Bet v $1_{142-156}$ is the dominant T-cell epitope of the major birch pollen allergen and important for cross-reactivity with Bet v 1–related food allergens

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Background: Individuals with birch pollen allergy frequently experience hypersensitivity reactions to certain foods, primarily because of IgE antibodies specific for the major birch pollen allergen Bet v 1 that cross-react with homologous food allergens.
Objective: We sought to characterize the major T-cell epitopes of Bet v 1 and to investigate their involvement in the cellular cross-reactivity with homologous food allergens.
Methods: T-cell epitope mapping of Bet v 1 was performed by testing Bet v 1–specific T-cell lines derived from 57 individuals with birch pollen allergy, with overlapping peptides representing the entire allergen. T-cell lines and T-cell clones were stimulated with Bet v 1–related major allergens from apple (Mal d 1), cherry (Pru av 1), hazelnut (Cor a 1), celery (Api g 1), carrot (Dau c 1), and soybean (Gly m 4) and with peptides deduced from the C-terminal amino acid sequences of these molecules.
Results: Bet v $1_{142-156}$, positioned in the highly conserved C-terminal region of Bet v 1, was identified as the major T-cell epitope recognized by 61% of individuals. Most T lymphocytes specific for Bet v $1_{142-156}$ were activated by one or more homologous food proteins or the respective peptides, as indicated by proliferation and cytokine production.
Conclusion: The major T-cell epitope of Bet v 1, Bet v $1_{142-156}$, plays an important role in the cellular cross-reactivity between this respiratory allergen and related food allergens. Thus T lymphocytes specific for Bet v $1_{142-156}$ might be activated by various Bet v 1–related food allergens *in vivo*, even out of the pollen season. (J Allergy Clin Immunol 2005;116:213-9.)

Key words: *Birch pollen allergy, food allergy, oral allergy syndrome, PR-10, Bet v 1, T-cell cross-reactivity*

Abbreviations used
APC: Antigen-presenting cell
OAS: Oral allergy syndrome
PR: Pathogenesis-related
SI: Stimulation index
TCC: T-cell clone
TCL: T-cell line

Birch pollen allergy is one of the main causes of allergy from spring to early summer in northern and central Europe. The major birch pollen allergen Bet v 1 is recognized by specific IgE antibodies in more than 95% of individuals with birch pollen allergy. Cloning and sequencing of Bet v 1 and its expression as a recombinant protein has facilitated the detailed characterization of this allergen on both the molecular and immunologic levels.[1-3] The 3-dimensional structure of Bet v 1 was determined by means of x-ray diffraction and nuclear magnetic resonance spectroscopy.[4] Immunologically, Bet v 1 contains conformational B-cell epitopes that have been defined by mAbs and peptide mimotopes,[5,6] whereas no linear B-cell epitopes have been described. Various linear T-cell epitopes within the Bet v 1 molecule were determined in individuals with birch pollen allergy, as well as in nonallergic individuals.[7,8] Hence Bet v 1 represents a very well-characterized allergen. Recently, novel treatment strategies for specific immunotherapy of patients with birch pollen allergy have been developed. Hypoallergenic variants of Bet v 1 with reduced IgE-binding capacities but retained T cell–activating properties have been created, either by using site-directed mutagenesis or through conversion of Bet v 1 into 2 fragments.[9,10] The latter already has been evaluated recently in clinical studies.[11]

Birch pollinosis in adolescents or adults is strongly associated with food allergy to certain fruits of the Rosaceae family (eg, apples, cherries, and apricots), tree nuts (eg, hazelnuts), and vegetables of the Apiaceae family (eg, celery and carrots).[12,13] Up to 70% of patients with birch pollen allergy exhibit a so-called oral allergy syndrome (OAS), a localized immediate-type hypersensitivity reaction characterized by irritation, itching, and

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TABLE I. Sequence alignment of Bet v $1_{142-156}$ with the corresponding amino acid regions in Bet v 1–related food allergens

