

Serological and skin-test diagnosis of birch pollen allergy with recombinant *Bet v* I, the major birch pollen allergen

G. MENZ, C. DOLECEK*, U. SCHÖNHEIT-KENN, F. FERREIRA†, M. MOSER‡, T. SCHNEIDER‡, M. SUTER§, G. BOLTZ-NITULESCU*, C. EBNER*, D. KRAFT* and R. VALENTA*

Hochgebirgsklinik Davos-Wolfgang, Davos, Switzerland, *Institute of General and Experimental Pathology, AKH, University of Vienna, Austria, †Institute of Genetics and Developmental Biology, University of Salzburg, ‡Swiss Institute of Asthma Research, Davos, Switzerland and §Institute of Virology, Zürich, Switzerland

Summary

Background Type I allergy represents a severe health problem in industrialized countries where up to 20% of the population suffer from allergic rhinitis, conjunctivitis and allergic asthma bronchiale and in severe cases from anaphylaxis, leading to death.

Objective The aim of this study was to evaluate recombinant *Bet v* I, the major birch pollen allergen for *in vivo* and *in vitro* diagnosis of birch pollen allergy.

Methods A group of 51 birch pollen allergic patients and eight non-allergic control individuals were tested for birch pollen allergy by skin-prick and intradermal testing, comparing commercial birch pollen extracts with recombinant *Bet v* I. Quantitative and qualitative serological testing was done with natural and recombinant allergens by radioallergosorbent test (RAST), enzyme-linked immunosorbent assay (ELISA) and immunoblotting.

Results Recombinant *Bet v* I allowed accurate *in vivo* and *in vitro* diagnosis of tree pollen allergy in 49/51 patients tested. No false positive results were obtained in any *in vitro* assay system (ELISA, Westernblot) or by skin testing (skin-prick, intradermal test) with recombinant *Bet v* I.

Conclusion Our results document that recombinant *Bet v* I produced in bacterial expression systems allows accurate *in vitro* and *in vivo* diagnosis of birch pollen allergy in >95% of birch pollen allergic patients.

Keywords: *in vitro*, *in vivo* diagnosis of birch pollen allergy, skin-prick testing, intradermal testing, recombinant birch pollen allergen *Bet v* I

Clinical and Experimental Allergy, Vol. 26, pp. 50–60. Submitted 13 February 1995; revised 19 May 1995; accepted 3 July 1995.

Introduction

Type I allergic diseases represent a health threat of increasing importance in industrialized countries [1] where up to 20% of the population suffers from allergic rhinitis, conjunctivitis and/or allergic asthma. Allergic effector reactions are due to the liberation of mediators such as histamine from allergic effector cells (mast cells

and basophils) upon crosslinking of specific IgE antibodies which are bound to high affinity Fcε receptors [2]. Tree pollen allergens, among them those from birch (*Betula verrucosqa*), belong to the potent and frequent elicitors of type I allergic symptoms in Middle and Northern Europe, Northern America and Australia [3].

For diagnosis of type I allergy so far crude allergen extracts were prepared from the allergen sources and used for serological assays and skin tests. These allergen extracts were however difficult to standardize regarding their contents of certain components, showed batch to

Correspondence: Dr R. Valenta, Institute of General and Experimental Pathology, AKH, University of Vienna, Währingergürtel 18–20, A-1090 Vienna, Austria.

batch variations and it proved to be extremely difficult to prepare undegraded extracts from sources which are rich in proteases (mites, fruits, vegetables). Although currently available allergen extracts allowed to diagnose whether a patient displayed IgE reactivity against a certain allergen source it was as yet impossible to identify specific IgE, directed against defined allergens. Effort has therefore been put in the molecular characterization of allergens aiming at the production of pure recombinant allergens which show similar immunological properties as their natural counterparts [4–6]. In a number of studies it has been demonstrated that recombinant allergens might be used for successful *in vitro* diagnosis of allergy [7,8]. The properties of recombinant allergens to trigger *in vitro* histamine release [9,10] and T-cell reactivity have been shown [11] and first results documented *in vivo* activity of recombinant allergens [12–14].

The present study reports the successful use of recombinant Bet v I, [15] the major birch pollen allergen for serological and skin diagnosis of birch pollen allergy in a representative number of patients.

Methods and patients

Characterization of patients and control individuals

Fifty-one tree pollen allergic patients with a skin-prick reaction of more than a 3 mm weal to commercial birch pollen extract (ALK, Horsholm, Denmark) and with a birch pollen specific IgE level of RAST class ≥ 2 (0.7–3.7 kU/L) were included in the study. At the time of the study all patients had stable lung function and there was no evidence of airway infection. Patients who had received corticosteroid therapy or anti-histamines or suffered from atopic dermatitis were excluded from the study. Eight non-allergic individuals (no evidence of elevated total IgE or specific IgE and without positive skin-prick test to commercial birch pollen extract) were selected according to the same criteria.

Expression and purification of recombinant Bet v I

The cDNA coding for Bet v I was subcloned into plasmid pKK223-3 transformed into *Escherichia coli* JM105 and induced with IPTG [15]. Recombinant Bet v I was purified to homogeneity as reported [16]. The purified recombinant allergen was tested for the absence of nucleic acids and endotoxins and was further subjected to basophil degranulation assays to demonstrate specific histamine release activity and lack of cell toxicity [10]. Purified recombinant Bet v I was stored lyophilized at -20°C until use.

