

Bet v 1, the major birch pollen allergen, initiates sensitization to Api g 1, the major allergen in celery: evidence at the T cell level

Barbara Bohle¹, Astrid Radakovics¹, Beatrice Jahn-Schmid¹, Karin Hoffmann-Sommergruber¹, Gottfried F. Fischer² and Christof Ebner¹

¹ Department of Pathophysiology, Division of Immunopathology, University of Vienna, Vienna, Austria

² Department of Blood Group Serology, University of Vienna, Vienna, Austria

Due to IgE cross-reactivity, birch pollen-allergic individuals frequently develop type I hypersensitivity reactions to celery tuber. We evaluated the T cell response to the major allergen in celeriac, Api g 1, and the cellular cross-reactivity with its homologous major allergen in birch pollen, Bet v 1. Api g 1-specific T cell lines (TCL) and clones (TCC) were established from peripheral blood mononuclear cells of allergic patients. Epitope mapping of Api g 1 with overlapping Api g 1-derived peptides revealed one dominant T cell-activating region, Api g 1_{109–126}. TCL and TCC generated with Api g 1 cross-reacted with the birch pollen allergen and, although initially stimulated with the food allergen, cellular responses to Bet v 1 were stronger than to Api g 1. Epitope mapping with Bet v 1-derived peptides revealed that T cells specific for several distinct epitopes distributed over the complete Bet v 1 molecule could be activated by Api g 1. Bet v 1_{109–126} was identified as the most important T cell epitope for cross-reactivity with Api g 1. This epitope shares 72% amino acid sequence similarity with the major T cell-activating region of the food allergen, Api g 1_{109–126}. Our data provide evidence that humoral as well as cellular reactivity to the major celery allergen is predominantly based on cross-reactivity with the major birch pollen allergen. The activation of Bet v 1-specific Th2 cells by Api g 1, in particular outside the pollen season, may have consequences for birch pollen-allergic individuals.

Key words: Human / T lymphocytes / Cytokines / Food allergy / Th1/Th2

Received	2/7/03
Revised	26/9/03
Accepted	6/10/03

1 Introduction

Birch pollen allergy is one of the main causes of allergic rhinitis from spring to early summer in Central Europe [1, 2]. However, besides the symptoms induced by inhalation of pollen allergens, up to 70% of birch pollen-allergic individuals experience type I hypersensitivity reactions when ingesting certain fruits and vegetables [3, 4]. In most of these cases, clinical reactions appear immediately after contact with the raw food and are characterized by oral itching, angioedema and irritation of the oral mucosa, summarized in the term “oral allergy syndrome” [5]. Immunological cross-reactivity between specific IgE Ab directed against allergens in birch pollen and the homologous proteins in these “birch pollen-related foods” was shown to cause this type of food allergy

[6–12]. The major birch pollen allergen is recognized by specific IgE Ab in more than 95% of patients allergic to birch pollen [13]. This allergen belongs to the pathogenesis-related protein family 10 and several members of this protein family have been identified in birch pollen-related food, e.g. Mal d 1 in apples, Cor a 1 in hazelnuts, Pyr c 1 in pears, Pru av 1 in cherries or Dau c 1 in carrots [14]. Recently, an allergen homologous to Bet v 1 was identified in soybean [15].

Celery tuber frequently induces food allergy in birch pollen-allergic patients [16, 17]. The major allergen in celeriac, Api g 1, is a Bet v 1-homologous protein [8, 18–21]. Other less frequently recognized proteins in celery tuber were demonstrated to share IgE epitopes with allergens in birch pollen, i.e. proteins in the molecular range of 46–60 kDa and Api g 4, celery profilin [19, 22, 23]. In addition, the existence of cross-reactive carbohydrate determinants was demonstrated [21, 24]. Api g 1 was cloned and expressed in recombinant form [25]. This molecule is 146 amino acids (aa) long and shares 41% aa sequence identity (61% similarity) with Bet v 1.

[DOI 10.1002/eji.200324321]

Abbreviations: TCL: T cell lines TCC: T cell clones SI: Stimulation index aa: Amino acid

Purified rApi g 1 was assessed for its applicability for *in vitro* and *in vivo* diagnosis of celery allergy and revealed accurate results in areas where birch trees are common [26].

Based on clinical observations and experiments on the IgE reactivity *in vitro*, a predominant role for Bet v 1 in the sensitization against Api g 1 was suggested [25, 26]. To further elucidate this theory we sought to characterize the T cell response to the major allergen in celery and to analyze the cellular cross-reactivity between Bet v 1 and Api g 1 in detail. We stimulated T lymphocytes with rApi g 1 and established T cell lines (TCL) and T cell clones (TCC). These cultures were analyzed for their reactivity with the complete rBet v 1 molecule as well as with synthetic Bet v 1-derived peptides in order to analyze cross-reactive T cell epitopes. In addition, Api g 1-specific TCL were tested with Api g 1-derived peptides to define relevant T cell epitopes of the food allergen. Our results confirm the hypothesis that Bet v 1 initiates the sensitization to Api g 1 at the humoral as well as the cellular level.