Allergen	Food	Amino acid sequence*	Peptide Similarity	Total protein Similarity
Bet v 1	–	TLLRAVESYLLAHSD	–	–
Cor a 1	Hazelnut	G**L***F***K****A****V****E***A***Y****L****L****A****H****P****D**	80	79
Gly m 4	Soybean	A**L***F***K****A***/***E***A***Y****L****L****A****H****P****D**	80	63
Api g 1	Celeriac	A**L***F***K****A****L****E***A***Y****L***/***A***N*	73	61
Dau c 1	Carrot	A**L***F***K****A***/***E***A***Y****L***/***A***N*	73	57
Mal d 1	Apple	G**L***F***K****L***/***E****S****Y****L***K***D****H****P****D**	60	71
Pyr c 1	Pear	G**L***F***K****L***/***E****S****Y****L***K***D****H****P****D**	60	70
Pru av 1	Cherry	N**L***F***K****L***/***E***T***Y****L***K***G****H****P****D**	60	74
Pru ar 1	Peach	N**L***F***K****L***/***E***T***Y****L***K***G****H****P****D**	60	72

Italics, conservative exchanges of amino acids; *bold*, identical amino acids.
*Amino acids are represented by 1-letter symbols.

swelling of lips and the oral mucosa on contact with raw fruits or vegetables containing allergens belonging to the pathogenesis-related (PR) protein family.[10,14-16] Systemic and more severe symptoms than OAS have been reported for patients with sensitization to Gly m 4 (SAM22; soybean).[17] The major birch pollen allergen plays a substantial role in the initiation of these food allergies, forming the so-called birch-fruit syndrome.[18] After initial sensitization to Bet v 1 by means of inhalation, Bet v 1–specific IgE antibodies cross-react with structurally related proteins in these foods, leading to clinical symptoms.

In addition to the humoral cross-reactivity between pollen and food allergens, cross-reactivity at the T-cell level has been demonstrated for homologues of Bet v 1 from apple and celery.[19,20] For both food allergens, a dominant T-cell epitope was identified that shares a high sequence homology with Bet v 1. In the present study we intended to evaluate important T-cell epitopes of Bet v 1 and their involvement in cross-reactivity with food allergens. T-cell epitopes were identified by means of peptide mapping of Bet v 1–specific T-cell lines (TCLs) derived from a large number of patients (n = 57) with associated food allergy. Bet v 1–specific TCLs and T-cell clones (TCCs) were stimulated with the Bet v 1–related food allergens Mal d 1 (apple), Pru av 1 (cherry), Cor a 1 (hazelnut), Api g 1 (celery), Dau c 1 (carrot), and Gly m 4 (SAM22; soybean) to determine cellular cross-reactivity.

METHODS

Patients

In total, 57 adults with birch pollen allergy and OAS to apple were included in the study. Type I allergy to birch pollen was documented on the basis of typical case history, positive skin prick test responses (Soluprick; ALK-Abello, Hørsholm, Denmark), and CAP-RAST scores of 3 or greater (Pharmacia, Uppsala, Sweden). In the sera of all patients, Bet v 1–specific IgE was demonstrated by means of immunoblotting (not shown). Skin prick test responses with fresh Golden Delicious apples (prick-to-prick method) were positive in all patients. The prick-to-prick tests were performed by pricking the freshly cut food with a disposable skin prick lancet (ALK-Abello) and then using this lancet to prick the skin on the volar forearm, according to the Dreborg and Foucard method.[21] Histamine (10 mg/mL) and 0.9% NaCl (Soluprick, ALK-Abello) were used as positive and negative controls, respectively. Reactions were recorded after 15 minutes. The test responses were considered positive if the wheal produced had a mean diameter of at least 3 mm. Moreover, skin prick tests with commercially available extracts for hazelnut and celery (Soluprick, ALK-Abello) and prick-to-prick tests with fresh celery, carrots, and hazelnuts were performed. Skin prick and prick-to-prick tests provided comparable results (concordance of 87%) but had a low predictive value for food allergy, as is known from other studies.[22,23] Adverse reactions to pollen-related foods were assessed by means of interrogation in standardized interviews. In addition to an OAS to apple, 76% of the individuals were stated to have OAS to peach, 69% to hazelnuts, 44% to carrots, 38% to cherries, 36% to pear, 27% to celery, and 7% to soybean. None of the patients had experienced symptoms more severe than OAS to any food.

