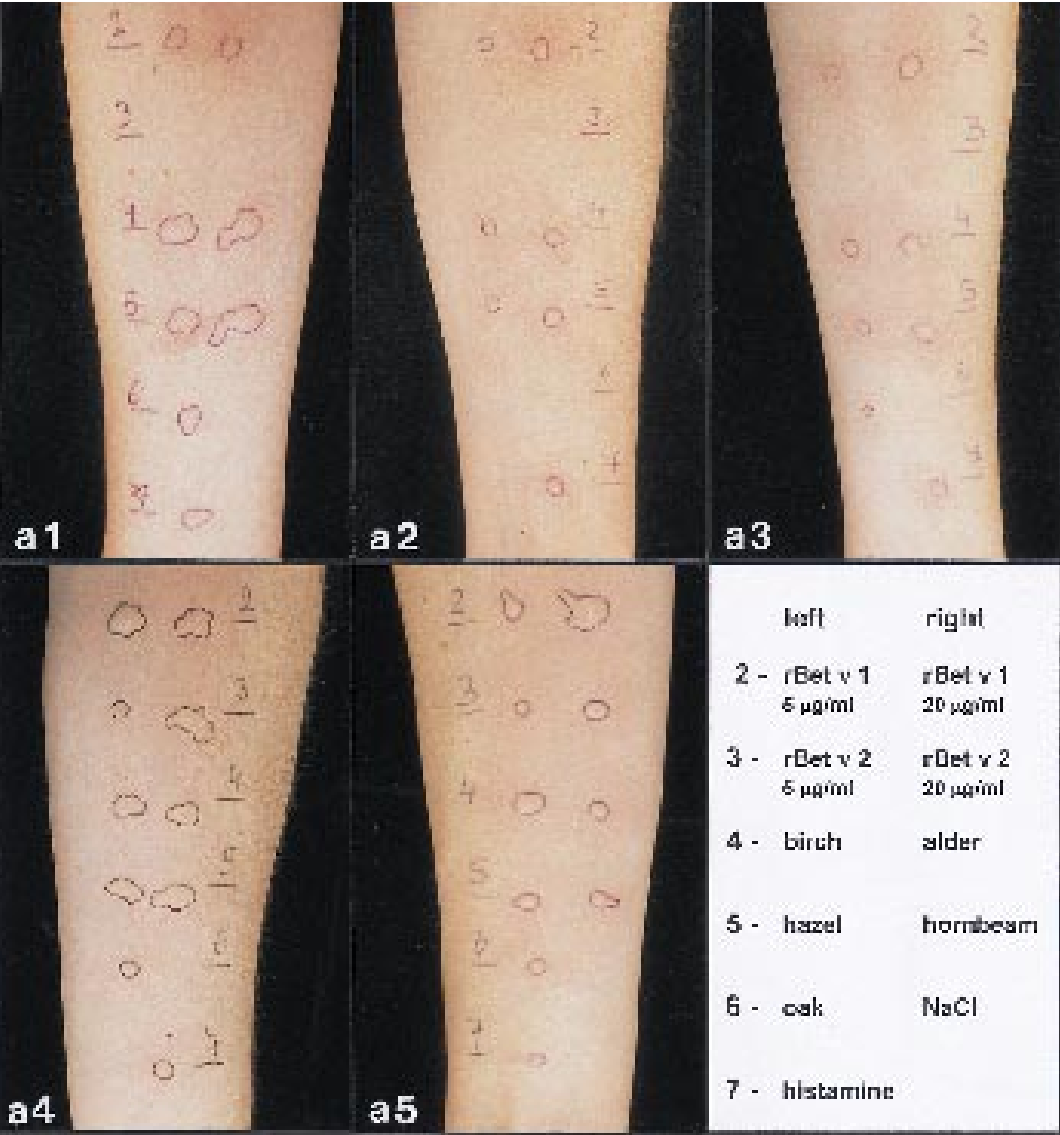


FIG 2. Photodocumentation of immediate-type skin reactions to recombinant birch pollen allergens and natural tree pollen extracts of 5 representative patients (*a1, a2, a3, a4, a5*; see Table III). Application order of test substances is indicated in *insert*.