2 Results

2.1 IgE inhibition determined by immunoblotting

The presence of IgE specific for rApi g 1 and rBet v 1 was demonstrated by immunoblotting (Fig. 1). All patients contained specific IgE against rApi g 1 (Fig. 1-I, lane 0) and rBet v 1 (Fig. 1-II, lane 0). Pretreatment of sera with rApi g 1 reduced IgE binding to Api g 1 (Fig. 1-I, lane A) but did not markedly influence the IgE reactivity to Bet v 1 (Fig. 1-II, lane A). In contrast, pre-incubation with rBet v 1 blocked IgE binding to both, Api g 1 and Bet v 1 (Fig. 1-I, II, lane B). Thus, all relevant IgE epitopes of Api g 1 are present in Bet v 1, but not vice versa. No signal was observed with a normal human serum pool (N; Fig. 1).

2.2 Proliferation assays

PBMC were stimulated with different concentrations of rApi g 1 and rBet v 1, respectively (data not shown). PBMC isolated from three nonallergic individuals did not proliferate in response to either allergen (data not shown). In allergic individuals stimulation of cells with 50 µg/ml of rApi g 1 and 25 µg/ml of rBet v 1 revealed strongest lymphoproliferative responses (Fig. 2). Stimulation of PBMC with rApi g 1 induced significantly less proliferation as with rBet v 1, although used at a higher concentration (Fig. 2; $p=0.001$).

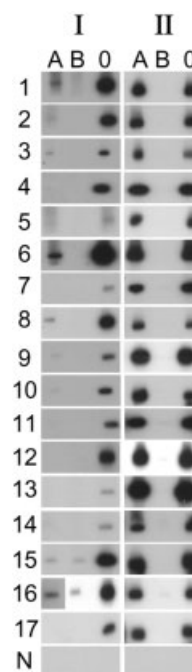


Fig. 1. IgE immunoblot. IgE reactivity of 17 different individuals to rApi g 1 (I, lane 0) and rBet v 1 (II, lane 0) is shown. Pre-incubation of sera with Api g 1 reduced IgE binding to Api g 1 (I, lane A) but not to Bet v 1 (II, lane A). Bet v 1 blocked IgE binding to both allergens (I and II, lane B). N = serum pool of nonallergic individuals.

2.3 TCL generated with Api g 1 are Bet v 1-specific

Twelve TCL generated with rApi g 1 were tested for their reactivity with rBet v 1 (Table 1). All Api g 1-specific TCL responded to incubation with the major birch pollen allergen. Overall, Api g 1-induced TCL showed significantly higher proliferative responses to Bet v 1 than to Api g 1 (Table 1; $p=0.005$). TCL11 and TCL12 had lost lymphoproliferative reaction to the initial stimulus Api g 1 but still responded to Bet v 1. These results indicate an enrichment of birch pollen-specific T cells in TCL generated with the celery allergen. Control TCL established without allergen showed no proliferative responses to either allergen (data not shown).

2.4 TCC generated with Api g 1 cross-react with Bet v 1

In total, 13 Api g 1-specific TCC from nine different patients were established (Table 2). When testing each TCC for proliferation in response to rBet v 1, 11/13 TCC reacted with the birch pollen allergen (Table 2). Ten of the 11 cross-reactive TCC responded more vigorously to

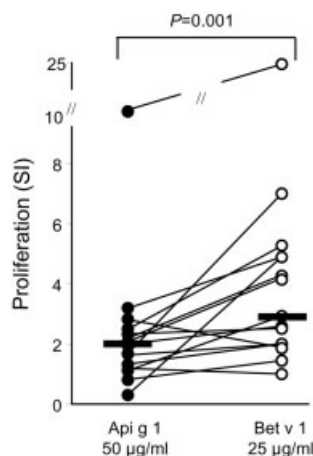


Fig. 2. Lymphoproliferation (SI) of PBMC derived from 13 allergic patients in response to stimulation with rApi g 1 (50 µg/ml, ●) and rBet v 1 (25 µg/ml, ○). The horizontal lines indicate the median value. Proliferative responses induced by Api g 1 were significantly lower than Bet v 1-induced proliferative responses (Wilcoxon Signed Ranks test, $p=0.001$).

Bet v 1 (Table 2). Only 2/13 TCC proliferated in response to Api g 1 exclusively. According to their cytokine pattern in response to stimulation with rApi g 1, 7/13 TCC were attributed to the Th2-like subset, 3/13 were Th1-like, and 3/13 produced similar levels of IL-4 and IFN- γ and were classified as Th0. All cross-reactive TCC synthesized the same cytokine pattern when stimulated with rBet v 1 (not shown).