Measurement of specific IgE and IgG antibodies

Antibodies were measured by radioallergosorbent test (RAST), immunoblotting and enzyme-linked immunosorbent assay (ELISA). RAST assays were done according to the instructions of the manufacturer (Pharmacia, Uppsala, Sweden). For the other assays total birch pollen protein extracts were prepared from *Betula verrucosa* pollen which had been purchased from Allergon, AB, Valinge, Sweden [17]. Approximately 1 mg total birch pollen extract was separated per 15 cm preparative sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [18] and blotted to nitrocellulose (Schleicher & Schuell, Dassel, Germany) [19]. A total of 30 μg recombinant Bet v I or recombinant Bet v II was separated per 15 cm preparative SDS-PAGE and transferred to nitrocellulose. Nitrocellulose strips were cut and incubated with 1:10 diluted sera from allergic patients or control individuals and bound IgE was detected with a ^{125}I -labelled anti-human IgE (Pharmacia, Uppsala, Sweden) as described [9]. For quantitative measurements of allergen specific IgE and IgG subclass reactivity, 96 well ELISA plates (Nunc, Roskilde, Denmark) were coated with 2 $\mu\text{g}/\text{mL}$ recombinant Bet v I dissolved in 0.1 M sodium carbonate buffer, pH 9.6 overnight at 4°C . Sera were diluted 1:10 for IgE and 1:50 for IgG subclasses in PBS/0.05% Tween 20/0.5% BSA and incubated overnight at 4°C . Bound IgE-antibodies and IgG1-IgG4 were detected with mouse monoclonal anti-human Ig antibodies (Pharmingen, San Diego, CA, USA) and a peroxidase-coupled sheep anti-mouse Ig (Amersham, UK).

Preparation of allergens and allergen extracts for skin testing

Commercial birch pollen extract for skin testing and intradermal testing was purchased from ALK, Horsholm, Denmark. For skin-prick testing 40 μL of crude extract were used and 100 SQ units for intradermal testing as recommended by the manufacturer. Recombinant Bet v I was dissolved at a concentration of 1 $\mu\text{g}/\mu\text{L}$ in 0.9% NaCl solution. Aliquots were stored at -20°C until use. For skin-prick testing 15 μL per drop of a 0.1 $\mu\text{g}/\mu\text{L}$ solution were used. Intradermal tests were done with 5 μg diluted serially in 0.9% NaCl in tenfold dilution steps and applied at dilutions ranging from 10^{-6} to 10^{-2} .

Skin testing

For skin-prick testing 15 μL of the test solution was placed on the patients forearms at a distance of more than 3 cm between individual application points. As controls 0.9% NaCl-negative control (Allergopharma,

Table 1. Characterization of birch pollen allergic individuals. Patients were numbered as in the immunoblots in Fig. 1. Sex, age, duration of asthma, rhinoconjunctivitis and asthma as well as previous hyposensitization treatment with birch pollen extract are displayed

Patients	Sex (male/female)	Age (years)	Asthma (years)	RCA (yes/no)	Hyposensitization (yes/no)
1	F	41	No asthma	Y	Y
2	F	29	No asthma	Y	N
3	F	35	17	Y	N
4	M	33	No asthma	Y	Y
5	M	41	5	Y	N
6	M	50	19	Y	Y
7	F	20	19	Y	Y
8	F	23	14	Y	Y
9	M	36	11	N	Y
10	M	40	5	Y	Y
11	F	38	14	Y	Y
12	M	22	3	Y	Y
13	M	66	9	Y	N
14	M	43	14	Y	N
15	F	29	14	Y	N
16	M	33	14	Y	N
17	F	27	27	Y	N
18	M	55	5	Y	N
19	F	61	31	Y	Y
20	M	56	4	Y	Y
21	M	36	7	Y	N
22	M	27	3	Y	N
23	M	25	12	Y	N
24	F	43	5	Y	N
25	M	48	40	Y	Y
26	F	37	2	Y	Y
27	F	41	6	Y	N
28	F	29	26	Y	Y
29	F	47	15	Y	N
30	M	42	No asthma	Y	N
31	F	54	14	Y	Y
32	M	49	46	Y	Y
33	F	23	3	Y	Y
34	M	55	14	Y	Y
35	F	41	23	Y	Y
36	F	58	28	Y	Y
37	F	53	5	Y	Y
38	F	53	10	Y	Y
39	F	38	5	Y	N
40	M	44	10	Y	N
41	F	67	15	Y	N
42	M	76	21	N	N
43	M	43	2	Y	N
44	M	33	14	Y	Y
45	M	31	26	Y	N
46	M	37	No asthma	Y	N
47	F	27	5	Y	Y
48	F	55	30	Y	N
49	M	31	9	Y	N
50	F	41	5	Y	Y
51	M	24	23	Y	N

Hamburg, Germany), histamine dihydrochloride-positive control (0.1%, Streuli & Co., Uznach, Switzerland) and a commercial birch pollen extract (ALK, Horsholm, Denmark) were used. The skin was pricked with sterile needles by entering and lifting the skin at 45° angle. The needles were discarded after the application to avoid possible cross-contamination. Reactions were recorded after 20 min by measuring the maximal longitudinal diameter of the weal. Weals of more than 3 mm diameter were regarded as a positive reaction. Skin-prick testing and intradermal testing was performed by the same investigator on the same day.