Allergens and peptides

Recombinant Bet v 1, Api g 1, and Dau c 1 were purchased from Biomay (Vienna, Austria). Recombinant Cor a 1, Pru av 1, and Gly m 4 were produced as previously described.[17,24,25] Synthetic peptides comprising the homologous amino acid region in several Bet v 1–related food allergens consisting of 13 to 15 amino acids (as listed in Table I) were purchased from Mimotopes (Biotrend, Köln, Germany).

T-cell epitope mapping

Short-term allergen-specific TCLs were generated from PBMCs of individuals with birch pollen allergy by means of initial stimulation with 10 μg/mL Bet v 1 according to protocols described previously.[8] In parallel, cultures without Bet v 1 served as controls. To map T-cell epitopes, Bet v 1–specific TCLs were stimulated with a panel of 50 overlapping, synthetic 12-mer peptides (Mimotopes, Biotrend) representing the complete amino acid sequence of Bet v 1. Incorporation of tritiated thymidine was measured after 48 hours, as previously described.[8] Stimulation indices (SIs) were calculated as the ratio between counts per minute obtained in cultures with T cells plus autologous antigen-presenting cells (APCs) plus peptide and counts per minute obtained in cultures containing T cells and APCs alone. A mean SI of 2.5 was defined as a positive T-cell proliferation after incubation with peptide.

T-cell cross-reactivity

Bet v 1–specific TCLs were stimulated in the presence of autologous APCs with homologous peptides, as listed in Table I, at 5 μg/mL to test cross-reactivity. In addition, Bet v $1_{142-156}$–specific TCCs established from Bet v 1–induced TCLs by mean of limiting dilution, as previously described,[8] were stimulated with recombinant proteins, which were used at optimum concentrations, as determined initially: Bet v 1, Cor a 1, Gly m 4, and Pru av 1 at 5 μg/mL; Mal d 1 at 25 μg/mL; and Api g 1 and Dau c 1 at 50 μg/mL. After 48 hours, supernatants of T-cell cultures were harvested for cytokine determination. Tritiated thymidine incorporation was measured after another 16 hours. All determinations were performed in duplicates, and mean counts per minute were calculated. A variance of 15% or less from the mean was accepted. If the mean of T cells plus autologous APCs plus allergen was higher than the mean of cultures containing T cells and APCs alone plus 5× SD, the response was considered positive. Consequently, for stimulations with peptides an SI of 2.5 and for proteins an SI of 2 was defined as the cutoff value.