2.5 Api g 1-specific T cell epitopes

To identify relevant T cell epitopes within the Api g 1 molecule, eight Api g 1-specific TCL were mapped using 48 overlapping dodecapeptides spanning the complete aa sequence of the food allergen [25]. Epitope recognition patterns of the different individuals are shown in Fig. 3. In total, seven distinct T cell epitopes of Api g 1 were identified. The peptides Api g 1_{1–12}, Api g 1_{28–39} and Api g 1_{139–150} were recognized by one individual each. The epitope located at Api g 1_{88–105} was recognized by

two individuals, peptides Api g 1_{10–24} and Api g 1_{121–138} were present in three TCL. Five of eight patients harbored T cells specific for epitopes contained in Api g 1_{109–126}, representing the major T cell-activating region of the Api g 1 molecule. In Fig. 4 the aa sequence of Api g 1_{109–126} is underlined within the Api g 1 molecule. Sequence alignment revealed that this dominant Api g 1-activating region shares 60% sequence identity (73% similarity) with the corresponding region of Bet v 1.

2.6 Cross-reactive Bet v 1-specific T cell epitopes

To define the T cell epitopes within Bet v 1 involved in the cross-reactivity, two TCL from the same patient were established using either rApi g 1 (A-TCL) or rBet v 1 (B-TCL) as initial stimulus. Subsequently, both TCL were subjected to epitope mapping using 50 overlapping peptides covering the complete sequence of Bet v 1 [27] and compared (Fig. 5). The peptides detected in B-TCL represented known T cell epitopes within the Bet v 1 molecule and very similar epitope recognition patterns were observed in the corresponding A-TCL [27]. Bet v 1_{61–75} and the epitope located within Bet v 1_{109–123} were recovered in all corresponding A-TCL. Peptides covering Bet v 1_{76–96} present in three B-TCL were found in two A-TCL. The N-terminal epitopes Bet v 1_{4–18} and Bet v 1_{16–33} were present in three A-TCL/five B-TCL and five A-TCL/seven B-TCL, respectively. The C-terminal T cell epitope covering Bet v 1_{142–156} present in eight B-TCL was detected in six A-TCL. Within these cross-reactive regions, some A-TCL responded to the Bet v 1 peptides whereas the corresponding B-TCL did not. On the other hand, the epitope contained in Bet v 1_{94–111} present in five B-TCL was recovered in only one A-TCL. Two patients responded to Bet v 1_{127–141} in A-TCL without reactivity in any B-TCL.

In summary, six prominent cross-reacting T cell epitopes within the Bet v 1 molecule were identified underlined in the sequence of Bet v 1 (Fig. 4). The sequence identity between Bet v 1 and Api g 1 at the respective positions are 27% for Bet v 1_{4–18}, 33% for Bet v 1_{16–33}, 33% for

Table 1. Proliferation of Api g 1-specific TCL induced by Api g 1 or Bet v 1

	1	2	3	4	5	6	7	8	9	10	11	12
Api g 1 50 µg/ml	3.2 ^{a)}	5.4	3.7	16.1	3.8	2.9	3.4	6.4	4.8	4.3	1.7	1.3
Bet v 1 5 µg/ml	3.8	7.8	5.6	20.9	3.7	4.6	2.0	6.8	24.1	18.7	7.3	3.4

^{a)} SI are shown.

Table 2. Characterization of Api g 1-specific TCC^{a)}

	TCC	IL-4 (pg/ml)	IFN- γ (pg/ml)	IL-10 (pg/ml)	Th subset	Phenotype	Api g 1 (SI)	Bet v 1 (SI)
1	C-A82	<9	>1000	33.1	1	CD4+ $\alpha\beta$ +	13.6	1
2	C-A106	20.7	35.2	14.2	0	CD4+ $\alpha\beta$ +	57.9	6.7
3	D-A21	1418	63.7	>1000	2	CD4+ $\alpha\beta$ +	6.8	359
4	DD-A91	88	<9.5	<4.7	2	CD4+ $\alpha\beta$ +	9.5	242
5	GR-A65	500	<9.5	11.1	2	CD4+ $\alpha\beta$ +	31.4	102
6	H-A78	116	15	233	2	CD4+ $\alpha\beta$ +	10.2	1
7	K-A110	<9	>1000	299	1	CD4+ $\alpha\beta$ +	4.3	6.7
8	SI-A25	774	<9.5	81.2	2	CD4+ $\alpha\beta$ +	53.7	720
9	S-A3	29.4	54.1	7.6	0	CD4+ $\alpha\beta$ +	7.6	733
10	S-A41	12.1	109	<4.7	1	CD4+ $\alpha\beta$ +	7.7	25.5
11	S-A49	1644	122.8	45	2	CD4+ $\alpha\beta$ +	15	50
12	S-A133	9.4	<9.5	<4.7	0	CD4+ $\alpha\beta$ +	9.8	189
13	W-A9	733	<9.5	576	2	CD4+ $\alpha\beta$ +	59	923

^{a)} For specific stimulation 5 μ g/ml Bet v 1 and 25 μ g/ml Api g 1 were used.

