

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

Ruth Fritsch, MD, Barbara Bohle, PhD, Ute Vollmann, Ursula Wiedermann, MD, PhD, Beatrice Jahn-Schmid, PhD, Monika Krebitz, PhD, Heimo Breiteneder, PhD, Dietrich Kraft, MD, and Christof Ebner, MD Vienna, Austria

Background: Food allergy to apple is frequent in individuals allergic to tree pollen. The major allergens of birch, Bet v 1, and apple, Mal d 1, have been cloned and sequenced and display a high degree of sequence identity, leading to IgE cross-reactivity.

Objective: We sought to investigate cross-reactivity between Bet v 1 and Mal d 1 at the level of allergen-specific T lymphocytes.

Methods: PBMCs of 13 patients allergic to birch pollen with oral allergy syndrome to apple were stimulated with rBet v 1 and rMal d 1, respectively, thereby establishing allergen-specific T-cell lines and T-cell clones. rMal d 1-specific T-cell cultures were tested for reactivity with rBet v 1, and rBet v 1-specific T cells were analyzed for reactivity with apple allergen. Cytokine production patterns in response to specific stimulation were evaluated. A selection of cross-reacting T-cell clones was mapped for epitope specificity by the use of overlapping Bet v 1-derived peptides.

Results: Nineteen Mal d 1-specific T-cell clones were produced, 79% of which cross-reacted with Bet v 1. Eight of 18 Bet v 1-specific T-cell clones cross-reacted with Mal d 1. Six peptides representing cross-reactive T-cell epitopes could be identified. The respective fragments from birch and apple displayed approximately 50% amino acid identity. Seventy percent of the cross-reactive T-cell clones revealed a T_{H2}-like cytokine production pattern.

Conclusion: The results indicate that cross-reactivity between apple and birch pollen leading to the clinical oral allergy syndrome occurs not only at the serologic, but also at the cellular level. (*J Allergy Clin Immunol* 1998;102:679-86.)

Key words: Oral allergy syndrome, birch pollen, apple, Bet v 1, Mal d 1, IgE, T helper lymphocyte

Since Tuft and Blumenstein¹ reported the association of pollinosis and allergy to plant-derived food for the first time in 1942, the occurrence of adverse reactions to food in patients with pollen allergy has been the topic of several studies.²⁻¹² The most frequent, and therefore best

Abbreviations used

BP:	Birch pollen
BPRF:	Birch pollen-related food
OAS:	Oral allergy syndrome
TCL:	T-cell line
TCC:	T-cell clone
SI:	Stimulation index

characterized, pollen-fruit syndrome is apple allergy in patients with tree pollen-induced pollinosis.^{4,5,10} Up to 70% of patients with birch pollen (BP) allergy exhibit the so-called *oral allergy syndrome* (OAS), an IgE-mediated food allergy characterized by oral itching and irritation of the oral mucosa on contact with raw fruits or vegetables.^{13,14} Systemic reactions to BP-related food (BPRF) have also been reported^{15,16}; however, in the case of apple, because of the chemical lability of the allergen,¹⁷ dangerous allergic reactions are rare. Bet v 1 and Mal d 1, the major allergens of birch and apple, are both proteins with a molecular mass of 17 kd. The proteins have been cloned, sequenced, and expressed in recombinant form in *Escherichia coli*.^{18,19} The proteins consist of 159 amino acids and share 64.5% amino acid sequence identity (55.6% identity at the nucleic acid level). Bet v 1 and Mal d 1 belong to a class of proteins expressed in plants in response to stress (ie, pathogenesis-related proteins).¹⁸ Mal d 1 is differentially expressed in apple strains. For example, high concentrations can be detected in common apples such as Granny Smith or Golden Delicious.¹⁹ Common IgE epitopes of Mal d 1 and Bet v 1 that lead to allergic cross-sensitization have been proven.^{10,20}

In recent years a substantial role for allergen-specific T helper (T_H) cells could be demonstrated in Type I allergy.²¹⁻²³ It could be shown that the majority of allergen-specific T_H cells produced a T_{H2}-like type of cytokine production (ie, a high IL-4/IFN- γ ratio) in response to specific stimulation. Moreover, molecular cloning techniques and the knowledge of amino acid sequences of atopic allergens made it possible to characterize T-cell epitopes.²⁴ Only limited information is available about allergen-specific T lymphocytes in food allergy; the respective publications deal with peanuts, milk, and egg.²⁵⁻²⁹

To our knowledge, this is the first investigation of the T-cell response underlying a food allergy based on cross-

From the Institute of General and Experimental Pathology, Department Immunopathology, University of Vienna, Vienna.