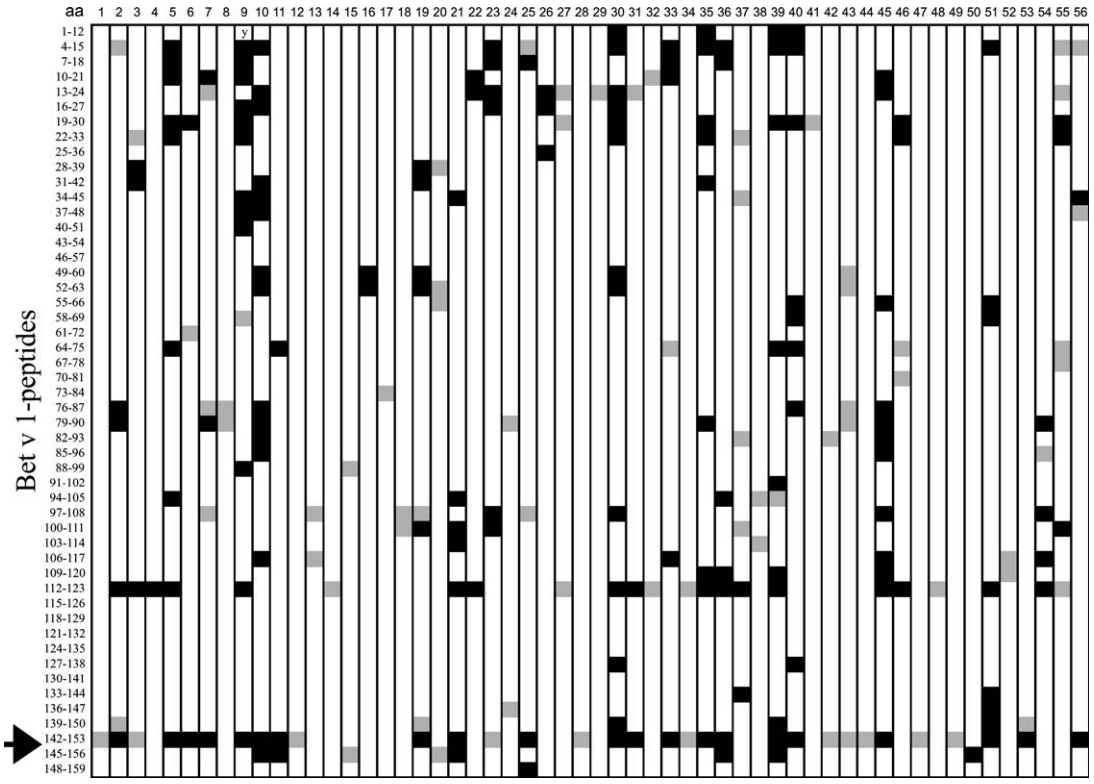

FIG 1. T-cell epitope mapping of Bet v 1. Proliferation of Bet v 1–specific TCLs from 57 different patients *(vertical lanes)* stimulated with 50 overlapping peptides spanning the amino acid sequence of Bet v 1 is shown. Peptides inducing an SI of greater than 5 are denoted in *black*, and SIs of 2.6 to 5 are denoted in *gray*. The most frequently recognized epitope, Bet v $1_{142\text{-}156}$, is indicated by an *arrow*.

Cytokine assays

Cytokine contents were determined by means of ELISA with Endogen Matched Antibody Pairs (Endogen, Woburn, Mass), according to the manufacturer's instructions (sensitivity limits: IL-4, 9 pg/mL; IL-5, 7.4 pg/mL; IFN-γ, 9.5 pg/mL). TCCs belonging to the T_H0 or T_H2 subset were used in this study. TCCs producing 10 times more IL-4 than IFN-γ were attributed to the T_H2 subset, and TCCs producing similar levels of IL-4 and IFN-γ were classified as T_H0. T_H0-like TCCs were SAZ 10IV, SUG B1, SD 266, GRÜ B24, RAD 47, and PAT 47, and T_H2-like TCCs were HPR 49II, SD 344, MAB 290, SD B58, and MAB 310.

Statistics

Pearson correlation coefficients were determined by using SPSS 10.0.7 (SPSS, Chicago, Ill).

RESULTS

Bet v $1_{142\text{-}156}$ represents the dominant T-cell epitope of Bet v 1

Epitope mapping of Bet v 1 was performed with 57 Bet v 1–specific TCLs (SI >2.5) obtained from different donors. These TCLs were stimulated with 50 overlapping 12-mer peptides covering the complete sequence of Bet v 1 (Fig 1). None of the control cultures generated in parallel without Bet v 1 proliferated in response to the pollen allergen. Confirming earlier data obtained with smaller numbers of patients,[7,8] several distinct T-cell epitopes within the Bet v 1 molecule were identified, and in 31 of 57 individuals, multiple T-cell specificities were observed. In total, 10 distinct T cell–activating regions within Bet v 1 were recognized by at least 10% of the individuals studied: 26% harbored T cells specific for Bet v $1_{4\text{-}18}$, 19% for Bet v $1_{13\text{-}24}$, 19% for Bet v $1_{19\text{-}33}$, 10% for Bet v $1_{34\text{-}48}$, 10% for Bet v $1_{52\text{-}63}$, 12% for Bet v $1_{64\text{-}75}$, 19% for Bet v $1_{79\text{-}93}$, 28% for Bet v $1_{94\text{-}111}$, and 40% for Bet v $1_{112\text{-}123}$. TCLs derived from 35 (61%) of 57 patients reacted with Bet v $1_{142\text{-}156}$, thus representing the dominant T-cell epitope of the major birch pollen allergen. This epitope was recognized as the sole epitope in 8 of 18 patients responding to a single epitope (Fig 1).