Bet v 1_{61–75}, 24% for Bet v 1_{76–96}, 60% for Bet v 1_{109–123} and 40% for Bet v 1_{142–156}.

3 Discussion

The cellular response to Api g 1, the Bet v 1-homologous allergen in celery tuber, was characterized and compared with the major birch pollen allergen. Our data indicate that predominantly birch pollen-specific T cells responded to the celery allergen and that birch pollen-related foods represent an important perennial stimulus for these lymphocytes.

Similar to our previous report on the T cell cross-reactivity between Bet v 1 and Mal d 1, the major allergen in apple, cellular reactivity with the celery homologue was mainly based on cross-reacting Bet v 1-specific T cells [28]. Consequently, we were interested to define the T cell epitopes of Bet v 1 responsible for reactivity with Api g 1. Six T cell epitopes distributed over the complete birch pollen molecule were found (Fig. 5). Hence, Api g 1 seems capable of activating T cells specific for several distinct Bet v 1 epitopes, although both allergens share only 41% aa sequence identity. In 9/12 TCL generated with Api g 1, the epitope Bet v 1_{109–126} was present indicating that this epitope primarily accounted for cross-reactivity with the celery allergen

(Fig. 5). Bet v 1_{109–126} shares 61% aa identity (72% similarity) with Api g 1_{109–126}, the T cell epitope which dominated the T cell response to Api g 1 (Fig. 3). The high aa identity in this part of both allergens may explain that Api g 1_{109–126} represented the major T cell-activating part of the celery allergen. No association between recognition of this particular T cell-activating domain and expression of special HLA class II molecules was found, which is consistent with previous results from our group (data not shown) [29].

In birch pollen-allergic individuals, Bet v 1-specific Th cells persist for a long time period in peripheral blood and skin [30]. However, CD4⁺ T cells do not seem to be intrinsically long-lived but seem to require intermittent and repeated contact with Ag to remain alive [31, 32]. The uptake of birch pollen-related food might activate Bet v 1-specific CD4⁺ T cells when patients do not encounter the pollen allergen. *In vivo* evidence for this hypothesis was provided by Reekers et al., who showed that ingestion of birch pollen-related food aggravated symptoms mediated by Bet v 1-specific T cells in the skin of atopic dermatitis patients [33].

In our opinion, the major celery allergen seems to play a particularly important role for activation of Bet v 1-specific T lymphocytes. The epitope Bet v 1_{109–123} mainly involved in cellular cross-reactivity between Api g 1 and

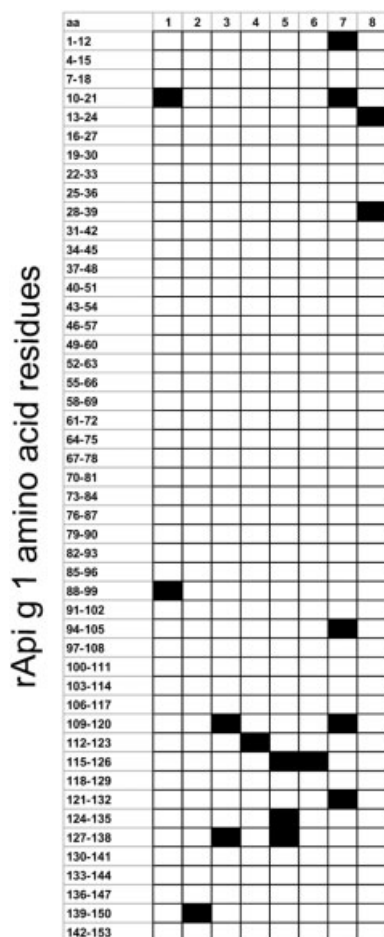


Fig. 3. Api g 1-specific T cell epitopes. TCL from eight patients were established with Api g 1 and proliferation was tested with overlapping peptides spanning the Api g 1 sequence. Proliferative responses (SI>3) are shown as black boxes.

Bet v 1 is recognized by the majority of birch pollen-allergic individuals (Fig. 5; [27, 29]). Recent observations from our laboratory showed that 45% of TCL generated with Bet v 1 responded to stimulation with Api g 1 whereas only 35% cross-reacted with the major apple allergen. Approximately 84% of birch pollen-allergic patients experience IgE-mediated symptoms after ingestion of apples but only 14% develop clinical food allergy to celery [20]. Therefore, a high number of symptom-free patients does not avoid this common food source and, as a consequence, continuously provides Ag to Bet v 1-specific T cells. The majority of Api g 1-specific TCC depicted in Table 2 was isolated from blood samples collected outside the pollen season and reacted with Bet v 1. In addition, 55% of these clones synthesized IL-4 in response to the food allergen. From these results we deduce that allergens in food might not only contribute to repeated activation of pollen-specific T cells but also to the typical maintenance of Bet v 1-specific IgE levels outside the season of pollination.

In conclusion, our analysis of the immune response to Api g 1 reveals that in addition to all relevant B cell epitopes the major birch pollen allergen contains the major T cell-activating region of Api g 1. This may finally confirm that Bet v 1 represents the pioneer in initializing an allergic response to the major celery allergen, which then plays a role in boosting the pollen-specific immune response.

