



New Bet v 1 isoforms including a naturally occurring truncated form of the protein derived from Austrian birch pollen[☆]

Roswitha Friedl-Hajek¹, Christian Radauer*, Gabriel O’Riordain, Karin Hoffmann-Sommergruber, Kerstin Leberl, Otto Scheiner, Heimo Breiteneder

Department of General and Experimental Pathology, University of Vienna, AKH-EBO-3Q, Waehringer Guertel 18-20, A-1090, Vienna, Austria

Accepted 25 May 1999

Abstract

Bet v 1, the major pollen allergen from white birch, displays a considerable degree of heterogeneity. Until now, all molecular and immunological characterization studies of Bet v 1 isoforms have been performed with commercially available pollen of Swedish origin. In regard to clinical studies with Austrian birch pollen allergic individuals, knowledge about the isoform repertoire in Austrian birch pollen was necessary. cDNAs coding for Bet v 1 isoforms from Austrian birch pollen were cloned by PCR amplification and sequenced. Besides the Austrian variants of the Swedish isoforms Bet v 1a (62% of the clones), LK167 (4%), and Bet v 1d/h, Bet v 1g, and Bet v 1l (24%), three sequences with a significantly lower homology to known isoforms and two Bet v 1a-homologous sequences with a 7 bp insertion coding for a truncated protein were detected. No Austrian variants of the majority of the Swedish isoforms were found. The isoforms coding for truncated proteins were expressed in *Escherichia coli* and tested by immunoblotting. They bound a polyclonal anti-Bet v 1 antibody but did not recognize birch pollen allergic patients’ serum IgE and two Bet v 1-specific monoclonal antibodies. The similarity of the Bet v 1 isoform patterns of Swedish and Austrian birch pollen justifies the use of Bet v 1 derived from Swedish pollen for clinical studies with birch pollen allergic individuals from outside Northern Europe. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Type I allergy; Bet v 1; Isoforms; IgE

1. Introduction

Birch is one of the major pollen sources of allergens

in developed countries of the northern hemisphere. A total of 96% of birch pollen allergic patients show IgE binding to the 17 kDa protein Bet v 1, the major allergen of birch pollen (Ipsen et al., 1988) and 60% of birch pollen allergic patients react exclusively with Bet v 1, indicating the importance of this protein in tree pollen allergy (Jarolim et al., 1989a). However, this protein displays a considerable degree of heterogeneity. Several charge variants have been detected by two-dimensional IgE-immunoblots and isoelectric focusing techniques (Rohac et al., 1991; Ferreira et al., 1993). In 1995, Swoboda et al. described thirteen different Bet v 1 isoforms, termed Bet v 1b to Bet v 1n. The previously isolated Bet v 1 cDNA was referred to as Bet v 1a (Breiteneder et al., 1989). Of these 14 cDNA sequences, three pairs of sequences (f/i, d/h, and m/n) each code for identical proteins and differ only by silent exchanges. N-terminal sequence analysis and

Abbreviations: PCR, polymerase chain reaction; aa, amino acids; bp, base pairs; cDNA, DNA complementary to RNA; mAb, monoclonal antibody; mRNA, messenger RNA; Taq polymerase, polymerase from *Thermus aquaticus*; TCC, T cell clone; Bet v 1, major birch pollen allergen; rBet v 1, recombinant Bet v 1; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; NBTC, 4-nitroblue tetrazolium chloride.

* All new nucleotide sequence data reported in this paper have been submitted to the EMBL nucleotide sequence database and have been assigned the accession nos. AJ002106–AJ002110, and AJ006903–AJ006915

* Corresponding author. Tel.: +43-1-40400-5116; fax: +43-1-40400-5130.

E-mail address: christian.radauer@akh-wien.ac.at (C. Radauer)

¹ The first two authors contributed equally to this paper.

mass spectroscopic analysis of purified natural Bet v 1 indicated that the isoforms a and b represented about 35% of pollen Bet v 1, whereas isoforms d and f seemed to represent about 10% each (Ferreira et al., 1996). Larsen et al. (1996) and Spangfort et al. (1996a) described another seven isoforms.