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Reprint requests: Christof Ebner, MD, Institute of General and Experimental Pathology, University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria.

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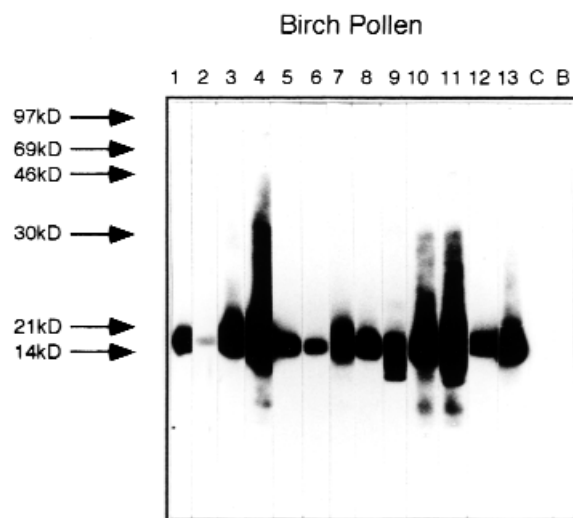


FIG 1. IgE immunoblot. Sera from 13 patients with BP allergy and OAS to apple were tested with blotted natural BP extract. All patients display IgE binding to Bet v 1 (17 kD). C, Normal human serum pool; B, buffer control.

reactivity to pollens. The aim of the study was to assess the capacity of apple allergens to induce lymphoproliferative responses and cytokine production in T cells from individuals allergic to BP with OAS to apple.

METHODS

Patients

Peripheral blood was collected from 13 patients allergic to BP (8 men and 5 women) with symptoms of OAS after ingestion of apple. Type I allergy to BP and apple was proven by typical case history and skin prick test. Furthermore, all patients displayed typical wheal-and-flare reactions in the prick-to-prick test with fresh Golden Delicious apples. The majority of the patients (70%) displayed adverse reactions to other BPRF in addition to apple (eg, nut, celery, peach, carrot, and kiwi). IgE reactivity with Bet v 1 and Mal d 1 was proven in immunoblot experiments.

Allergen extracts and recombinant allergens

Extraction of proteins from Golden Delicious apples and BP extracts was performed according to methods described previously.^{10,12} Purified recombinant proteins (rBet v 1 and rMal d 1) were purchased from Biomay/Biotechnik (Fa. Mayerhofer, Linz, Austria).

Immunoblots

Western Blots were performed as described previously.³⁰ Briefly, patients' sera were diluted 1:4 and incubated with nitrocellulose strips coated with either apple or BP extract, rBet v 1, or rMal d 1. Bound IgE was detected with ¹²⁵I-labeled rabbit anti-human IgE antibody (Pharmacia Diagnostics, Uppsala, Sweden). A serum pool of nonallergic donors and buffer alone was used as negative control.

Proliferation assays

Peripheral venous blood was collected in preservative-free heparin-coated vials. PBMCs were separated by density gradient centrifugation on Ficoll Hypaque (Pharmacia, Uppsala, Sweden)

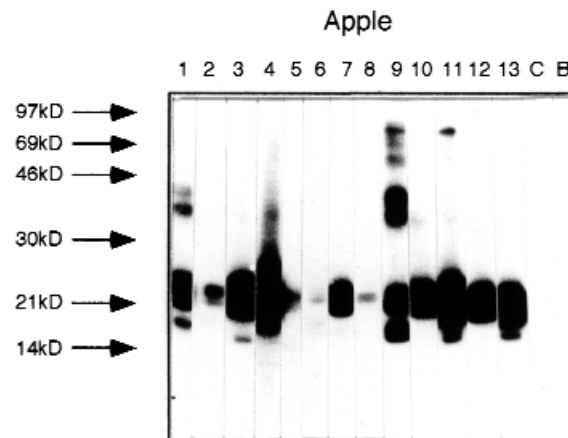


FIG 2. IgE Immunoblot. Sera from 13 patients with BP allergy and OAS to apple were tested with blotted natural apple (Golden Delicious) extract. All patients display IgE binding to Mal d 1 (17 kD). C, Normal human serum pool; B, buffer control.

and resuspended in culture medium (Ultra Culture Medium; Bio Whittaker, Walkersville, Md) supplemented with 2 mmol/L glutamine and 2×10^{-5} mol/L 2-mercaptoethanol. Cells (2×10^5 PBMCs) were cultured in triplicates in 96-well plates (200 μ L) in the presence of 25, 12.5, 6.25, and 3.125 μ g rBet v 1 and rMal d 1 per milliliter for 6 days at 37°C in a 5% CO₂ humidified atmosphere. In the last 16 hours of culture ³H-thymidine (Amersham; 0.5 μ Ci/well) was added, and the incorporated radioactivity was measured by scintillation counting.