Intradermal tests were done on the patients back. The test sites were placed at least 5 cm apart to avoid false positive results. Histamine dihydrochloride (Allergopharma, Hamburg, Germany) was used at a concentration of 0.01% as a positive control and 0.9% NaCl (Allergopharma, Hamburg, Germany) served as negative control. The solutions were injected intradermally with a syringe until a weal with a diameter of approximately 5 mm was obtained. The reactions were recorded after 20 min and tests were regarded as positive if the size was at least half the size of the skin reaction induced by the histamine control. Tests were considered negative when up to dilutions of 10^{-2} no reaction was observed. Dermal reactions were recorded with a ball-point pen and transferred with a scotch tape to paper. The surface of the weal was calculated according to the formula $[(D1 + D2)/2]^2$ where D1 represents the maximal longitudinal diameter and D2 the maximal transversal diameter. For nine patients the surface could not be calculated (ND = not done; Table 2) because retrospective measurements showed that the test had been stopped as being clinically positive before half of the histamine weal or cut-off concentration was reached. Sera for subsequent serological analysis were obtained before skin testing and stored at -20°C until use.

Statistical analysis of correlations

Statistical analysis was performed with the Pearson's regression analysis and with the Mann-Whitney *U*-test. Correlation coefficients and statistical significance were determined with Spearman regression analysis. Correlations associated with probability values of $P = 0.05$ were considered as significant.

Results

IgE-reactivity of birch pollen allergic patients and control individuals with natural birch pollen allergens and recombinant Bet v I

For the quantitative and qualitative analysis of antibody

reactivities to birch pollen allergens three different assays were used. Birch pollen specific IgE was measured by RAST, ELISA and immunoblotting. The immunoblots done with natural birch pollen extract (Fig. 1A), with recombinant Bet v I (Fig. 1B), and recombinant Bet v II (Fig. 1C) showed that 49 of 51 tree pollen allergic patients displayed IgE reactivity against recombinant Bet v I among which 34 patients reacted exclusively with Bet v I. Sixteen out of the 51 tree pollen allergic patients reacted with Bet v I and had IgE antibodies specific for additional allergens of a higher and/or lower molecular weight than Bet v I (17 kDa). Ten patients reacted with Bet v II [20] at 14 kDa among which eight patients were positive with recombinant Bet v I and Bet v II and two patients reacted exclusively with recombinant Bet v II. None of the non-allergic control individuals displayed IgE-reactivity with natural or recombinant birch pollen allergens in any of the *in vitro* assay systems.

Comparing RAST values with the reactivity patterns of patients with nitrocellulose blotted natural birch pollen allergens it appeared that high birch RAST scores were mostly due to IgE reactivity to Bet v I because the ELISA results indicated that those patients with high birch RAST scores had a high titre of IgE directed against recombinant Bet v I ($r = 0.9432$, $P = 0.000$) (Table 2). When sera of patients with high IgE titres against recombinant Bet v I were tested with nitrocellulose blotted recombinant Bet v I, intense reactivity was observed (Fig. 1B).

High anti-Bet v I titres in the IgG subclasses were not paralleled by high IgE-reactivity and even in patients without detectable IgE-reactivity to recombinant Bet v I specific IgG antibodies could be detected (#23, #50 in Table 2). No significant association of elevated IgG1-IgG4 or IgE reactivity to recombinant Bet v I was observed (Table 2) when patients had received hyposensitization treatment (Mann-Whitney *U*-test: IgE: $P = 0.478$; IgG1: $P = 0.29$; IgG2: $P = 0.15$; IgG3: $P = 0.1$; IgG4: $P = 0.8$). High as well as low Bet v I specific IgE levels were observed regardless whether patients had received previous hyposensitization treatment.

Skin-test activity of recombinant Bet v I

Table 1 summarizes patients data: age, sex, clinical symptoms and hyposensitization treatment. In Table 2 total serum IgE levels, birch pollen RAST values, results of skin testing and serological measurements are displayed for the birch pollen allergic patients whereas Table 3 shows the non-allergic individuals (control group). All 51 birch pollen allergic patients tested had positive skin-prick results with a commercially available