In 1996, by using recombinant proteins, Ferreira et al. showed that the Bet v 1 isoforms differed in their ability to bind IgE from Austrian birch pollen-allergic patients. Bet v 1a, e and j exhibited the highest IgE-binding activity, whereas Bet v 1d, g and l turned out to be low IgE-binders. These isoforms were tested in further experiments for their ability to activate allergen-specific T lymphocytes. Bet v 1a, the isoform with the highest IgE-binding capacity, had the lowest score for T cell proliferation, whereas Bet v 1d, a low IgE-binder, showed the highest score. This led to the idea to use isoform d for immunotherapy. Using Bet v 1d, a sufficient T cell stimulation could be obtained and IgE-mediated anaphylactic side effects would be avoided.

For all previous experiments (RNA extraction, cDNA library construction, testing of patients' sera with birch pollen extract) commercially available Swedish birch pollen was used. The analysis of Bet v 1 isoforms was also done with a birch pollen extract from this commercially available pollen. This means that until now individuals that have been sensitized with birch pollen of local origin were tested and partially treated with allergens derived from a different geographic and climatic area. It is known that there are significant genetic variations between different birch trees even within the same habitat (Larsen, 1995). The immunological significance of these variations is underlined by analyses of Bet v 1-specific T cells (Ebner et al., 1993; Sparholt et al., 1997) which showed that the most frequently recognized Bet v 1 T cell epitopes differ between the Austrian and the Danish population (Van Neerven et al., 1999). In order to design universally applicable tools for diagnosis or immunotherapy, it is desirable to define panels of recombinant isoallergens which contain a minimum number of different proteins, but all relevant B or T cell epitopes, respectively. A step towards this goal is the analysis of the local variability in isoallergen composition.

In order to shed light on this issue, this study was aimed at determining the isoform repertoire of Bet v 1 in birch pollen of Austrian origin. Possible sequence variants of Bet v 1 derived from Austrian birch pollen were compared to known Swedish isoforms. Thus, we wanted to determine to what extent the various isoforms (high and low IgE-binders) are present in an Austrian population of birch trees.

2. Methods

2.1. First-strand cDNA synthesis and polymerase chain reaction (PCR) amplification

Pollen was collected from seven birch trees (*Betula verrucosa*) located near the city of Salzburg, both young (height 2 m) and mature trees. Total RNA was prepared from 2 g of pollen using a phenol-chloroform extraction. Single-stranded cDNA synthesis was carried out on 2 µg of total RNA primed with oligo(dT) using avian myeloblastosis virus reverse transcriptase. PCR was performed by amplification of the birch cDNA with oligonucleotide primers complementary to bases 1–27 (HB22) and 456–480 (HB20) of Bet v 1.

HB22: 5'-ATG GGT GTT TTC AAT TAC GA/ACT GAG-3' (sense)

HB20: 5'-TTA GTT GTA GGC ATC GGA GTC TGC CAA GAG G-3' (antisense)

Reaction mixtures were subjected to the following conditions: denaturation at 94°C for 30 s, annealing at 55°C for 35 s, and extension at 72°C for 45 s. After 20 cycles, a final extension time of 7 min was added.

2.2. Cloning and sequencing of Bet v 1-coding fragments

The obtained fragment was cloned into a vector appropriate for sequencing. Individual clones, 50, were sequenced in both directions by the Sanger dideoxy method (Sanger et al., 1977) and analyzed by a LI-COR model 4000L DNA sequencer (LI-COR, Lincoln, Nebraska, USA) using fluorescent labeled primers. The sequences were then compared to sequences in EMBL and GenBank databases using the GCC program package (Genetics Computer Group, Madison, Wisconsin, USA).

2.3. Expression of recombinant truncated Bet v 1 isoforms

*Nde*I and *Eco*RI restriction sites were introduced at the 5'- and 3'-end of the cDNA encoding isoforms Bet v 1at14 and Bet v 1at87 by PCR. Fragments were cloned into the *Nde*I/*Eco*RI-digested pMW175 expression plasmid which is under control of the T7 promoter (Hoffmann-Sommergruber et al., 1997a). Competent *E. coli* BL21(DE3) cells were transformed with the resulting plasmid. The transformed cells were grown at 37°C to an OD₆₀₀ of 0.8. Induction of protein expression was performed by addition of 1 mM IPTG. Incubation was carried out for additional 4 h. Afterwards, cells were harvested by centrifugation and lysed by boiling them in SDS-PAGE sample buffer.