Bet v $1_{142\text{-}156}$ shares a high sequence homology with the C-terminal ends of Bet v 1–related food allergens

The immunodominant T-cell epitope Bet v $1_{142\text{-}156}$ is located within the C-terminus, a highly conserved region among the different isoforms of Bet v 1, as well as Bet v 1–related tree pollen allergens.[26,27] To evaluate the amino acid similarity with Bet v 1–related food allergens, Bet v $1_{142\text{-}156}$ was compared with the corresponding regions in apple (Mal d $1_{141\text{-}155}$), pear (Pyr c $1_{141\text{-}155}$), cherry (Pru av $1_{142\text{-}156}$), peach (Pru ar $1_{142\text{-}156}$), hazelnut (Cor a $1_{143\text{-}157}$), celery (Api g $1_{141\text{-}153}$), carrot (Dau c $1_{141\text{-}153}$), and soybean (Gly m $4_{141\text{-}155}$; Table I). The sequence

TABLE II. Proliferation of Bet v $1_{142-156}$–specific TCLs with corresponding peptides from homologous food allergens

	Stimulating peptide derived from:						
TCLs	**Bet v 1**	**Cor a 1**	**Gly m 4**	**Api g 1**	**Dau c 1**	**Pyr c 1/Mal d 1**	**Pru ar 1/Pru av 1**
MAH	**8.8***	**42.6**	NT	**22.0**	**40.5**	NT	NT
VMA	**39.9**	1.2	NT	1.6	1.1	NT	NT
HST	**22.0**	1.0	NT	0.8	**3.1**	NT	NT
HEM	**4.3**	**2.6**	NT	1.8	**2.9**	NT	NT
PST	**5.8**	**2.5**	NT	**3.3**	**4.1**	NT	NT
PMA	**2.9**	**2.5**	1.4	0.8	**2.5**	1.3	1.5
OLT	**12.6**	1.5	**2.6**	1.2	1.5	0.9	0.7
NEM	**6.3**	**9.0**	0.3	1.2	1.1	**2.8**	**4.9**
KPE	**3.3**	**4.6**	**2.7**	**3.4**	**11.1**	0.6	0.9
HOR	**15.5**	2.1	1.0	1.7	1.2	0.7	0.7
MAB	**41.6**	**17.4**	**5.6**	**3.9**	**3.6**	**2.6**	0.9
SUG	**5.9**	**6.4**	0.9	**12.2**	1.5	**2.5**	1.1
HIR	**2.9**	1.2	**2.5**	1.3	1.2	1.2	1.2
BER	**4.1**	1.0	0.5	1.0	1.0	0.5	0.5
MIM	**2.5**	1.1	0.7	0.6	1.3	0.8	0.7
Positive TCL (%)	100	53	40	33	47	30	10

Background counts ranged from 210 to 16,386 cpm (median, 1708 cpm). An SI of 2.5 or greater was considered positive *(bold numbers)*.
NT, Not tested.
*Mean SI of duplicates are shown.

similarities to Bet v $1_{142-156}$ ranged from 60% to 80%, demonstrating that the C-terminal ends are also highly conserved among Bet v 1–related food proteins. Identical amino acid sequences were found to be present within the respective peptides of Mal d 1 and Pyr c 1, as well as in Pru av 1 and Pru ar 1.

Bet v $1_{142-156}$–reactive TCLs cross-react with C-terminal peptides of Bet v 1–related food allergens

TCLs from 15 different patients reactive with Bet v $1_{142-156}$ were stimulated with peptides representing the corresponding regions in Bet v 1–related food allergens in a first approach to investigate the involvement of the dominant T-cell epitope Bet v $1_{142-156}$ in cross-reactivity with homologous food allergens (Table I). As shown in Table II, 10 (71%) of 14 TCLs proliferated with one or more food allergen–derived peptides (Table I). Cor a $1_{143-157}$ induced proliferation in 53% of tested TCLs, followed by peptides derived from Dau c $1_{141-153}$ in 47%, Gly m $4_{141-155}$ in 40%, Api g $1_{141-153}$ in 33%, Mal d 1/Pyr c $1_{141-155}$ in 30%, and Pru av 1/Pru ar $1_{142-156}$ in 10% of TCLs. In 4 TCLs food-derived peptides from Cor a 1, Api g 1, or Dau c 1 elicited more pronounced proliferative responses than Bet v $1_{142-156}$.