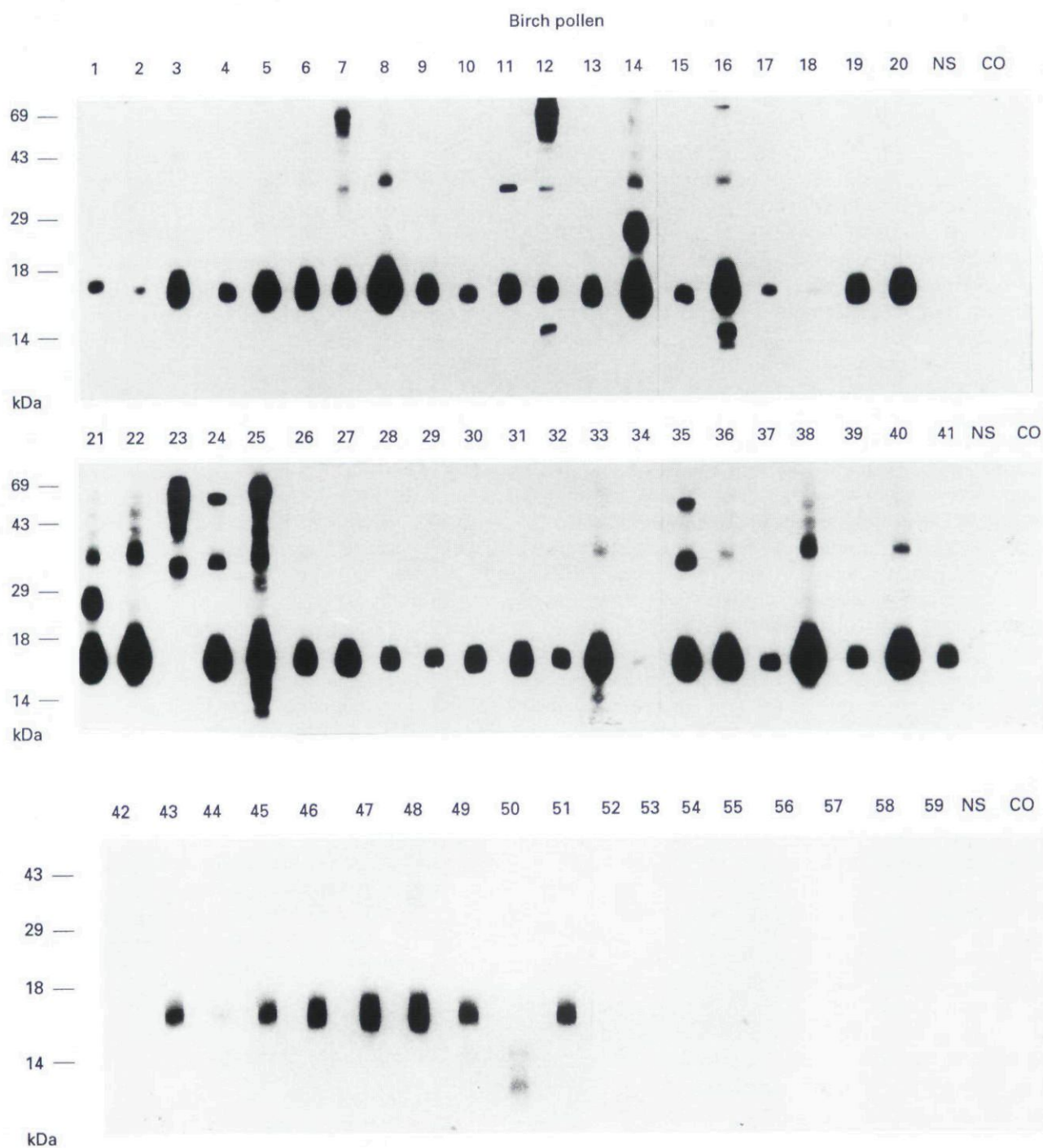


Fig. 1. IgE-reactivity of birch pollen allergic patients and control individuals with nitrocellulose blotted natural birch pollen allergens (a), recombinant *Bet v I* (b) and recombinant *Bet v II* (c). Nitrocellulose strips containing natural birch pollen extract (birch pollen), recombinant *Bet v I* (rec. *Bet v I*) or recombinant *Bet v II* (rec. *Bet v II*) were probed with serum IgE from 51 birch pollen allergic patients (lanes 1–51), nine non-allergic individuals (52–59, NS) and buffer without addition of serum (lane CO). Bound IgE was detected with a ^{125}I -labelled anti-human IgE antibody.