	1					60
Bet v 1a	MGVFNYETET	TSVIPAAARLF	KAFILDGDNL	FPKVAPQAIS	SVENIEGNNGG	PGTIKKISFP
Bet v 1at42	-----	-----	-----	-----	-----	-----
Bet v 1at5	-----	-----	-----	i-----	-----	-----
Bet v 1at2	-----	-----	-----	v-----	-----	-----n--
Bet v 1at76	-----	-----	-----	v-----	-----	-----n--
Bet v 1d	-----i--	-----	-----	v-----	-----	-----n--
Bet v 1at10	-----	-----	-----	v-----	-----	-----n--
Bet v 1at37	-----	-----	-----	-----	-----	-----
Bet v 1at8	-----	-----	-----	-----	-----	-----
Bet v 1at14	-----	-----	-----	-----	-----	-----
Bet v 1at87	-----	-----	-----	-----	-----	-----
Bet v 1at12	-----	-----	-----	-----	-----	-----
Bet v 1at26	-----a	-----	-----	-----	-----	-----
Bet v 1at15	-----	-----	-----	v-----	-----	-----
Bet v 1at50	-----	--l-----	r-----	-----	-----	-----
Bet v 1at45	-----	-----	-----	-----	-----	-----n--
Bet v 1at7	-----	-----	-----e--	i-----	-----	-----t--
Bet v 1at59	-----	-----	-----e-t-	i-----	-----	-----t--
	61					120
Bet v 1a	EGFPFKYVKD	RVDEV DHTNF	KYNYSVIEGG	PIGDTLEKIS	NEIKIVATPD	GGSILKISNK
Bet v 1at42	-----	-----	-----	-v-----	----l-----	-----
Bet v 1at5	-----	-----	-----	-v-----	---l-----	-----e----
Bet v 1at2	-----	-----	-----	-v-----	-----	-----cv-----
Bet v 1at76	-----	-----	-----	-v-----	-----	-----cv-----
Bet v 1d	-----	-----	-----	-v-----	-----	-----cv-----
Bet v 1at10	-----	-----	-----	-----	-----	-----
Bet v 1at37	-----	-----	-----	-----	-----	-----cv-----
Bet v 1at8	-----	-----	-----	-v-----	-----t--	-----cv-----
Bet v 1at14	-----	-----	-----	-----	-----	-----n
Bet v 1at87	-----	-----	-----	-m-----	-----	-----n
Bet v 1at12	-----	-----	-----	-m-----	-----	-----
Bet v 1at26	-----	-----	-----	-----	-----	-----
Bet v 1at15	-----	-----	-----	-----	-----	-----
Bet v 1at50	-----	-----	-----	-m-----	-----	-----
Bet v 1at45	-----	-----	-----	-m-----	-----	-----
Bet v 1at7	--s-----	-----	-----	-v-----	-----	-----cv-----
Bet v 1at59	--s-----e	-----a--	--s-m----	al-----c	-----	-----cv-----
	121					160
Bet v 1a	YHTKGDHEVK	AEQVKASKEM	GETLLRAVES	YLLAHS DAYN		
Bet v 1at42	-----m-	--hm--i--k	-----	-----	-----	-----
Bet v 1at5	-----m-	--hm--i--k	--a-----	-----	-----	-----
Bet v 1at2	-----n----	-----	-----	-----	-----	-----
Bet v 1at76	-----	-----	-----	-----	-----	-----
Bet v 1d	-----n----	-----	-----	-----	-----	-----
Bet v 1at10	-----	-----	-----g	-----	-----	-----
Bet v 1at37	-----n----	-----	-----	-----	-----	-----
Bet v 1at8	-----n----	-----	-----	-----	-----	-----
Bet v 1at14	-----	-----	-----	-----	-----	-----
Bet v 1at87	-----	-----	-----	-----	-----	-----
Bet v 1at12	-----	-----	-----	-----	-----	-----
Bet v 1at26	-----	-----	-----	-----	-----	-----
Bet v 1at15	-----	-----	-----	-----	-----	-----
Bet v 1at50	-----	-----	-----	-----	-----	-----
Bet v 1at45	-----n----	-----	-----	-----	-----	-----
Bet v 1at7	-----n----	-----	-----	-----	-----	-----
Bet v 1at59	-----n----	-----	-----	-----	-----	-----

Fig. 1. Comparison of the 16 new peptide sequences found among 50 clones containing a Bet v 1 fragment from Austrian birch pollen. The sequences are compared to Bet v 1a, the most frequent isoform in Swedish birch pollen and Bet v 1d, a low IgE-binding Swedish isoform. dashes indicate residues identical to Bet v 1a, dots indicate gaps inserted during the alignment.