4 Materials and methods

4.1 Allergic patients

Type I allergy to birch pollen and celery was documented by typical case histories and positive skin prick tests. In addition, each patient displayed typical wheal-and-flare reactions to fresh celery root (prick to prick test). None of the

Bet	1	<u>GVFN</u> YETETTSVIPAA <u>RLFKAF</u> ILDGDNLFPKVAPQAISSVENIEGNGGPGTIKKISFP	59
		GV + E TS + A ++F+ F++D D + PK AP A SVE I+G+GGPGT+K I+ P	
Api	1	GVQTHVLELTSSVSAEKIFQGFVIDVDTVLPKAAPGAYKSVE- <u>IKGDGGPGTLKIITLP</u>	59
Bet	60	<u>EGFP</u> FKYVKDRVDEVDTNFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISN	118
		+G P + R+D V+ ++YSVI+G + +E I N + +V T DGGSI K +	
Api	60	DGGPITMTLRIDGVNKEALTFDYSVIDGDILLGFIESIENHVVLVPTA <u>DGGSICKTTA</u>	118
Bet	119	<u>KYHT</u> KGDEHVKAQVKASKEMGETLLRAVESYLLAHSDAYN	159
		+HTKGD V E +K + E L +A+E+YL+A+	
Api	119	<u>IFHT</u> KGDAVVPEENIKYANEQNTALFKALEAYLIAN	154

Fig. 4. Sequence alignment of Bet v 1 and Api g 1. Italic letters indicate sequence identity, + marks conservative exchanges. The immunodominant T cell-activating region of Api g 1 is underlined in the Api g 1 sequence. T cell epitopes of Bet v 1 involved in the cross-reactivity between Bet v 1 and Api g 1 are underlined in the Bet v 1 sequence.

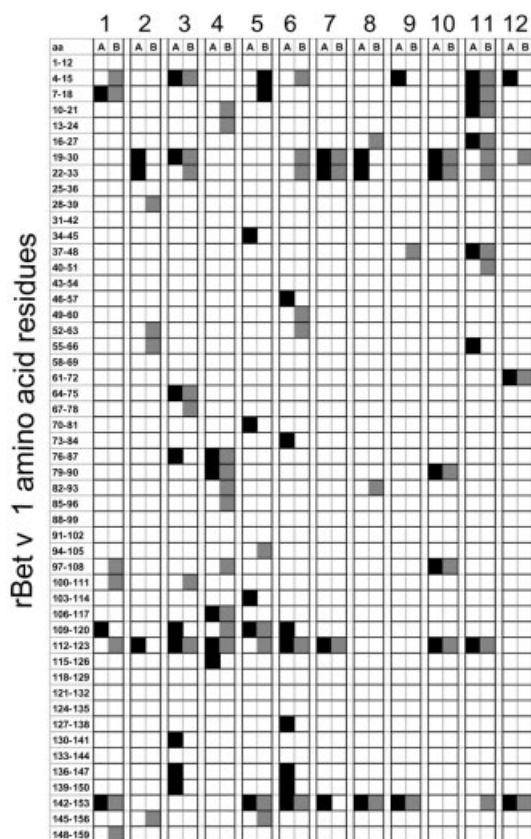


Fig. 5. Bet v 1-specific T cell epitopes in rApi g 1-induced (A) and rBet v 1-induced (B) TCL. Both TCL from 12 different patients were stimulated with a panel of 50 peptides representing the aa sequence of Bet v 1. Peptides inducing an SI > 3 are shown as black boxes in A-TCL and as gray boxes in B-TCL.

patients had developed more severe symptoms to celery than oral allergy syndromes. All patients possessed IgE directed against rBet v 1 and rApi g 1 in their sera (Fig. 1). Molecular HLA typings for HLA-DRB and -DQB alleles were performed according to methods described [34].

4.2 Allergens

rApi g 1 and rBet v 1 were obtained from Biomay (Vienna, Austria). Bacterial endotoxin was detected using the *Limulus* amoebocyte lysate assay (BioWhittaker, Walkersville, MD). Endotoxin levels in both allergens were below 30 pg/μg.

4.3 Immunoblots and inhibition assays

Immunoblotting of patients' sera was performed as previously described [35]. Briefly, rApi g 1 (2 μg per lane) or rBet v 1 (1 μg per lane) were separated by 12% SDS-PAGE and blotted onto a nitrocellulose membrane. The membrane

was incubated with patients' sera overnight at 4°C. After incubation with a ¹²⁵I-labeled anti-human IgE Ab (IBL, Hamburg, Germany), bound IgE was visualized by autoradiography. A serum pool obtained from five donors without birch pollen allergy served as a negative control. For inhibition experiments the serum of each patient was incubated overnight at room temperature with either rApi g 1 (5 μg/ml) or rBet v 1 (5 μg/ml). As control, the same concentration of BSA was added.