Allergen-specific T-cell lines

Allergen-specific T-cell lines (TCLs) were obtained by a technique previously described.³¹ Briefly, 2×10^6 PBMCs were stimulated with the (individually determined) optimal concentration of rMal d 1 (in the range of 10 to 50 μ g/mL) or rBet v 1 (5 to 20 μ g/mL) in 24-well, flat-bottomed culture plates (3524 Costar, Cambridge, Mass) for 5 days. Subsequently, human rIL-2 (10 U/mL; Boehringer Mannheim, Mannheim, Germany) was added, and the culture was continued for an additional 7 days. Viable T-cell blasts were then separated by density centrifugation. TCLs produced with rMal d 1 were tested for their proliferative response on stimulation with rMal d 1 (25 μ g/mL) and rBet v 1 (10 μ g/mL) adding 10^5 autologous irradiated PBMCs as antigen-presenting cells. Proliferation was assessed by a ³H-thymidine incorporation assay.

Allergen-specific T-cell clones

T-cell blasts from rMal d 1 and rBet v 1 specific TCLs were cloned by limiting dilution in 96-well plates as described³¹ (Nunc-clone, Nunc, Roskilde, Denmark) in the presence of 3×10^5 irradiated (5000 rad) allogenic PBMCs as feeder cells, 0.5% PHA vol/vol (Life Technologies, Grand Island, NY), and 20 U rIL-2/mL in the above-described medium. Growing microcultures were then expanded at weekly intervals with fresh feeder cells and rIL-2. The specificity of the T-cell clone (TCC) was assessed by proliferation assays incubating 2×10^4 T-cell blasts in the presence of 10^5 autologous irradiated PBMCs with rBet v 1 (10 μ g/mL) or rMal d 1 (25 μ g/mL). Microtiter plates were incubated for 48 hours at 37°C in a 5% CO₂ humidified atmosphere. After pulsing for 16 hours with ³H-thymidine, cells were harvested, and the incorporated radioactivity was measured by scintillation counting.

TABLE I. Characteristics (epitope recognition, proliferation, and T_H subset) of TCCs produced with rMal d 1 as initial stimulus

TCC	Epitope (Bet v 1-AA)	rMal d 1 (SI)	rBet v 1 (SI)	IFN- γ (pg/mL)	IL-4 (pg/mL)	T _H subset
WDm48*	ND	1.4	4.5	0	107.0	2
WDm160*	ND	1.1	8.4	0	253.4	2
RRm24*	ND	2.0	44.0	0	1308.2	2
RRm78*	ND	1.6	14.6	0	40.4	2
RRm175*	142-156	1.4	10.3	17.5	1982.4	2
RRm212*	ND	1.3	5.9	30.0	1126.4	2
WDm8*	—	3.6	10.8	35.0	34.4	0
WDm17*	142-156	3.0	5.8	0	2022.0	2
WDm19*	55-66	3.8	11.1	20.0	1962.0	2
WDm38*	55-66	3.3	5.7	15.0	1964.0	2
WDm46*	142-153	4.0	9.3	0	14.2	2
RRm29*	139-156	2.2	5.5	45.0	1966.0	2
RRm95*	ND	3.3	13.9	70.0	495.0	0
RRm107*	ND	3.3	11.0	90.0	1611.0	2
MAHm22*	ND	10.4	6.4	0	19.2	2
RRm79†	—	6.6	1.3	2135	0	1
RRm169†	—	3.1	1.2	175	126.7	0
CHKm12†	—	275	2	10	46.4	0
GEMm170†	ND	14	1.3	0	0	0

ND, Not done.

*TCCs displaying cross-reactivity between Bet v 1 and Mal d 1.

†Mal d 1-specific TCCs.