Table 1
Similarities of Austrian Bet v 1 sequences to Swedish isoforms^a

Austrian isoform	% of clones	Most similar Swedish isoform					
		DNA level			Protein level		
		Isoform	%Identity	Exchanges	Isoform	%Identity	Exchan
A—Bet v 1a-related isoforms							
Bet v 1at10	2%	ALK224	99.0	5	ALK2229	98.1	3
Bet v 1at12	4%	Bet v 1a	99.8	1	Bet v 1a	99.4	1
Bet v 1at22	4%	ALK224	99.6	2			
Bet v 1at15	4%	ALK224	99.8	1	ALK2229	99.4	1
Bet v 1at17	34%	Bet v 1a	100	0			
Bet v 1at21	2%	Bet v 1a	99.4	3	Bet v 1a	100	0
Bet v 1at33	6%	ALK224	100	0			
Bet v 1at26	2%	ALK224	98.1	9	Bet v 1a	99.4	1
Bet v 1at45	2%	ALK224	98.8	6	Bet v 1a	98.1	3
Bet v 1at50	2%	ALK224	99.0	5	Bet v 1a	98.1	3
Total	62%						
B—ALK167-related isoforms							
Bet v 1at8	2%	ALK167	99.6	3	ALK167	99.4	1
Bet v 1at37	2%	ALK167	99.0	5	ALK167	99.4	1
Total	4%						
C—Bet v 1d-related isoforms							
Bet v 1at2	18%	Bet v 1h	99.4	3	Bet v 1d	99.4	1
Bet v 1at7	2%	Bet v 1g	98.3	8	Bet v 1g	98.1	3
Bet v 1at76	4%	Bet v 1h	99.0	5	Bet v 1d	98.8	2
Total	24%						
D—new isoforms							
Bet v 1at5	2%	Bet v 1a	96.9	15	Bet v 1a	94.4	9
Bet v 1at14	2%	ALK224	100	0 ^b	Bet v 1a	99.6	1
Bet v 1at42	2%	ALK224	97.3	13	ALK184	96.3	6
Bet v 1at59	2%	Bet v 1k	97.1	14	Bet v 1b	94.4	9
Bet v 1at87	2%	ALK224	99.8	1 ^b	Bet v 1a	98.3	2
Total	10%						

^a Bet v 1a: Austrian isoforms, Bet v 1a–n: Swedish isoforms (Swoboda et al., 1995), ALK: Swedish isoforms (Larsen et al., 1996; Spangfort et al., 1996a).

^b The identities and nucleic acid exchanges refer to the sequences without their 7 base pair insertion after nucleotide 354.

2.4. SDS-PAGE and immunoblotting

Proteins from crude *E. coli* lysates containing recombinant proteins were separated by 12% SDS-PAGE and blotted to nitrocellulose (NC) sheets. As positive control, 1 µg of purified recombinant Bet v 1a was run and blotted, the negative control was an *E. coli* lysate of untransformed cells. The sheets were tested with either (a) a pool of patients' sera, whose IgE all recognized only the Bet v 1 band in an aqueous extract of birch pollen (17 kDa pool), (b) a serum from a patient recognizing several proteins in a birch pollen extract (SS), (c–d) two Bet v 1-specific monoclonal antibodies raised against birch pollen proteins (BIP1 and BIP4; Jarolim et al., 1989b), or (e) a polyclonal rabbit-anti-Bet v 1 antibody. The patients of the 17 kDa pool, all of them living in Austria, were established birch pollen

allergics defined by case history, skin prick test, a RAST.

Strips of NC sheets incubated with patients' sera were shaken in an 1:10 diluted ¹²⁵I-labelled rabbit anti-human-IgE antibody (Pharmacia, Uppsala, Sweden) overnight and subjected to autoradiography. Blots with the anti-Bet v 1 antibodies were detected with alkaline phosphatase-coupled secondary antibodies and developed with BCIP and NBTC.