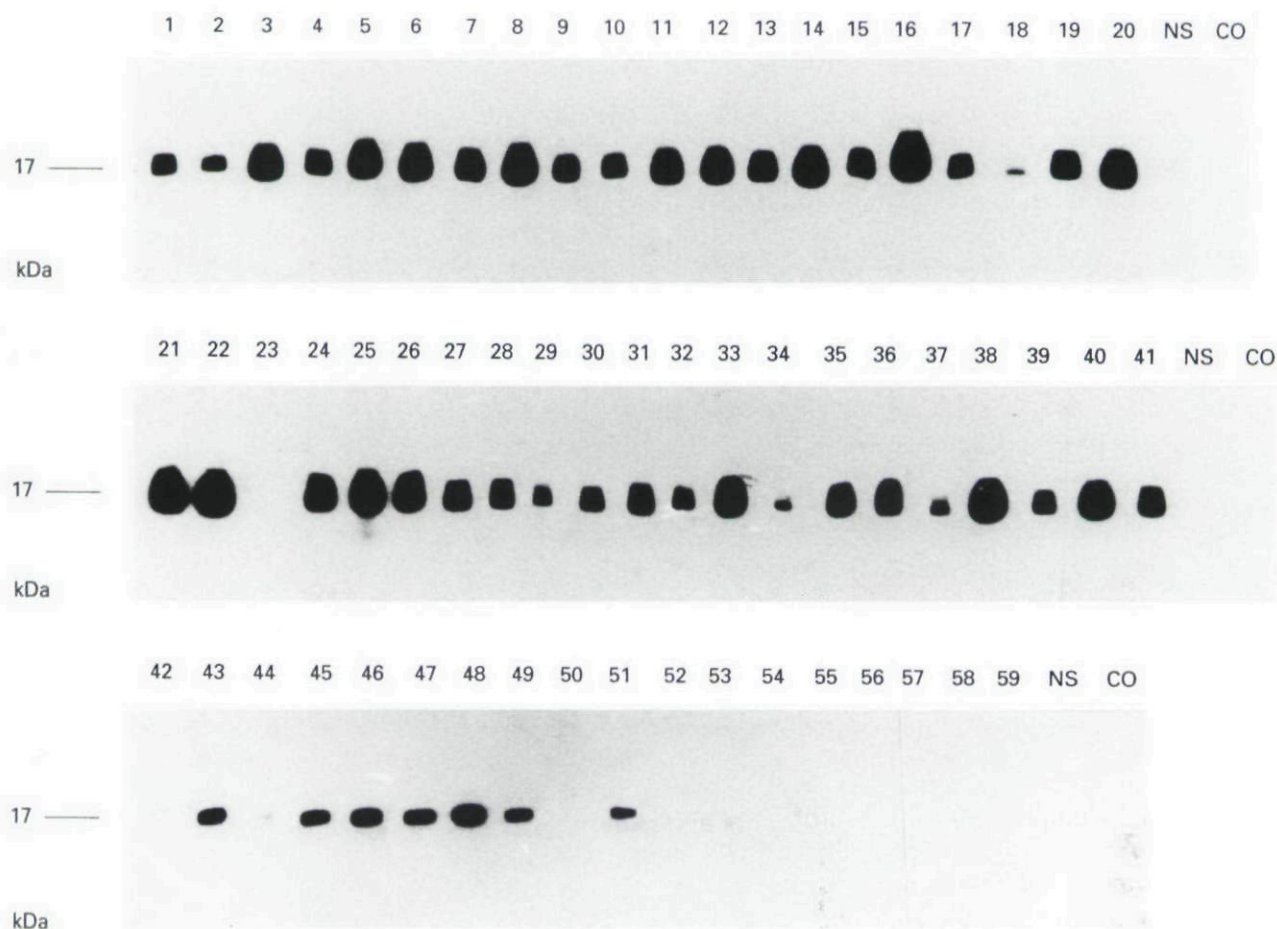


Fig. 1. (Continued).

birch pollen extract. Using recombinant *Bet v I* alone 49/51 patients gave positive skin-prick reactions. Two out of the three negative patients were however positive with recombinant *Bet v II* [20,7] (data not shown). By intradermal testing with natural birch pollen extract all 51 patients could be diagnosed. In the test series with recombinant *Bet v I*, nine patients could not be evaluated because testing was stopped before half of the histamine weal was reacted. Forty out of the remaining 42 patients were positive with recombinant *Bet v I*. The patient who was negative in the intradermal test was also negative in skin-prick testing with recombinant *Bet v I*. This patient reacted positively to recombinant *Bet v II* and *Bet v II* specific IgE could be detected in the serum (data not shown).

Comparison of in vitro and in vivo tests

Table 3 displays the results of the serological testing obtained by RAST, immunoblot and ELISA as well as the results obtained by skin testing of the patients with

natural birch pollen extract and recombinant *Bet v I*. Table 3 shows the results for the control group of healthy individuals. As mentioned above, high birch pollen RAST levels were mostly associated with high IgE titers against recombinant *Bet v I* ($r = 0.927$, $P = 0.000$). In contrast there was little correlation ($r = 0.4331$, $P = 0.001$) of *Bet v I* specific IgE levels with elevated total IgE. All 51 birch pollen allergic patients could be diagnosed by skin testing and serological testing using natural birch pollen extracts. Using recombinant *Bet v I*, the major birch pollen allergen alone, the percentage of *in vitro* diagnosed patients was 96% and skin testing with recombinant *Bet v I* allowed to diagnose 96% of the patients. Comparing the intensity of the skin reactions obtained with natural birch pollen extract or recombinant *Bet v I* with the amounts of specific IgE, high IgE titres were not associated with elevated skin sensitivity (*Bet v I* specific IgE-skin-prick *rBet v I*: $r = 0.096$, $P = 0.502$; *Bet v I* specific IgE-skin-prick ALK: $r = 0.160$, $P = 0.26$). If recombinant *Bet v II*