TABLE V. Average percentage inhibition of IgE binding

Sera	Natural tree pollen extracts					Mean
	Betula verrucosa (%)	Alnus glutinosa (%)	Carpinus betulus (%)	Corylus avellana (%)	Quercus alba (%)	
Austria (n = 38)	91	88	76	77	69	80
France (n = 27)	90	89	78	83	76	83
Sweden (n = 19)	92	80	67	75	60	75
Switzerland (n = 18)	96	96	89	84	84	90
Mean (n = 102)	92	88	77	80	72	82

The mean percentages of inhibition for each country and extract are summarized in the right column and bottom line, respectively.

reacted with the related Fagales pollen allergens but also contained most of their IgE epitopes. On the basis of the results of qualitative and quantitative measurements, those components against which patients are sensitized can be selected for specific immunotherapy. Because rBet v 1 and rBet v 2 account for most of the Fagales pollen-specific IgE, specific immunotherapy with recombinant birch pollen allergens alone may be considered, instead of with mixtures of natural tree pollen extracts, which, in addition to the desired allergens, contain undefined components. The idea that immunotherapy of Fagales pollen allergy can be performed with recombinant birch pollen allergens alone is supported by a previous study that documents that birch pollen extract was equally effective in treating Fagales pollen allergy compared with a mixture containing alder, birch, and hazel pollen extracts.³⁶⁻³⁸ We are of course aware that our serologic competition study just defines 2 recombinant birch pollen allergens that contain most of the Fagales pollen-specific IgE epitopes as possible candidate molecules for specific immunotherapy of Fagales pollen allergy. Clinical therapy studies will certainly be needed to determine whether immunotherapy performed with defined recombinant birch pollen allergens may be effective. On the other hand, we believe that the careful selection of allergens defined according to the patient's IgE reactivity profile may contribute to the improvement of specific immunotherapy because it will help to avoid therapy-induced sensitization to novel components.⁵⁴