Peptides

A panel of 50 peptides synthesized according to the Bet v 1 sequence was produced by Cambridge Research Biochemicals Limited. The dodecapeptides were overlapping for 3 amino acids (neighbors sharing 9 residues). The concentration used in the proliferation assays was 1 μ g/well (5 μ g/mL). Two peptides located near the C-terminus of Bet v 1 (MGETLLRAVESYLLAHS, amino acids 139-156) and Mal d 1 (GLFKLIESYLKEHQD, amino acids 141-155) were synthesized separately (MWG-Biotech, Munich, Germany), purified to homogeneity (>95%), and checked by HPLC and MALDI-TOF analysis. The peptides were dissolved in medium and used in experiments with TCCs in concentrations of 1 μ g/mL.

Cytokine production

Clone cells (1×10^5) were incubated with 10^5 autologous irradiated antigen-presenting cells in the presence of 10 μ g/mL rBet v 1 or 25 μ g/mL rMal d 1. Supernatants were collected after 24 hours of incubation, and cytokine levels were measured by using ELISA systems (IL-4 sensitivity threshold, 2 pg/mL; IFN- γ sensitivity threshold, 1.5 pg/mL; Medgenix Diagnostix, Fleurus, The Netherlands). Supernatants derived from antigen-presenting cells/antigen alone served as negative controls.

RESULTS

Skin prick tests and IgE immunoblots

All patients had positive skin prick test responses to BP and positive prick-to-prick test responses to fresh apple. All patients showed IgE binding to allergens in natural BP and apple extract and reacted with the major allergens Bet v 1 and Mal d 1 (Figs 1 and 2). Testing for IgE reactivity with rMal d 1 showed that 11 of 13 patients had IgE binding. Two patients did not react with the recombinant molecule, obviously because of reactivity with another isoform variant (not shown). rBet v 1-spe-

cific IgE was present in all sera tested. Control subjects did not show IgE binding to any of the tested extracts or recombinant proteins.

Proliferation assays with PBMCs and rMal d 1-induced TCLs

PBMCs and 10 rMal d 1-specific TCLs were tested for reactivity with rMal d 1 and rBet v 1, respectively. Primary stimulation assays with freshly isolated PBMCs revealed similar, but weak, stimulation indexes (SIs) for both Bet v 1 and Mal d 1 (rBet v 1: SI = 1 to 8.4, mean 2.7 ± 1.9 ; rMal d 1: 1.3 to 5.6, mean: 2.8 ± 1.5). However, when restimulated after enrichment of specific cells, Mal d 1-induced TCLs showed a significantly higher response to rBet v 1 than to rMal d 1 (rBet v 1: mean SI: 8.2 ± 13.9 ; rMal d 1: mean SI: 1.8 ± 0.9 ; $P < .05$, Mann-Whitney test), indicating an activation of birch-reactive T lymphocytes by stimulation with recombinant apple allergen. PBMCs isolated from nonallergic individuals or from patients allergic to BP without sensitization to fruits did not respond significantly to stimulation with rMal d 1 (not shown).

TCCs induced with rMal d 1 and rBet v 1

rMal d 1-induced TCLs were established from the peripheral blood of 10 individuals. By limiting dilution, depending on the quality of TCLs, 19 specific rMal d 1-specific TCCs (derived from 5 different donors) could be isolated. These TCCs were tested for reactivity with rMal d 1 and rBet v 1. Fifteen of 19 TCCs (79%) reacted with both rBet v 1 and rMal d 1, and the remaining 4 TCCs proliferated in response to rMal d 1 exclusively (Table I). The majority of the cross-reactive TCCs (14 of 15) proliferated more vigorously in response to rBet v 1 than to