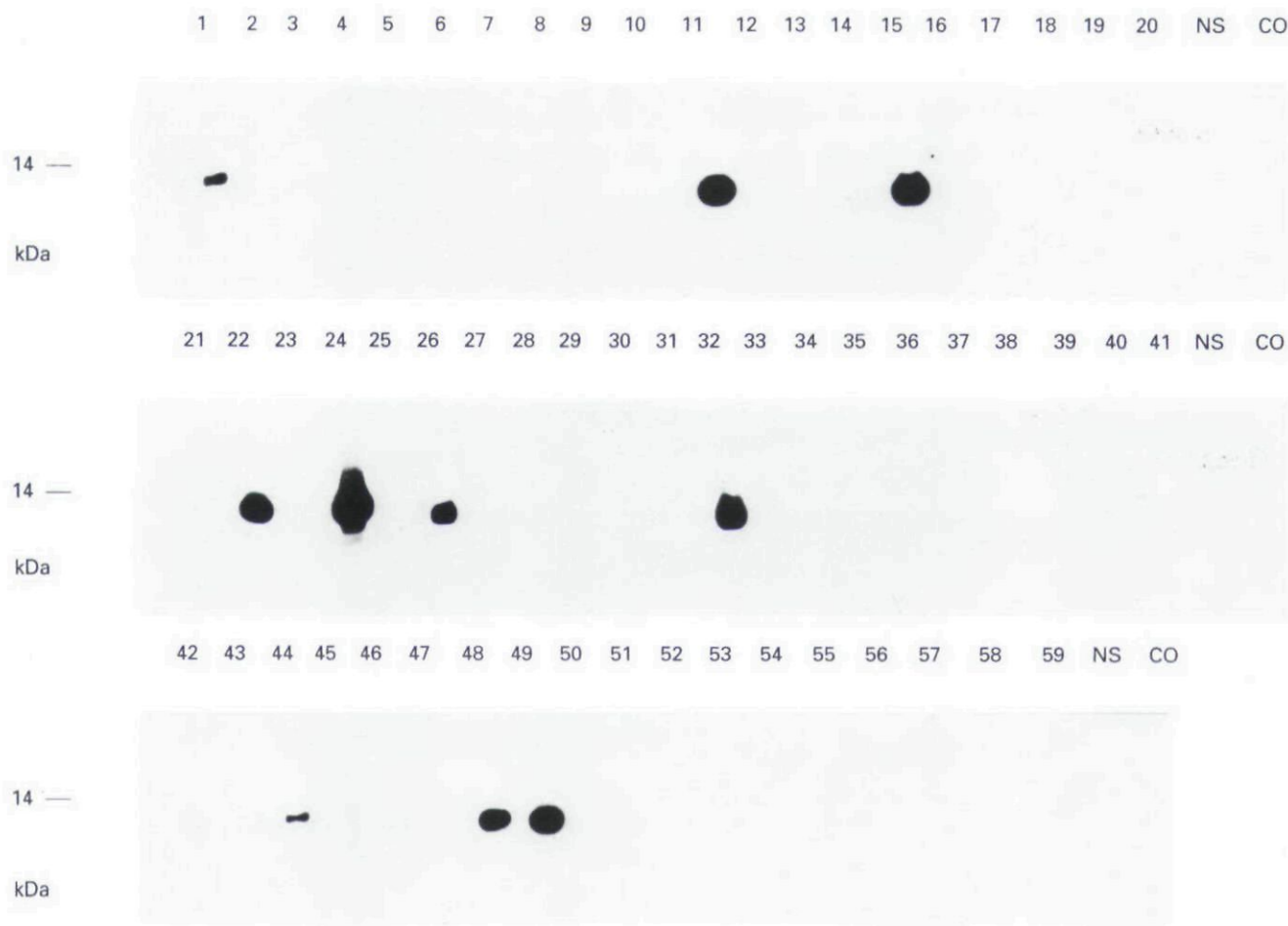


Fig. 1. (Continued).

was included in serological assays and skin tests all birch pollen allergic patients were diagnosed (data not shown).

Discussion

By the application of cDNA cloning techniques the progress in the field of allergen characterization has rapidly increased in the recent years [4,5]. A number of cDNAs coding for plant, mite, fungal, mammalian, and venom allergens has been isolated and expressed in *E. coli* [6]. In first studies the IgE-binding capacity of recombinant allergens has been demonstrated. It was shown that a relative small panel of recombinant allergens can be sufficient for diagnosis of tree [7] and grass pollen allergy [8]. It was further demonstrated that recombinant tree and grass pollen allergens lead to specific and dose-dependent release of histamine from basophils of allergic patients [10] and induced specific T-

cell proliferation [11]. First studies in experimental animal models also indicated that recombinant allergens induce IgE responses which are directed against the natural counterparts and homologous allergens [21,22] and evidence was provided that recombinant allergens might have similar skin-test activity as natural allergens [12–14]. The promising results regarding the similar properties of natural and recombinant allergens have subsequently led to a serious consideration of using recombinant allergens for *in vitro* and *in vivo* diagnosis as well as specific therapy of type I allergies [23,24].

In the present study the *in vitro* and skin-test activity of recombinant *Bet v* I, the major birch pollen allergen [15] was evaluated and compared with commercial natural birch pollen extracts. Recombinant *Bet v* I has been characterized extensively *in vitro* and in animal models [21,22]. It has been shown that recombinant *Bet v* I shared immunological properties with its natural counterparts [16] and it was further demonstrated that

Table 2. Comparison of serological and skin testing results. Results from a group of birch pollen allergic patients (patients 1–51) and non-allergic control individuals are displayed. Total IgE levels, birch pollen specific RAST, *Bet v I* specific IgE and IgG, skin-prick (SPT) and intradermal tests (IDT) with birch pollen extract (ALK) or recombinant *Bet v I* (r*Bet v I*) are shown