3. Results

3.1. Bet v 1 isoform variants in Austrian birch pollen

Fifty individual clones were sequenced in both directions and could be assigned to 20 different nucleotide sequences encoding 17 different proteins (Fig. 1);

clones (Bet v 1at17; 34%) were identical to Bet v 1a, the dominant isoform of Swedish pollen. Three additional clones (Bet v 1at33) identical to ALK224 (EMBL accession no. Z80098) and 1 clone (Bet v 1at21) with 3 base exchanges compared to Bet v 1a gave rise to a total of 21 clones (42%) which shared their protein sequences with Bet v 1a (Table 1A). Another 7 individual sequences (10 clones, 20%) differed from Bet v 1a in up to 9 positions on the nucleic acid level, but only in up to 3 positions in their peptide sequences (Table 1A). Two clones (4%, Table 1B) were similar to ALK167 (EMBL accession no. Z80100), a Bet v 1a-related Swedish isoform (97.7% identity between ALK 167 and Bet v 1a on the DNA-level). Three different sequences (12 clones, 24%) showed the highest homology to Bet v 1d/h, Bet v 1g, and Bet v 1l, the low IgE binders (Table 1C). Of the remaining 5 clones (10%) 3 (Bet v 1at5, Bet v 1at42, Bet v 1at59) displayed sequences showing no particular similarity to any of the Swedish isoforms known so far, they showed 13–15 base exchanges compared to the most similar isoforms. Two clones (Bet v 1at14, Bet v 1at87) had a truncated open reading frame. Therefore, these five clones were regarded as new Austrian isoforms (Table 1D).

None of the DNA sequences was identical to the coding region of one of the 10 genomic Bet v 1 sequences obtained from Austrian birch leaf DNA (Hoffmann-Sommergruber et al., 1997b), nevertheless one clone (Bet v 1at26) shares its peptide sequence with bvgc11 and bvgc31.

3.2. Truncated variants of Bet v 1 in Austrian birch pollen

Two new isoforms with a striking feature were detected. The clones Bet v 1at14 and Bet v 1at87 had a 7 bp insertion (AACAACT) following base 354 of the Bet v 1 sequence. This resulted in a stop codon right after this insertion. In this way, the open reading frame was reduced to 360 bp compared to 480 of the full-length-version, coding for a protein of 120 amino acids (13.2 kDa). These two clones differed only at one position from each other (position 276: A or G). The resulting proteins had the highest homology to Bet v 1a aa 1–119 with an additional asparagine (N) at aa 120 (see Fig. 1).

3.3. Immunoblotting of the two recombinant truncated isoforms

To determine the influence of the truncation of the protein on its B cell epitopes, recombinant protein of the two truncated isoforms at14 and at87 were expressed in *E. coli* BL21(DE3) and blotted onto nitrocellulose. A positive control of full-length rBet v 1a