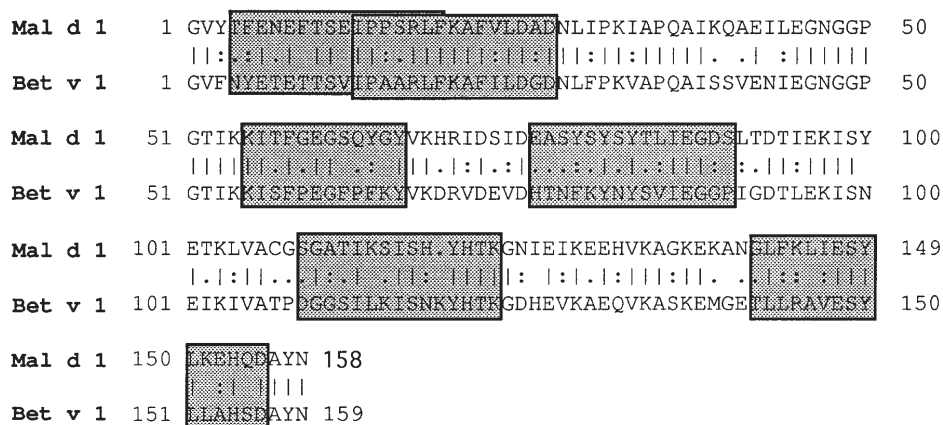


FIG 3. Sequence alignment of Bet v 1 and Mal d 1. Vertical bars indicate sequence identity, colons mark conservative exchanges, and dots represent gaps produced by the aligning program. T-cell epitopes recognized by cross-reactive TCCs are marked.

TABLE II. Characteristics (epitope recognition, proliferation, and T_H subset) of TCCs produced with rBet v 1 as initial stimulus

TCC	Epitope (Bet v 1-AA)	Bet v 1 (SI)	Mal d 1 (SI)	IFN- γ (pg/mL)	IL-4 (pg/mL)	T_H subset
WDb4*	4-18	79.0	7.0	2300.0	0	1
HPRb248*	13-27	8.5	46.0	700.0	0	1
WDb535*	13-27	148.0	175.0	10.0	1966.0	2
MAHb5*	76-90	243.0	5.4	15.0	218.0	2
HCB33*	109-123	17.0	3.4	1245.0	493.0	0
HPRb231*	142-156	622.0	45.0	0	502.0	2
SAZb10*	142-156	15.0	9.5	1500.0	1.5	1
HPRb77*	142-156	306.0	28.0	100.0	856.0	0
PStb33†	28-42	21.3	1.0	60.0	674.0	2
WDb147†	52-69	286.0	3.0	200.0	794.0	0
WFb29†	79-93	20.0	1.1	130.0	1442.0	2
WFb14†	82-93	15.0	1.3	215.0	3404.0	2
SDb2†	94-105	39.0	1.9	0	116.0	2
MAHb17†	100-111	46.0	1.9	15.0	0	1
WFb24†	112-123	71.0	0.6	213.0	1346.0	0
GZb9†	127-138	28.0	1.3	80.0	120.0	0
GZb10†	127-138	38.6	1.2	80.0	560.0	0
WDb514†	142-156	35.0	1.8	0	0	0

*TCCs displaying cross-reactivity between Bet v 1 and Mal d 1.

†Bet v 1-specific TCCs.

rMal d 1, and we observed that the proliferative responses to rMal d 1 decreased with time. In some rMal d 1-induced TCCs the Mal d 1 reactivity was practically undetectable after several rounds of restimulation, whereas the same TCCs still reacted with rBet v 1 (Table I).

Eighteen TCCs derived from 9 different individuals were established by using rBet v 1 as the initial stimulus, and these were investigated for their reactivity with rMal d 1. Eight of 18 TCCs (44.4%) proliferated on incubation with rMal d 1. The remaining 10 rBet v 1-specific TCCs (55.6%) did not react with rMal d 1. Again, the majority of cross-reactive TCCs showed a markedly stronger proliferation when stimulated with rBet v 1 than with rMal d 1 (Table II). In 2 clones the proliferative response

induced by incubation with rMal d 1 was higher than the proliferation evoked by rBet v 1 (Table II).

Epitope mapping of TCCs

To investigate cross-reactive T-cell epitopes of Bet v 1 and Mal d 1, 10 rMal d 1-induced TCCs were subjected to epitope mapping by using 50 overlapping peptides covering the complete sequence of rBet v 1. Seven of these TCCs were reactive with both rBet v 1 and rMal d 1 (Table I). Two cross-reactive epitopes could be distinguished by this approach: 4 TCCs (derived from 2 different patients) recognized peptides near the carboxy-terminus (amino acids 139-156) and 2 TCCs (from the same individual) reacted with sequence parts (amino acids

55-66). One cross-reactive TCC did not react with any of the tested peptides (Table I). Three Mal d 1-specific TCCs did not react with the rBet v 1 protein. In these clones, as could be expected, no epitope could be mapped with Bet v 1 sequence-derived peptides.