Patient	Total IgE (kU/L)	RAST birch (kU/L)	SPT		IDT		ELISA r <i>Bet v I</i> IgE	Immunoblot		ELISA r <i>Bet v I</i>			
			ALK	r <i>Bet v I</i>	ALK	r <i>Bet v I</i>		r <i>Bet v I</i> IgE	r <i>Bet v II</i> IgE	IgG1	IgG2	IgG3	IgG4
1	58.7	6.65	+	+	+	+	0.12	+	–	0.19	0.23	0.00	0.35
2	149	4.05	+	+	+	+	0.04	+	+	0.92	0.31	0.00	0.21
3	230	26.5	+	+	+	+	0.92	+	–	1.24	0.50	0.00	0.75
4	29.1	6.41	+	+	+	+	0.10	+	–	0.84	0.60	0.36	0.60
5	263	60.9	+	+	+	+	1.66	+	–	0.50	0.22	0.00	0.70
6	125	29.4	+	+	+	+	0.74	+	–	1.10	1.40	0.14	0.92
7	289	17.4	+	+	+	+	0.43	+	–	1.42	1.18	0.81	2.50
8	325	100	+	+	+	+	2.06	+	–	1.64	1.17	0.03	1.50
9	192	14.5	+	+	+	ND	0.17	+	–	0.57	0.13	2.18	0.24
10	36	6.16	+	+	+	–	0.07	+	–	1.19	0.77	0.00	0.02
11	689	14.5	+	+	+	+	0.49	+	–	0.72	0.42	0.01	1.06
12	842	36	+	–	+	+	0.27	+	+	0.86	0.12	0.02	0.12
13	186	26.8	+	+	+	+	0.16	+	–	0.53	0.26	0.08	1.61
14	359	83.4	+	+	+	+	1.86	+	–	1.52	1.77	0.07	2.50
15	87.2	14.9	+	+	+	+	0.14	+	–	0.47	0.10	0.00	0.36
16	1251	85.4	+	+	+	+	2.05	+	+	0.32	0.77	0.08	0.56
17	118	5.07	+	+	+	+	0.05	+	–	0.86	0.17	0.04	0.28
18	199	4.16	+	+	+	+	0.05	+	–	0.09	0.25	1.03	0.43
19	115	13	+	+	+	+	0.18	+	–	1.47	2.50	0.80	2.50
20	127	33.5	+	+	+	ND	0.46	+	–	1.15	1.71	0.40	2.50
21	556	73.2	+	+	+	+	2.27	+	–	1.23	0.44	0.00	1.26
22	475	100	+	+	+	+	2.50	+	–	1.39	0.70	0.00	2.16
23	1958	12.8	+	+	+	ND	0	–	+	0.03	0.31	0.00	0.01
24	1083	38.4	+	+	+	+	1.12	+	–	0.98	0.37	0.00	1.73
25	1500	100	+	+	+	ND	1.51	+	+	1.60	2.50	0.40	2.50
26	987	37.2	+	+	+	+	0.45	+	–	0.20	0.02	0.25	0.00
27	197	25.8	+	+	+	+	0.42	+	+	0.58	0.14	0.08	0.10
28	193	4.95	+	+	+	+	0.09	+	–	1.28	0.13	0.00	0.10
29	275	6.61	+	+	+	+	0.06	+	–	0.61	0.13	0.00	0.29
30	91.3	6.64	+	+	+	+	0.09	+	–	0.08	0.34	0.00	0.47
31	88	7.3	+	+	+	+	0.15	+	–	0.36	0.29	0.00	0.86
32	646	8.55	+	+	+	ND	0.07	+	–	0.77	0.81	0.24	0.68
33	1037	53.6	+	+	+	+	1.65	+	+	0.98	0.58	0.00	1.67
34	14.5	3.5	+	+	+	+	0.03	+	–	0.12	0.11	0.00	0.01
35	181	35.5	+	–	+	ND	0.72	+	–	1.61	2.50	0.18	2.50
36	468	41.3	+	+	+	+	0.86	+	–	1.18	0.58	0.37	0.98
37	83.8	4.71	+	+	+	+	0.03	+	–	0.62	0.45	0.11	0.35
38	286	82.5	+	+	+	ND	1.54	+	–	0.94	1.02	0.06	1.75
39	33.4	3.6	+	+	+	ND	0.02	+	–	0.41	0.04	0.02	0.62
40	332	59.1	+	+	+	+	1.99	+	–	1.31	1.56	1.45	1.33
41	108	10.5	+	+	+	+	0.10	+	–	0.50	0.23	0.15	1.09
42	385	3.6	+	+	+	+	0.03	+	–	0.91	0.59	1.69	1.54
43	393	28.6	+	+	+	+	0.84	+	–	1.15	1.26	1.55	1.08
44	186	7.53	+	+	+	ND	0.05	+	–	0.32	0.36	0.11	2.02
45	337	47.7	+	+	+	+	0.57	+	+	0.71	0.23	0.20	0.52
46	291	51.1	+	+	+	+	1.31	+	–	0.42	0.08	0.00	0.88
47	332	100	+	+	+	+	2.41	+	–	0.90	0.84	0.50	2.50

Table 2. (Continued)

Patient	Total IgE (kU/L)	RAST birch (kU/L)	SPT		IDT		ELISA rBet v I IgE	Immunoblot		ELISA rBet v I			
			ALK	rBet v I	ALK	rBet v I		rBet v I IgE	rBet v II IgE	IgG1	IgG2	IgG3	IgG4
48	1320	100	+	+	+	+	2.41	+	—	0.82	1.37	0.40	1.95
49	442	41.3	+	+	+	+	0.93	+	+	1.10	0.55	0.06	1.84
50	663	19.8	+	—	+	—	0	—	+	0.30	0.02	0.05	0.01
51	314	25.1	+	+	+	+	0.56	+	—	0.87	0.36	0.09	1.03

a combination of *Bet v I* together with *Bet v II* might be sufficient for accurate *in vitro* diagnosis of birch pollen allergy [7].