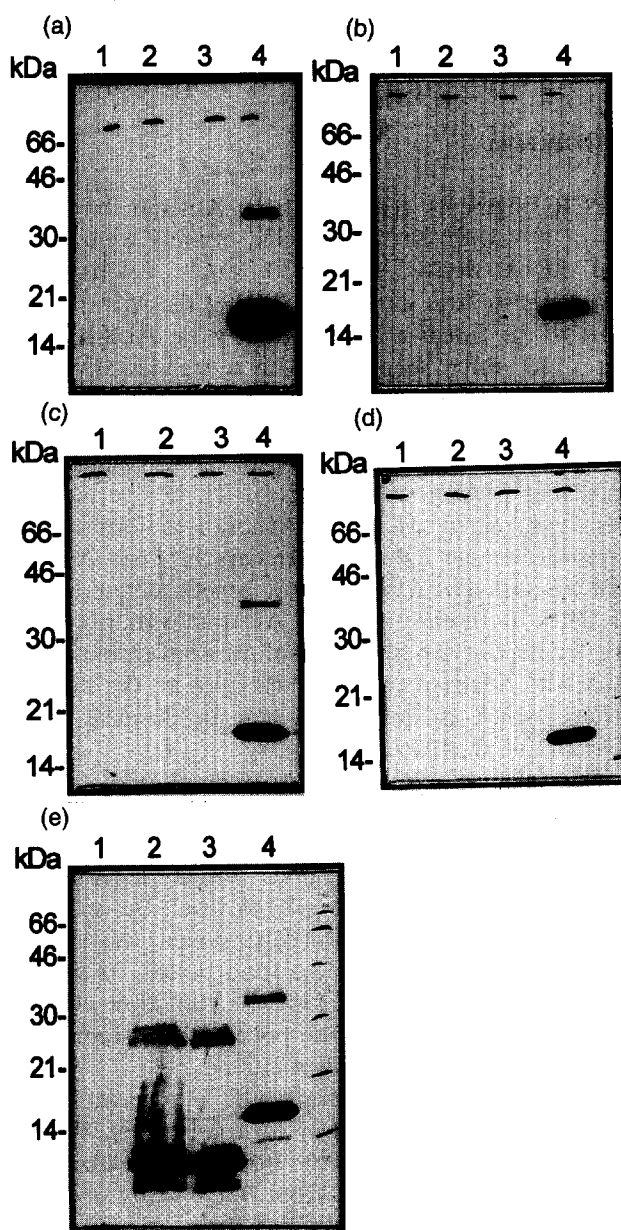


Fig. 2. Immunoblots of crude *E. coli* lysates containing recombinant truncated Bet v 1 isoforms. Lane 1: *E. coli* host without a plasmid (negative control), lane 2: rBet v 1at87, lane 3: rBet v 1at14, lane 4: 1 µg of purified rBet v 1a (positive control). The membranes were incubated with (A) a pool of sera from birch pollen allergic patients who all recognize solely the 17 kDa protein in a birch pollen protein extract; (B) serum of donor SS; (C) the monoclonal anti-Bet v 1 antibody BIP1; (D) the monoclonal anti-Bet v 1 antibody BIP4; (E) a polyclonal IgG-rabbit-anti-Bet v 1 antibody.

and a negative control of *E. coli* lysate without a recombinant protein were also blotted. The incubation with serum and antibodies revealed that rBet v 1at14 and rBet v 1at87 bound a polyclonal rabbit anti-Bet v 1 antibody (Fig. 2E), but did not bind the monoclonal anti-Bet v 1 antibodies BIP1 and BIP4 as well as birch

pollen allergics' serum IgE (Fig. 2A–D). The positive control bound to all tested antibodies and sera.

4. Discussion

PCR fragments generated from Austrian birch pollen cDNA with Bet v 1-specific primers were cloned and DNA derived from 50 single colonies was sequenced in both directions. Besides the Austrian variants of the Swedish isoforms Bet v 1a, ALK167, and Bet v 1d, three sequences with a significantly lower homology to known isoforms (94.4–95.6% identity on the protein level to Bet v 1a) and two Bet v 1a-homologous sequences with a 7-bp-insertion coding for a truncated protein were detected. These five isoforms were designated as novel Austrian isoforms. They demonstrate the habitat differences between Austrian and Swedish birch pollen by a genetic polymorphism of Bet v 1. Interestingly, no Austrian variants of the greater part of the Swedish isoforms have been found, especially Austrian isoforms belonging to the Bet v 1b group, which is quite abundant in Swedish pollen (Ferreira et al., 1996), are missing.

The relative amounts of isoforms a and d determined by mass spectroscopy and N-terminal sequence analysis of purified Bet v 1 from Swedish pollen (Ferreira et al., 1996) could be roughly corroborated in Austrian pollen on the mRNA level. Bet v 1a, the most frequent isoform in Swedish pollen, was also the most frequent isoform in Austrian pollen, its amount seems to be even higher. Likewise, the level of Bet v 1 d-like isoforms in Austrian pollen (24%) seems to be higher than in Swedish pollen (10%).

Additionally, two isoforms with a truncated open reading frame were found in Austrian pollen. The isoforms Bet v 1at14 and Bet v 1at87 differed from each other at one position and from Bet v 1a by one silent mutation. Bet v 1at87 differed from the Bet v 1a sequence in two positions at the DNA level. The truncated isoforms represented 4% of the Bet v 1 isoform repertoire analyzed. This may reflect the level of the natural occurrence of the truncated protein in Austrian birch pollen, although there is no proof of the expression of truncated Bet v 1 in Austrian birch pollen. We have done Western blots of protein extracts from the pollen that was used for cDNA synthesis. After detection with a polyclonal anti-Bet v 1 antibody several weak bands below 17 kDa were seen, one of them at 13 kDa, the calculated molecular weight of truncated Bet v 1 (data not shown). However, this is no evidence for the expression of truncated proteins since this method cannot distinguish between truncated isoforms and degradation products. An alternative method for the detection of truncated Bet v 1 could be Northern blotting. An objection to its use is the diffi-

culty to distinguish between RNAs of 483 and 490 nucleotides and the fact that it is impossible to design a probe specific for the truncated isoforms.

The truncated version of the protein (120 aa) comprised 75% of the full-length-version (160 aa including the starting methionine). Eleven T cell epitopes of Bet v 1 have been described (Ebner et al., 1993; 1995), three of which are immunodominant, which means nearly every donor displays TCCs reactive with this epitope. Eight T cell epitopes are still intact on the truncated Bet v 1. Yet, two of the missing epitopes are immunodominant. Although the truncated isoforms would produce a T cell stimulation as well, this response would be incomplete.