4.4 Proliferation assays

PBMC were obtained from peripheral blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia Diagnostics, Uppsala, Sweden). PBMC (2 × 10⁵) were cultured in triplicates in 96-well plates (Nunc, Denmark) in 200 μl of serum-free Ultra Culture Medium (BioWhittaker) supplemented with 2 mM/l glutamine and 2 × 10⁻⁵ M 2-ME in the presence of either allergen for 6 days at 37°C and 5% CO₂ in a humidified atmosphere. rApi g 1 was titrated in the concentration range of 12.5 to 100 μg/ml, rBet v 1 in the range of 3.18 to 25 μg/ml. During the last 16 h of culture, [³H]thymidine (0.5 μCi per well) was added and the incorporated radioactivity was measured by scintillation counting.

4.5 Allergen-specific TCL

Allergen-specific short-term TCL were obtained by a technique previously described [36]. Briefly, PBMC (1.5 × 10⁶/ml) were stimulated with either 20 μg/ml of purified rApi g 1 or 10 μg/ml of purified rBet v 1a in 24-well flat-bottomed culture plates (3524; Costar, Cambridge, MA) under conditions as described above. After 5 days, 10 U/ml of human rIL-2 (Boehringer, Mannheim, Germany) was added. At day 7, T cell blasts were enriched by density gradient centrifugation and TCC were established by limiting dilution. Remaining T cell blasts were expanded at weekly intervals with fresh allogeneic irradiated feeder cells and rIL-2. Cells of expanded TCL (5 × 10⁴) were restimulated with rApi g 1 (50 μg/ml) and rBet v 1 (5 μg/ml) in the presence of 1 × 10⁵ autologous irradiated (60 Gy) PBMC. TCL were mapped for T cell epitopes using a panel of 48 dodecapeptides representing the complete aa sequence of Api g 1 [25] (5 μg/ml of each peptide; Mimotopes/Biotrend, Köln, Germany) and a panel of 50 dodecapeptides representing the complete aa sequence of Bet v 1a (Mimotopes/Biotrend) [27]. After 48 h, [³H]thymidine was added and cell proliferation was evaluated as described above. The stimulation index (SI) was calculated as ratio between cpm obtained in cultures with TCL plus autologous PBMC plus Ag and cpm obtained in cultures containing TCL and PBMC. Peptides inducing an SI > 3 were considered as positive.

4.6 Api g 1-specific TCC

TCC were established according to published protocols [36]. Briefly, T cell blasts from Api g 1-specific TCL were seeded by limiting dilution technique in 96-well round-bottom plates (Nunc) in the presence of 2×10^5 irradiated allogeneic PBMC, 0.5% (v/v) PHA (Gibco, Grand Island, NY) and rIL-2 (4 U/well) in the medium described above. Growing microcultures were expanded at weekly intervals with fresh allogeneic irradiated PBMC and rIL-2. The specificity of TCC was assessed in proliferation assays as soon as the cell number reached 2×10^5 . Api g 1-specific TCC were thereafter expanded by alternating turns of stimulation with autologous irradiated PBMC and allergen, or with allogeneic feeder cells and rIL-2. The phenotype of TCC was analyzed by FCM as previously described [36].

4.7 Measurement of cytokine patterns of TCC

TCC were washed and incubated with irradiated autologous PBMC in the presence of rApi g 1 (50 µg/ml) or rBet v 1 (5 µg/ml) for 24 h. Cytokine levels in SN were measured by ELISA using matched Ab pairs (Endogen, Woburn, MA) according to instructions by the manufacturers (sensitivity limits: IL-4: 9 pg/ml, IFN-γ: 9.5 pg/ml, IL-10: 4.7 pg/ml). Cultures containing TCC and PBMC alone served as negative controls. TCC were distinguished into Th subsets according to their cytokine pattern in response to allergen-specific stimulation. TCC synthesizing more than five times pg/ml IL-4 than pg/ml IFN-γ were attributed to the Th2-like subset, TCC producing five times more pg/ml IFN-γ than pg/ml IL-4 to the Th1-like subset. TCC producing similar levels of IL-4 and IFN-γ were classified as Th0.

4.8 Statistical analysis

Statistical significance of differences was determined by the Wilcoxon Signed Ranks test. Differences were considered statistically significant for $p < 0.05$.

Acknowledgements: This work was supported by Grant SFB F018-07 from the Fonds zur Förderung der Wissenschaftlichen Forschung, Vienna, Austria, and by Biomay, Vienna, Austria. The authors wish to thank Ingrid Fae for excellent technical support.