For skin testing and serological assays the cDNA coding for *Bet v I* [15] was expressed in *E. coli* and the recombinant protein was purified to homogeneity. Fifty-one tree pollen allergic patients were tested in parallel with natural allergen extracts and purified recombinant *Bet v I* by skin-prick testing and intradermal testing. It was shown that recombinant *Bet v I* was sufficient to diagnose *in vitro* and by skin testing birch pollen allergy in more than 95% of the allergic individuals. None of the non-allergic individuals displayed IgE reactivity to recombinant *Bet v I* or gave positive skin reactions. Comparing *in vivo* testing with *in vitro* serological assays no association of allergen specific IgE titres with elevated skin reactivity was observed indicating that in addition to the levels of specific IgE other parameters such as the sensitivity of the effector cells may be of importance. High birch pollen RAST values were however significantly associated with high titers of IgE directed against recombinant *Bet v I*, indicating that a

high percentage of birch pollen specific IgE is bound by recombinant *Bet v I*. The skin-test activity of recombinant *Bet v I* was in good agreement with earlier studies indicating that recombinant *Bet v I* can induce specific histamine release from patients basophils *in vitro* [9,10] and hence possesses biological activity.

In conclusion, the present study demonstrates that >95% of birch pollen allergic patients could be diagnosed *in vitro* and *in vivo* using a single recombinant birch pollen allergen, *Bet v I*. No false positive results were obtained in any patient or control individual. It has to be assumed that single recombinant allergens or even combinations of recombinant allergens might be less efficient to screen for a sensitization against a certain allergen source. The present study reflects this fact because 2/51 tree pollen allergic patients could not be diagnosed with recombinant *Bet v I* alone. However, when recombinant *Bet v II* was included in the test assays all patients were diagnosed. Hence, it will be necessary to determine panels of recombinant allergens suitable for the diagnosis of certain allergies which in view of the

Table 3.

Patient	Total IgE (kU/L)	RAST birch (kU/L)	SPT		IDT		ELISA rBet v I IgE	Immunoblot		ELISA rBet v I			
			ALK	rBet v I	ALK	rBet v I		rBet v I IgE	rBet v II IgE	IgG1	IgG2	IgG3	IgG4
52	33.4	<0.35	—	—	—	—	0	—	—	0.04	0.33	0.00	0.00
53	2	<0.35	—	—	—	—	0	—	—	0.05	0.02	0.12	0.00
54	41.7	<0.35	—	—	—	—	0	—	—	0.01	0.01	0.00	0.00
55	33.4	<0.35	—	—	—	—	0	—	—	0.00	0.39	0.01	0.00
56	50.2	<0.35	—	—	—	—	0	—	—	0.00	0.03	0.39	0.04
57	18.5	<0.35	—	—	—	—	0	—	—	0.00	0.00	0.00	0.00
58	2	<0.35	—	—	—	—	0	—	—	0.00	0.00	0.02	0.00
59	59.5	<0.35	—	—	—	—	0	—	—	0.00	0.00	0.03	0.04

tremendous progress that has been made in the field of allergen characterization appears feasible. If the recombinant allergens which are necessary to cover more complex systems such as grass pollen allergy and mite allergy can be produced, *in vivo* diagnosis of type I allergy will be markedly improved in the future.

The use of recombinant allergens for *in vitro* and *in vivo* diagnosis offers a number of advantages compared with current allergy diagnosis. Recombinant allergens such as Bet v I can be produced as biologically active molecules in heterologous expressions systems in large amounts, in suitable purity and batch consistency. Recombinant technologies will allow to produce Bet v I at lower costs and due the batch to batch consistency recombinant Bet v I is expected to represent a more safe product for immunotherapy. Using recombinant allergens it is possible to determine the reactivity pattern of allergic patients to defined allergens and to measure the amount of IgE directed against these molecules [25,26]. The present study shows that a high percentage of birch pollen specific IgE is directed against recombinant Bet v I. Most patients with high birch pollen RAST scores had high IgE titres against recombinant Bet v I.

Based on the knowledge that allergic crossreactivities are due to IgE crossreactivities against allergens of similar primary structure present in different sources it is possible to explain clinically observed crossreactivity phenomena. Bet v I homologous proteins in pollen of other trees such as hazel, alder, hornbeam, oak, chestnut as well as Bet v I homologous proteins was always associated with IgE-crossreactivity to Bet v I [27–35]. Hence a positive reaction with recombinant Bet v I together with allergic symptoms towards sources containing Bet v I homologous proteins makes it highly likely that Bet v I represents the cross-sensitizing agent for these patients.

The possibility to exactly determine patients IgE-reactivity patterns with defined recombinant allergens might be considered as a first step towards the preparation of hyposensitization cocktails according to the patients needs. Hyposensitization treatment according to the patients reactivity pattern (allergograms) may help to reduce adverse reactions or even sensitization to substances against which the patients were originally not sensitized but which are still components of the currently available hyposensitization extracts. Allergen specific diagnosis may then form the molecular basis for allergen specific immunotherapy [36]. Based on the encouraging results obtained with recombinant Bet v I for *in vivo* testing of tree pollen allergy, hyposensitization strategies which are based on recombinant Bet v I may be seriously considered.

Acknowledgements

This study was supported by grant S06703 of the Austrian Science Foundation and by a grant from the Hochgebirgsklinik Davos-Wolfgang. All serological and *in vivo* studies were approved by the local ethics commission and done after informed consent was obtained from patients and control individuals.

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