In 1996, the three-dimensional structure of Bet v 1 was published (Spangfort et al., 1996b; Gajhede et al., 1996). The amino acids that are missing in the truncated isoforms constitute the complete C-terminal α -helix, a loop region and a small part of β -strand 7 of the intact protein. In the cited studies, the hydrophobic core that is responsible for the integrity of the Bet v 1 structure was identified. This core includes residues from the N-terminal part of the C-terminal α -helix. If the complete C-terminal helix is missing, as is the case for the truncated isoforms, the hydrophobic core will be disrupted. Therefore, it is highly unlikely that the structure of the truncated Bet v 1 will resemble that of the full-length protein (M.D. Spangfort, personal communication).

The truncated isoforms Bet v 1at14 and Bet v 1at87 were generated as recombinant proteins and immunoblots were done. In contrast to IgE from patients' sera and the monoclonal anti-Bet v 1 antibodies BIP1 and BIP4 that did not bind to Bet v 1at14 and at87, a polyclonal rabbit anti-Bet v 1 IgG antibody reacted with the two truncated isoforms. These data demonstrate that a polyclonal antiserum contains IgG antibodies directed against epitopes that are not recognized by IgE antibodies. Those epitopes could be linear epitopes or conformational epitopes formed by locally limited structural elements. The inability of the monoclonal antibodies BIP1 and BIP4 to bind the truncated Bet v 1 isoforms indicates that these monoclonal IgG antibodies are dependent on the availability of epitopes formed by the intact structure of the complete protein.

Our data are in agreement with the results of Vrtala et al. (1997), who demonstrated T cell stimulation using two recombinant fragments of Bet v 1a. The fragments represent the two halves of the Bet v 1 protein. These halves were random-coiled, as shown by circular dichroism. Polyclonal mouse IgG against these fragments was generated and reacted also with complete recombinant Bet v 1. Patients' serum did not react with the Bet v 1 halves.

This study demonstrates the differences in the Bet v

1 isoallergen repertoire between Swedish and Austrian birch pollen. Although some Swedish isoforms are found in Austrian pollen, most of them are missing and some Austrian isoforms not found in Swedish pollen were detected. The immunological significance of this variability remains to be investigated.

Acknowledgements

This work was supported by grant S06707-MED of the “Fonds zur Foerderung der Wissenschaftlichen Forschung”, Vienna, Austria and by grant 7627/1 of the “Jubilaeumsfonds der Oesterreichischen Nationalbank”, Vienna, Austria.