REFERENCES

- Emberlin JC. Grass, tree and weed pollens. In: Kay AB, editor. Allergy and allergic diseases. Oxford: Blackwell Science; 1997. pp 835-57.
- Allergy. Which allergens? Pharmacia: Västra Aros, Västerås; 1985.
- Valenta R, Niederberger V, Fischer S, Jäger S, Spitzauer S, Kraft D. Tree pollen allergens. In: Lockey RF, editor. Allergen immunotherapy. 2nd ed. New York: Marcel Dekker; 1998. In press.
- Eriksson NE, Wihl JA, Arrendal H, Strandhede SO. Tree pollen allergy. II. Sensitization to various tree pollen allergens in Sweden. A multicentre study. Allergy 1984;39:610-7.
- Gassner M, Kurtz M, Wüthrich B. Zur Prävalenz der Pollinosis bei Schulkindern. Allergologie 1996;19:403-8.
- Eriksson NE. Allergy to pollen from different deciduous trees in Sweden. An investigation with skin tests, provocation tests and the radioallergen sorbent test (RAST) in springtime hay fever patients. Allergy 1978;33:299-309.
- Viander M, Fraki J, Djupsund BM, Laine S. Antigens and allergens in birch pollen extract. Allergy 1979;34:289-302.
- Ipsen H, Loewenstein H. Isolation and immunochemical characterization of the major allergen of birch pollen (*Betula verrucosa*). J Allergy Clin Immunol 1983;72:150-9.
- Vik H, Elsayed S. Comparative studies on tree pollen allergens. X. Further purification and N-terminal amino acid sequence analyses of the major allergen of birch pollen (*Betula verrucosa*). Int Arch Allergy Appl Immunol 1986;80:17-25.
- Ipsen H, Hansen OC. Structural similarities among major allergens of tree pollens. In: Sehon AH, Kraft D, Kunkel G, editors. Epitopes of atopic allergens. Brussels: The UCB Institute of Allergy; 1990. pp 3-8.
- Jarolim E, Rumpold H, Endler AT, Ebner H, Breitenbach M, Scheiner O, et al. IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verrucosa*. Allergy 1989;44:385-95.
- Rohac M, Birkner T, Reimtzter I, Bohle B, Steiner R, Breitenbach M, et al. The immunological relationship of epitopes on major tree pollen allergens. Mol Immunol 1991;28:897-906.
- Eriksson NE, Formgren H, Svenonius E. Food hypersensitivity in patients with pollen allergy. Allergy 1982;37:437-43.
- Halmepuro L, Vuontela K, Kalimo K, Björkstén F. Cross-reactivity of antibodies with allergens in birch pollen, fruits and vegetables. Int Arch Allergy Appl Immunol 1984;74:235-40.
- Calkhoven PG, Aalbers M, Koshte VL, Pos O, Oei HD, Aalberse RC. Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. Allergy 1987;42:382-90.
- Ebner C, Birkner T, Valenta R, Rumpold H, Scheiner O, Kraft D. Common epitopes of birch pollen and apples: studies by Western and Northern blot. J Allergy Clin Immunol 1991;88:588-94.
- Hirschwehr R, Valenta R, Ebner C, Ferreira F, Sperr WR, Valent P, et al. Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. J Allergy Clin Immunol 1992;90:927-36.
- Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). J Allergy Clin Immunol 1995;95:962-9.
- Valenta R, Duchene M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, et al. Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. Science 1991;253:557-60.
- Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, et al. Profilins constitute a novel family of functional plant pan-allergens. J Exp Med 1992;175:377-85.
- Vallier P, Dechamps C, Valenta R, Vial O, Deviller P. Purification and characterization of an allergen from celery immunologically related to an allergen present in several other plant species. Identification as a profilin. Clin Exp Allergy 1992;2:774-82.
- van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. Int Arch Allergy Immunol 1992;98:97-104.
- Valenta R, Ball T, Vrtala S, Duchêne M, Kraft D, Scheiner O. cDNA cloning and expression of timothy grass (*Phleum pratense*) pollen profilin in *Escherichia coli*: comparison with birch pollen profilin. Biochem Biophys Res Commun 1994;199:106-18.
- Vrtala S, Wiedemann P, Mittermann I, Eichler HG, Sperr WR, Valent P, et al. High-level expression in *Escherichia coli* and purification of recombinant plant profilins: comparison of IgE-binding capacity and allergenic activity. Biochem Biophys Res Commun 1996;226:42-50.
- Breiteneder H, Pettenburger K, Bito A, Valenta R, Kraft D, Rumpold H, et al. The gene coding for the major birch pollen allergen, Bet v 1, is highly homologous to a pea disease resistance response gene. EMBO J 1989;8:1935-8.
- Valenta R, Duchene M, Vrtala S, Birkner T, Ebner C, Hirschwehr R, et al. Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. J Allergy Clin Immunol 1991;88:889-94.
- Valenta R, Sperr WR, Ferreira F, Valent P, Sillaber C, Tejkl M, et al. Induction of specific histamine release from basophils with partially purified natural and recombinant birch pollen allergens. J Allergy Clin Immunol 1993;91:88-97.
- Ebner C, Ferreira F, Szephalusi Z, Jilek A, Valenta R, Parronchi P, et al. Identification of multiple T-cell epitopes on Bet v 1, the major birch pollen allergen using specific T cell clones and overlapping peptides. J Immunol 1993;2150:1047-54.
- Ferreira F, Mayer P, Sperr WR, Valent P, Seiberler S, Ebner C, et al. Induction of IgE antibodies with predefined specificity in rhesus monkeys with recombinant birch pollen allergens, Bet v 1 and Bet v 2. J Allergy Clin Immunol 1996;97:95-103.
- Vrtala S, Mayer P, Ferreira F, Susani M, Sehon AH, Kraft D, et al. Induction of IgE antibodies in mice and rhesus monkeys with recombinant birch pollen allergens: different allergenicity of Bet v 1 and Bet v 2. J Allergy Clin Immunol 1996;98:913-21.
- Menz G, Dolecek C, Schönheit-Kenn U, Ferreira F, Moser M, Schneider T, et al. Serological and skin-test diagnosis of birch pollen allergy with recombinant Bet v 1, the major birch pollen allergen. Clin Exp Allergy 1996;26:50-60.
- Pauli G, Oster JP, Deviller P, Heiss S, Bessot JC, Susani M, et al. Skin testing with recombinant allergens rBet v 1 and birch profilin, rBet v 2: diagnostic value for birch pollen and associated allergies. J Allergy Clin Immunol 1996;97:1100-9.
- Godnic-Cvar J, Susani M, Breiteneder H, Berger A, Havelic L, Waldhör T, et al. Recombinant Bet v 1, the major birch pollen allergen, induces