TCCs specific for Bet v $1_{142-156}$ cross-react with Bet v 1–related food allergens

Eleven TCCs from 8 different donors specific for Bet v $1_{142-156}$ were stimulated with the entire food proteins Mal d 1, Api g 1, Dau c 1, Cor a 1, Pru av 1, and Gly m 4 to prove at the clonal level that Bet v $1_{142-156}$ is involved in cross-reactivity with Bet v 1–related food allergens (Table III). In total, 10 of 11 TCCs proliferated in response to least one of these 6 food allergens. Three Bet v $1_{142-156}$–specific TCCs were activated by one Bet v 1–related food allergen. Another 3 TCCs reacted to stimulation with 2 different food proteins. Two TCCs responded to 3 food allergens, and 1 TCC each responded to 4 or 5 food allergens. Api g 1 was recognized by 63.6%, Cor a 1 by 54.5%, Dau c 1 by 45.5, Gly m 4 by 44.4%, Pru av 1 by 11.1%, and Mal d 1 by 9.1% of the tested TCCs. A positive correlation between these percentages of T-cell responses with the respective peptide similarities (Table I) was calculated ($r = 0.856$, $P = .30$). Similar to TCLs, in 6 TCCs the stimulation with food allergens (Cor a 1, Gly m 4, Api g 1, Dau c 1, or Mal d 1) resulted in a higher proliferation than was induced with Bet v 1.

In addition to proliferation, cytokine production (IL-4, IL-5, and IFN-γ) was measured in supernatants from 8 of 11 TCCs. These comprised 5 T_H2- and 3 T_H0-like TCCs. The food allergens that induced proliferation also induced cytokine synthesis. The resulting cytokine patterns were similar to those observed with Bet v 1. Two representative TCCs with a T_H2 phenotype are shown in Fig 2.

DISCUSSION

Food allergy associated with birch pollinosis is primarily caused by Bet v 1–specific IgE antibodies that cross-react with related food allergens of the PR-10 protein family.[18,28] Thus far, the antigen-specific T-lymphocyte response underlying this food allergy has not been extensively investigated. In the present study we identified Bet v $1_{142-156}$ as the immunodominant T-cell epitope of the major birch pollen allergen in a large number (n = 57) of patients with birch pollen allergy and concomitant food allergy. In addition, we demonstrated that T cells specific

TABLE III. Proliferation of Bet v $1_{142-156}$–specific TCCs with total proteins of homologous food allergens

	Stimulation with:							
TCCs	**$Bet_{142-153}$**	**Bet v 1**	**Cor a 1**	**Gly m 4**	**Api g 1**	**Dau c 1**	**Mal d 1**	**Pru av 1**
SAZ 10 IV	**3.2***	**3.3**	1.0	0.8	**3.7**	0.7	0.4	0.8
HPR 49 II	**688.0**	**268.0**	0.8	0.9	**64.4**	0.7	0.7	0.7
SUG B1	**444.0**	**2.7**	**2.1**	NT	1.6	1.0	**3.2**	NT
SD 266	**21.7**	**85.0**	0.9	1.1	1.6	**90.0**	0.8	0.9
SD 334	**9.6**	**8.4**	0.5	**4.0**	**10.0**	**7.8**	0.9	0.5
SD B58	**42.4**	**15.2**	0.8	0.9	0.8	0.7	0.8	0.9
MAB 290	**182.5**	**116.0**	**2.6**	1.0	**33.0**	1.0	1.8	1.0
MAB 310	**23.8**	**16.7**	**6.7**	**21.4**	**8.6**	**15.7**	0.5	0.4
GRÜ B 24	**16.8**	**14.3**	**30.5**	NT	1.1	**25.0**	NT	NT
RAD 47	**26.6**	**22.2**	**2.7**	**15.2**	**10.6**	**20.8**	1.6	**2.1**
PAT 47	**2.9**	1.7	**7.1**	**3.0**	**3.5**	1.7	1.0	1.0
Positive TCCs (%)	100	91	55	44	64	46	9	11

An SI of 2 or greater was considered positive *(bold numbers)*. Background counts ranged from 88 to 8147 cpm (median, 701 cpm).
NT, Not tested.
*Mean SI of duplicates are shown.

for Bet v $1_{142-156}$ are activated by different Bet v 1–related food allergens to proliferate and synthesize cytokines, including IL-4 and IL-5, a fact that might have implications for the allergic status of the patient.