References

- 1 Wuthrich, B., Epidemiology of allergies in Switzerland. *Ther. Umsch.* 2001. **58**: 253–258.
- 2 Arshad, S. H., Karmaus, W., Matthews, S., Mealy, B., Dean, T., Frischer, T., Tsitoura, S., Bojarskas, J., Kuehr, J. and Forster, J., Association of allergy-related symptoms with sensitisation to common allergens in an adult European population. *J. Investig. Allergol. Clin. Immunol.* 2001. **11**: 94–102.
- 3 Dreborg, S. and Foucard, T., Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983. **38**: 167–172.
- 4 Wuthrich, B., Birch pollen allergy in relation with hypersensitivity for various kinds of fruit. *Hautarzt* 1990. **41**: 583–584.
- 5 Ortolani, C., Ispano, M., Pastorello, E., Bigi, A. and Ansaloni, R., The oral allergy syndrome. *Ann. Allergy* 1988. **61**: 47–52.
- 6 Calkhoven, P. G., Aalbers, M., Koshte, V. L., Pos, O., Oei, H. D. and Aalberse, R. C., 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**: 382–390.
- 7 Ebner, C., Birkner, T., Valenta, R., Rumpold, H., Breitenbach, M., Scheiner, O. and Kraft, D., Common epitopes of birch pollen and apples – studies by Western and Northern blot. *J. Allergy Clin. Immunol.* 1991. **88**: 588–594.
- 8 Ebner, C., Hirschwehr, R., Bauer, L., Breiteneder, H., Valenta, R., Ebner, H., Kraft, D. and Scheiner, O., 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**: 962–969.
- 9 van Ree, R., Fernandez-Rivas, M., Cuevas, M., van Wijngaarden, M. and Aalberse, R. C., Pollen-related allergy to peach and apple: an important role for profilin. *J. Allergy Clin. Immunol.* 1995. **95**: 726–734.
- 10 Asero, R., Relevance of pollen-specific IgE levels to the development of Apiaceae hypersensitivity in patients with birch pollen allergy. *Allergy* 1997. **52**: 560–564.
- 11 Asero, R., Detection and clinical characterization of patients with oral allergy syndrome caused by stable allergens in Rosaceae and nuts. *Ann. Allergy Asthma Immunol.* 1999. **83**: 377–383.
- 12 Scheurer, S., Wangorsch, A., Nerkamp, J., Skov, P. S., Ballmer-Weber, B., Wuthrich, B., Hausteiner, D. and Vieths, S., Cross-reactivity within the profilin panallergen family investigated by comparison of recombinant profilins from pear (Pyr c 4), cherry (Pru av 4) and celery (Api g 4) with birch pollen profilin Bet v 2. *J. Chromatogr. B Biomed. Sci. Appl.* 2001. **756**: 315–325.
- 13 Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O. and Breitenbach, M., The gene coding for the major birch pollen allergen Betv1, is highly homologous to a pea disease resistance response gene. *EMBO J.* 1989. **8**: 1935–1938.
- 14 Breiteneder, H. and Ebner, C., Molecular and biochemical classification of plant-derived food allergens. *J. Allergy Clin. Immunol.* 2000. **106**: 27–36.
- 15 Kleine-Tebbe, J., Vogel, L., Crowell, D. N., Hausteiner, U. F. and Vieths, S., Severe oral allergy syndrome and anaphylactic reactions caused by a Bet v 1-related PR-10 protein in soybean, SAM22. *J. Allergy Clin. Immunol.* 2002. **110**: 797–804.
- 16 Wuthrich, B., Stager, J. and Johansson, S. G., Celery allergy associated with birch and mugwort pollinosis. *Allergy* 1990. **45**: 566–571.
- 17 Ballmer-Weber, B. K., Vieths, S., Luttkopf, D., Heuschmann, P. and Wuthrich, B., Celery allergy confirmed by double-blind, placebo-controlled food challenge: a clinical study in 32 subjects with a history of adverse reactions to celery root. *J. Allergy Clin. Immunol.* 2000. **106**: 373–378.
- 18 Helbling, A., Lopez, M., Schwartz, H. J. and Lehrer, S. B., Reactivity of carrot-specific IgE antibodies with celery, apiaceous spices, and birch pollen. *Ann. Allergy* 1993. **70**: 495–499.
- 19 Bauer, L., Ebner, C., Hirschwehr, R., Wuthrich, B., Pichler, C., Fritsch, R., Scheiner, O. and Kraft, D., IgE cross-reactivity