- hypersensitivity reactions equal to those induced by natural Bet v 1 in the airways of patients allergic to tree pollen. *J Allergy Clin Immunol* 1997;99:100-6.
34. Valenta R, Kraft D. Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr Opin Immunol* 1995;7:751-6.
 35. Valenta R, Steinberger P, Duchene M, Kraft D. Immunological and structural similarities among allergens: prerequisite for a specific and component-based therapy of allergy. *Immunol Cell Biol* 1996;74:187-94.
 36. Peterson BN, Janniche H, Munch EP, Wihl JA, Bowadt H, Ipson H, et al. Immunotherapy with partially purified and standardized tree pollen extracts. I. Clinical results from a three-year double blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel. *Allergy* 1988;43:353-62.
 37. Wihl JA, Ipson H, Petersen BN, Munch EP, Janniche H, Loewenstein H. Immunotherapy with partially purified and standardized tree pollen extracts. II. Results of skin prick tests and nasal provocation tests from a three-year double-blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel. *Allergy* 1988;43:363-9.
 38. Ipson H, Schwartz B, Wihl JA, Petersen BN, Munch EP, Janniche H, et al. Immunotherapy with partially purified and standardized tree pollen extracts. III. Specific IgE response to the major allergens of alder, birch and hazel pollen during immunotherapy. *Allergy* 1988;43:370-7.
 39. Twardosz A, Hayek B, Seiberler S, Vangelista L, Elfman L, Grönlund H, et al. Molecular characterization, expression in *Escherichia coli* and epitope analysis of a two EF-hand calcium-binding birch pollen allergen, Bet v 4. *Biochem Biophys Res Commun* 1997;239:197-204.
 40. Vrtala S, Grote M, Duchêne M, van Ree R, Kraft D, Scheiner O, et al. Properties of tree and grass pollen allergens: reinvestigation of the linkage between solubility and allergenicity. *Int Arch Allergy Immunol* 1993;102:160-9.
 41. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
 42. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
 43. Hoffmann-Sommergruber K, Susani M, Ferreira F, Jertschin P, Ahorn H, Steiner R, et al. High-level expression and purification of the major birch pollen allergen, Bet v 1. *Protein Expr Purif* 1997;9:33-9.
 44. Susani M, Jertschin P, Dolecek C, Sperr WR, Valent P, Ebner C, et al. High level expression of birch pollen profilin (Bet v 2) in *Escherichia coli*: purification and characterization of the recombinant allergen. *Biochem Biophys Res Commun* 1995;215:250-63.
 45. Towbin H, Staehelin T, Gordon T. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979;76:4350-4.
 46. Breiteneder H, Hoffmann-Sommergruber K, Ebner C, Reikerstorfer A, Duchêne M, Ferreira F, et al. cDNA cloning and expression in *Escherichia coli* of Aln g 1, the major allergen in pollen of alder (*Alnus glutinosa*). *J Allergy Clin Immunol* 1992;90:909-17.
 47. Larsen JN, Stroman P, Ipsen H. PCR-based cloning and sequencing of isogenes encoding the tree pollen major allergen Car b 1 from *Carpinus betulus*, hornbeam. *Mol Immunol* 1992;29:703-11.
 48. Breiteneder H, Ferreira F, Hoffmann-Sommergruber K, Ebner C, Breitenbach M, Kraft D, et al. Four recombinant isoforms of Cor a 1, the major allergen of hazel pollen, show different IgE binding properties. *Eur J Biochem* 1993;212:355-62.
 49. Engel E, Richter K, Obermeyer G, Briza P, Kungl AJ, Simon B, et al. Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. *J Biol Chem* 1997;272:28630-7.
 50. Fischer S, Heiss S, Hess M, Duchêne M, Kraft D, Valenta R. cDNA cloning and characterization of a highly crossreactive birch pollen allergen. *Allergy Clin Immunol Int* 1997;Suppl 4:146.
 51. Valenta B, Breiteneder H, Pettenburger K, Breitenbach M, Rumpold H, Kraft D, et al. RNA- and DNA-sequence similarities of the major allergens of birch, alder, hazel, and hornbeam pollens. In: Sehon AH, Kraft D, Kunkel G, editors. *Epitopes of atopic allergens*. Brussels: The UCB Institute of Allergy; 1990. pp 73-6.
 52. Swoboda I, Jilek A, Ferreira F, Engel E, Hoffmann-Sommergruber K, Scheiner O, et al. Isoforms of Bet v 1, the major birch pollen allergen, analyzed by liquid chromatography, mass spectrometry, and cDNA cloning. *J Biol Chem* 1995;270:2607-13.
 53. Ferreira F, Hirtenlehner K, Jilek A, Godnik-Cvar J, Breiteneder H, Grimm R, et al. Dissection of the immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. *J Exp Med* 1996;183:599-608.
 54. van Ree R, van Leeuwen WA, Dieges PH, van Wijk RG, de Jong N, Brewczynski PZ, et al. Measurement of IgE antibodies against purified grass pollen allergens (Lol p 1, 2, 3 and 5) during immunotherapy. *Clin Exp Allergy* 1997;27:68-74.