Although Bet v 1 contains a variety of different T-cell epitopes (Fig 1), the C-terminus of the major birch pollen allergen appears to contain a particularly immunogenic determinant. In the patient population analyzed, 61% of the individuals harbored T cells specific for a peptide located at amino acid residues 142-156 (Fig 1). The same amino acid region also represents an important T-cell epitope for patients with birch pollen allergy but without food allergy (not shown) and healthy individuals and has been identified as the immunodominant T-cell epitope for BALB/c mice.[8,29] This strong immunogenicity might be due to the fact that the C-terminus of Bet v 1 is highly conserved and identical among all isoforms of Bet v 1 known so far.[25,30] Furthermore, this part of the molecule shares a high amino acid sequence similarity with various Bet v 1–related tree pollen allergens, such as Aln g 1 (alder) or Car b 1 (hornbeam) and Cor a 1 (hazel).[27,31,32] Bet v $1_{142-156}$–specific TCCs were shown to cross-react with several different isoforms of the major allergen in hazel pollen.[33] In addition, Bet v $1_{142-156}$–specific TCCs cross-reacted with the major apple allergen Mal d 1.[19] Because the C-termini of Mal d 1 and other important Bet v 1–related food allergens are highly homologous to Bet v $1_{142-156}$ (Table I), it is plausible that T lymphocytes specific for this peptide generally are important for cellular cross-reactivity with PR-10–like proteins in food.

Oligoclonal Bet v $1_{142-156}$–reactive TCLs were tested with the corresponding homologous peptides of PR-10–like allergens from apple, peach, pear, cherry, hazelnut, celery, carrot, and soybean to investigate this hypothesis (Table II). Monoclonal Bet v $1_{142-156}$–specific TCCs were stimulated with entire Bet v 1–related food allergens to demonstrate that Bet v $1_{142-156}$–specific T lymphocytes can be activated by peptides derived from these proteins

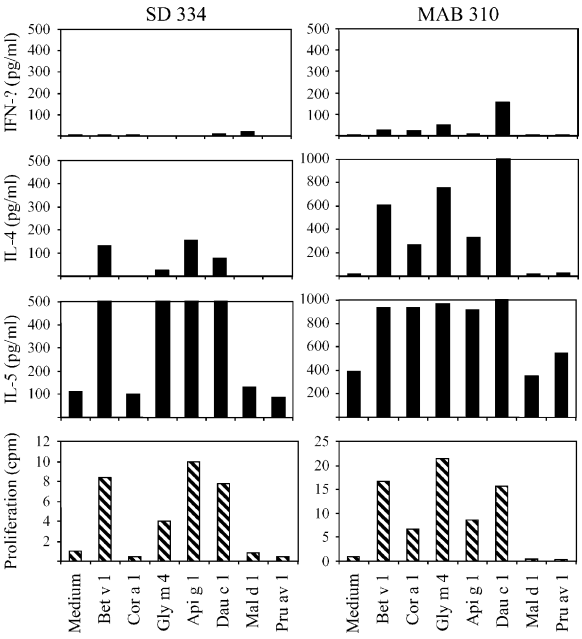

FIG 2. Activation of pollen-specific TCCs by food allergens. Two representative TCCs specific for $Bet_{142-156}$ were stimulated with Bet v 1 and homologous food allergens. Proliferation *(hatched columns)* and cytokine responses *(filled columns)* are shown.

after natural antigen processing (Table III). The major allergens of hazelnut (Cor a 1) and of vegetables of the Apiaceae family (Api g 1 and Dau c 1) represented the most stimulating proteins for T cells specific for the immunodominant epitope of Bet v 1, whereas homologous proteins from Rosaceae fruits were less effective stimulators. The extent of T-cell cross-reactivity with Bet v 1–related food allergens corresponds with the degree of amino acid sequence homology of their C-termini with Bet v $1_{142-156}$ (Table I), namely 73% to 80% for Api g 1, Dau c 1, and Cor a 1 versus 60% similarity for the fruit