References

- Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O., Breitenbach, M., 1989. The gene coding for the major birch pollen allergen Bet v 1, is highly homologous to a pea disease resistance response gene. *EMBO J.* 8, 1935–1938.
- Ebner, C., Szépfalusi, Z., Ferreira, F., Jilek, A., Valenta, R., Parronchi, P., Maggi, E., Romagnani, S., Scheiner, O., Kraft, D., 1993. 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.* 150, 1047–1054.
- Ebner, C., Schenk, S., Najafian, N., Siemann, U., Steiner, R., Fischer, G.W., Hoffmann, K., Szépfalusi, Z., Scheiner, O., Kraft, D., 1995. Nonallergic individuals recognize the same T cell epitopes of Bet v 1, the major birch pollen allergen, as atopic patients. *J. Immunol.* 154, 1932–1940.
- Ferreira, F.D., Hoffmann-Sommergruber, K., Breiteneder, H., Pettenburger, K., Ebner, C., Sommergruber, W., Steiner, R., Bohle, B., Sperr, W.R., Valent, P., Kungl, A.J., Breitenbach, M., Kraft, D., Scheiner, O., 1993. Purification and Characterization of recombinant Bet v 1, the Major Birch Pollen Allergen: immunological equivalence to natural Bet v 1. *J. Biol. Chem.* 268, 19,574–19,580.
- Ferreira, F., Hirtenlehner, K., Jilek, A., Godnik-Cvar, J., Breiteneder, H., Grimm, R., Hoffmann-Sommergruber, K., Scheiner, O., Kraft, D., Breitenbach, M., Rheinberger, H.J., Ebner, C., 1996. Dissection of 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.* 183, 599–609.
- Gajhede, M., Osmark, P., Poulsen, F.M., Ipsen, H., Larsen, J.N., van Neerven, R.J.J., Schou, C., Lowenstein, H., Spangfort, M.D., 1996. X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. *Nat. Struct. Biol.* 3, 1040–1045.
- Hoffmann-Sommergruber, K., Susani, M., Ferreira, F., Jertschin, P., Ahorn, H., Steiner, R., Kraft, D., Scheiner, O., Breiteneder, H., 1997a. High-level expression and purification of the major birch pollen allergen, Bet v 1. *Protein Expr. Purif.* 9, 33–39.
- Hoffmann-Sommergruber, K., Vanek-Krebitz, M., Radauer, C., Wen, J., Ferreira, F., Scheiner, O., Breiteneder, H., 1997b. Genomic characterization of members of the Bet v 1 family: genes coding for allergens and pathogenesis-related proteins share intron positions. *Gene* 197, 91–100.
- Ipsen, H., Horn, N., Hansen, D.C., 1988. Structural similarities between amentiferous tree pollen major allergens. *J. Allergy Clin. Immunol.* 81, 265.
- Jarolim, E., Rumpold, H., Endler, A.T., Ebner, H., Breitenbach, M., Scheiner, O., Kraft, D., 1989a. IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verrucosa*. *Allergy* 44, 385–395.
- Jarolim, E., Tejkl, M., Rohac, M., Schlerka, G., Scheiner, O., Kraft, D., 1989b. Monoclonal antibodies against birch pollen allergens: characterization by immunoblotting and use for single-step affinity purification of the major allergen Bet v 1. *Int. Arch. Allergy Appl. Immunol.* 90, 54–60.
- Larsen, J.N., 1995. Isoallergens—significance in allergen exposure and response. *ACI News* 7, 141–160.
- Larsen, J.N., Sparholt, S.H., Ipsen, H., 1996. Epitope Structure of Recombinant Isoallergens of Bet v 1. In: Kraft, D., Schon, A. (Eds.), *New Horizons in Allergy Immunotherapy*. Plenum Press, New York, pp. 243–249.
- Rohac, M., Birkner, T., Reimitzer, I., Bohle, B., Steiner, R., Breitenbach, M., Kraft, D., Scheiner, O., Gabl, F., Rumpold, H., 1991. The immunological relationship of epitopes on major tree pollen allergens. *Mol. Immunol.* 28, 897–906.
- Sanger, F., Nicklen, S., Coulsen, A.R., 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
- Spangfort, M.D., Ipsen, H., Sparholt, S.H., Aasmus-Olsen, S., Osmark, P., Poulsen, F.M., Larsen, M., Mortz, E., Roepstorff, P., Larsen, J.N., 1996a. Characterization of recombinant isoforms of birch pollen allergen Bet v 1. In: Kraft, D., Schon, A. (Eds.), *New horizons in Allergy Immunotherapy*. Plenum Press, New York, pp. 251–254.
- Spangfort, M.D., Larsen, J.N., Gajhede, M., 1996b. Crystallization and preliminary X-ray investigation at 2.0 Å resolution of Bet v 1, a birch pollen protein causing IgE-mediated allergy. *Proteins* 26, 358–360.
- Sparholt, S.H., Larsen, N.J., Ipsen, H., Schou, C., van Neerven, R.J.J., 1997. Crossreactivity and T cell epitope-specificity of Bet v 1-specific T cells suggests the involvement of multiple isoallergens in the sensitization to birch pollen. *Clin. Exp. Allergy* 27, 932–941.
- Swoboda, I., Jilek, A., Ferreira, F., Engel, E., Hoffmann-Sommergruber, K., Scheiner, O., Kraft, D., Breiteneder, H., Pittenauer, E., Schmid, E., Vicente, O., Heberle-Bors, E., Ahorn, H., Breitenbach, M., 1995. Isoforms of Bet v1, the major birch pollen allergen, analyzed by liquid chromatography, mass spectrometry and cDNA cloning. *J. Biol. Chem.* 270, 2607–2613.
- Van Neerven, R.J.J., Larsen, J.N., Wissenbach, M., Würtzen, P.A., 1999. T cell responses to pollen allergens—reactivity patterns and clinical relevance. *ACI International* 11, 37–42.
- Vrtala, S., Hirtenlehner, K., Vangelista, L., Pastore, A., Eichler, H.G., Sperr, W.R., Valent, P., Ebner, C., Kraft, D., Valenta, R., 1997. Conversion of the major birch pollen allergen, Bet v 1, into two nonanaphylactic T cell epitope-containing fragments. *J. Clin. Invest.* 99, 1673–1681.