- between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens: immunoblot investigation of the birch-mugwort-celery syndrome. *Clin. Exp. Allergy* 1996. **26**: 1161–1170.
- 20 Jankiewicz, A., Aulepp, H., Baltes, W., Bogl, K. W., Dehne, L. I., Zuberbier, T. and Vieths, S., Allergic sensitization to native and heated celery root in pollen-sensitive patients investigated by skin test and IgE binding. *Int. Arch. Allergy Immunol.* 1996. **111**: 268–278.
 - 21 Luttkopf, D., Ballmer-Weber, B. K., Wuthrich, B. and Vieths, S., Celery allergens in patients with positive double-blind placebo-controlled food challenge. *J. Allergy Clin. Immunol.* 2000. **106**: 390–399.
 - 22 Ganglberger, E., Radauer, C., Grimm, R., Hoffmann-Sommergruber, K., Breiteneder, H., Scheiner, O. and Jensen-Jarolim, E., N-terminal sequences of high molecular weight allergens from celery tuber. *Clin. Exp. Allergy* 2000. **30**: 566–570.
 - 23 Scheurer, S., Wangorsch, A., Haustein, D. and Vieths, S., Cloning of the minor allergen Api g 4 profilin from celery (*Apium graveolens*) and its cross-reactivity with birch pollen profilin Bet v 2. *Clin. Exp. Allergy* 2000. **30**: 962–971.
 - 24 Fotisch, K., Altmann, F., Haustein, D. and Vieths, S., Involvement of carbohydrate epitopes in the IgE response of celery-allergic patients. *Int. Arch. Allergy Immunol.* 1999. **120**: 30–42.
 - 25 Breiteneder, H., Hoffmann-Sommergruber, K., O'Riordain, G., Susani, M., Ahorn, H., Ebner, C., Kraft, D. and Scheiner, O., Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur. J. Biochem.* 1995. **233**: 484–489.
 - 26 Hoffmann-Sommergruber, K., Demoly, P., Crameri, R., Breiteneder, H., Ebner, C., Laimer Da Camara Machado, M., Blasler, K., Ismail, C., Scheiner, O., Bousquet, J. and Menz, G., IgE reactivity to Api g 1, a major celery allergen, in a Central European population is based on primary sensitization by Bet v 1. *J. Allergy Clin. Immunol.* 1999. **104**: 478–484.
 - 27 Ebner, C., Szepefalusi, Z., Ferreira, F., Jilek, A., Valenta, R., Parronchi, P., Maggi, E., Romagnani, S., Scheiner, O. and Kraft, D., 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. **150**: 1047–1054.
 - 28 Fritsch, R., Bohle, B., Vollmann, U., Wiedermann, U., Jahn-Schmid, B., Krebitz, M., Breiteneder, H., Kraft, D. and Ebner, C., Bet v 1, the major birch pollen allergen, and Mal d 1, the major apple allergen, cross-react at the level of allergen-specific T helper cells. *J. Allergy Clin. Immunol.* 1998. **102**: 679–686.
 - 29 Ebner, C., Schenk, S., Najafian, N., Siemann, U., Steiner, R., Fischer, G. W., Hoffmann, K., Szepefalusi, Z., Scheiner, O. and Kraft, D., Nonallergic individuals recognize the same T cell epitopes of Bet v 1, the major birch pollen allergen, as atopic patients. *J. Immunol.* 1995. **154**: 1932–1940.
 - 30 Bohle, B., Schwihla, H., Hu, H. Z., Friedl-Hajek, R., Sowka, S., Ferreira, F., Breiteneder, H., Bruijnzeel-Koomen, C. A., de Weger, R. A., Mudde, G. C., Ebner, C. and Van Reijssen, F. C., Long-lived Th2 clones specific for seasonal and perennial allergens can be detected in blood and skin by their TCR-hypervariable regions. *J. Immunol.* 1998. **160**: 2022–2027.
 - 31 Matsushita, S., Kohsaka, H. and Nishimura, Y., Evidence for self and nonself peptide partial agonists that prolong clonal survival of mature T cells *in vitro*. *J. Immunol.* 1997. **158**: 5685–5691.
 - 32 Nakamura, Y., Takagi, S., Suzuki, M., Ito, H., Murakami, S. and Ohta, N., Survival of memory T cells specific for Japanese cypress pollen allergen is maintained by cross-stimulation of putative pectate lyases from other plants. *Allergy* 2001. **56**: 385–392.
 - 33 Reekers, R., Busche, M., Wittmann, M., Kapp, A. and Werfel, T., Birch pollen-related foods trigger atopic dermatitis in patients with specific cutaneous T cell responses to birch pollen antigens. *J. Allergy Clin. Immunol.* 1999. **104**: 466–472.
 - 34 Fischer, G. F., Fae, I., Petrasek, M., Haas, H. and Mayr, W. R., An HLA-DR11 variant (HLA-DRB1*1115) segregating in a family of Turkish origin. *Tissue Antigens* 1995. **45**: 143–144.
 - 35 Jarolim, E., Rumpold, H., Endler, A. T., Ebner, H., Breitenbach, M., Scheiner, O. and Kraft, D., IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verrucosa*. *Allergy* 1989. **44**: 385–395.
 - 36 Bohle, B., Wagner, B., Vollmann, U., Buck, D., Niggemann, B., Szepefalusi, Z., Fischer, G., Scheiner, O., Breiteneder, H. and Ebner, C., Characterization of T cell responses to Hev b 3, an allergen associated with latex allergy in spina bifida patients. *J. Immunol.* 2000. **164**: 4393–4398.

Correspondence: Barbara Bohle, Department of Pathophysiology, Division of Immunopathology, Waehringer Guertel 18–20, A-1090 Vienna, Austria
 Fax: +43-1-40400-5130
 e-mail: barbara.bohle@akh-wien.ac.at