allergens. A significant positive correlation between activation of Bet v 1–specific TCCs ($r = 0.856$, $P = .03$) and C-terminal sequence homology was found. A similar correlation was obtained for TCLs ($r = 0.811$, $P = .05$). Variations in the ranking of cross-reactivity obtained in TCLs and TCCs (eg, for Api g 1) might be explained by the different HLA background of the patients in these experiments. It is worth noting that the extent of cross-reactivity between Bet v 1 and homologous food allergens seems strikingly different at the T-cell and B-cell levels. It is known that Bet v 1–specific IgE antibodies react preferentially with PR-10–like allergens in Rosaceae fruits and less with those present in vegetables of the Apiaceae family.[34] In accordance, all patients in this study had experienced IgE-mediated reactions to apple, 76% of these to peach, but only 27% to celery and 44% to carrot. By contrast, the major allergens from celery and carrot, Api g 1 and Dau c 1, induced much stronger responses in Bet v 1–specific TCLs and TCCs than the major allergens from apple and peach, Mal d 1 and Pru ar 1 (Tables II and III). Hence the cross-reactivity of IgE between the major birch pollen and related food allergens correlated roughly with the overall amino acid similarity of the entire proteins (Table I), which is the basis for the formation of similar conformational IgE epitopes. On the other hand, T-cell cross-reactivity depended on amino acid similarity restricted to parts of Bet v 1 that contain relevant, linear T-cell epitopes. Consequently, foods that less frequently induce immediate hypersensitivity reactions can represent very potent activators of pollen-specific T cells. In the case of the major hazelnut allergen Cor a 1, the entire protein, as well as its C-terminal part, possess a high degree of amino acid similarity to Bet v 1. Accordingly, both a high prevalence of OAS against hazelnuts (69%) in individuals with birch pollen allergy and a high potency of Cor a 1 to stimulate Bet v $1_{142\text{-}156}$–specific T cells was observed.

A high percentage of individuals with birch pollen allergy harbor T cells specific for Bet v $1_{142\text{-}156}$ in their blood (Fig 1), and most T lymphocytes specific for this dominant epitope belong to the T_H2 subset.[7,19,33] The induction of proliferation in Bet v $1_{142\text{-}156}$–specific TCCs by related food allergens was accompanied by the synthesis of IL-4 and IL-5 (Fig 2). Because Bet v 1–specific T cells were considerably activated by related food allergens *in vitro*, a similar cross-stimulation might also occur *in vivo*. In this respect the uptake of pollen-related food with a strong T cell–activating capacity might amplify the allergic immune response, particularly at times when patients are not exposed to pollen. Circulating Bet v 1–specific T_H2 cells have been demonstrated in patients with birch pollen allergy outside the pollen season.[35] In addition to IL-7, T-cell receptor signaling induced by means of antigen recognition is involved in the maintenance memory $CD4^+$ T cells.[36] The survival of pollen-specific T_H cells might be accomplished by means of intermittent stimulation with peptides derived from related food allergens, as has been shown for T cells specific for the Japanese cypress pollen allergen Cha o 1 *in vitro*.[37] Long-lived Bet v 1–specific memory T cells have been traced in the peripheral blood and skin of individuals with birch pollen allergy.[38] Actual evidence for the *in vivo* relevance of birch pollen–related food allergens has been provided by the fact that their ingestion can lead to an exacerbation of eczema in patients with birch pollen allergy and atopic dermatitis. These late-phase reactions seemed to be mediated by Bet v 1–specific T_H cells.[39] Vice versa, an improvement of eczema has been observed after a respective elimination diet.[40]

In conclusion, we identified an immunodominant T-cell epitope in Bet v 1 important for the cellular cross-reactivity with pollen-related foods. Although the major allergens of celery and carrot induced food allergy less often, these proteins represented the most potent activators of Bet v $1_{142\text{-}156}$–specific T lymphocytes. Thus the symptom-free ingestion of these foods might perennially boost the pollen-specific T-cell response, which potentially causes the typically remaining IgE levels out of the pollen season. On the other hand, the continuous activation of pollen-specific T lymphocytes by food allergens could also lead to peripheral tolerance. Therefore the consequences of food uptake for the allergic status of the patients need to be investigated *in vivo*.

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