Eighteen rBet v 1-specific TCCs were subjected to epitope mapping. Epitopes located at positions corresponding to amino acids 4-18, 13-27, 52-69, 76-93, 94-105, 100-111, 109-123, 127-138, and 142-156 were recognized, representing published T-cell stimulating areas within the Bet v 1 molecule (Table II).^{31,32} Analyzing the epitope recognition of the rBet v 1-induced TCCs cross-reacting with rMal d 1 revealed again specificity for the C-terminal epitope (amino acids 142-156) in 3 TCCs derived from 2 different patients. Two TCCs (derived from different individuals) recognized an epitope located at amino acid 13-27; peptides representing amino acid positions 4-18, 76-90, and 109-123 were recognized by one TCC each (Table II).

In total 6 cross-reacting epitopes could be identified (Fig 3): amino acids 4-18 (sequence identity between rBet v 1 and rMal d 1 at this position: 53%), 13-27 (73%), 55-66 (50%), 76-90 (33%), 109-123 (53%), and 142-156 (46%).

Cross-reactive peptides

Five Bet v 1-specific TCCs derived from Bet v 1-induced TCLs were epitope mapped and displayed reactivity with the epitope Bet v 1 139-156. All these TCCs recognized the peptide at the corresponding position of the rMal d 1 molecule Mal d 1 (Fig 4). On the other hand, 4 TCCs produced by using Mal d 1 as the initial stimulus reacted with Mal d 1 141-155 and with the corresponding Bet v 1 peptide (Fig 4). Unspecific activation of T cells by the peptides can be excluded because Bet v 1/Mal d 1 nonspecific TCCs did not proliferate in response to the peptides.

Cytokine production of specific TCCs

Sixty-nine percent of rMal d 1-induced TCCs displayed a T_{H2} -like cytokine production pattern, 26% could be attributed to the T_{H0} subset, and only 1 TCC (5%) was identified as T_{H1} . Focusing on the cross-reactive TCCs, the ratio $T_{H2}:T_{H0}:T_{H1}$ revealed 87:13:0, indicating that all these TCCs produced IL-4 (Table I). The cytokine pattern of rBet v 1-induced TCCs allowed a distribution of TCCs into 39% T_{H2} -, 39% T_{H0} -, and 22% T_{H1} -like clones. The ratio for the cross-reactive Bet v 1-specific TCCs amounted to 37.5:25:37.5 (Table II). Evaluating all cross-reactive TCCs derived from Mal d 1 and Bet v 1 TCLs ($n = 23$), 70% were of the T_{H2} type, 17% displayed a T_{H0} -like cytokine production pattern, and 13% were T_{H1} -like TCCs.

DISCUSSION

A number of clinical syndromes caused by cross-reactivity of IgE antibodies in patients with inhalant allergy have been described.²⁻¹² The most frequent among these syndromes is the association of food allergy to fruits, nuts, and vegetable food in patients with pollen allergy during

the spring (the flowering period of trees). In the majority of cases, OAS to apple in individuals allergic to BP is caused by IgE cross-reactivity of the major allergens Bet v 1 (*Betula verrucosa*) and Mal d 1 (*Malus domestica*).¹⁰ A smaller group of patients with OAS is allergic to profilins, a group of broadly cross-reactive allergens.³³ It is well established that this clinical phenomenon depends on IgE-mediated inflammatory processes; however, the underlying T-lymphocyte response has not been characterized. The importance of allergen-specific T lymphocytes in the pathogenesis of Type-I allergy became clear in the last years.²¹⁻²³ The analysis of the quantity and the quality of the T-cell responses to Mal d 1, the Bet v 1-homologous protein in apple, allowed us to speculate about the relevance of T-cell responses to BPRF in the maintenance and/or induction of the allergic state.

PBMCs were isolated from the peripheral blood of patients allergic to BP (Fig 1) with OAS and displaying IgE binding to apple (Fig 2) and other BPRFs. Obviously because of the low precursor frequency of allergen-reactive T cells, proliferative responses to both Mal d 1 and Bet v 1 were low in PBMCs. Therefore allergen-reactive oligoclonal (TCL) and clonal T-cell cultures (TCC) were produced by restimulation of PBMCs with allergen. Throughout these experiments stimulation with rBet v 1 led to stronger lymphoproliferative responses (Tables I and II). This observation was independent of whether the T cells were selected and the cultures established by using rMal d 1 or rBet v 1 as initial stimulus, indicating that Bet v 1-specific T lymphocytes were activated by rMal d 1, a protein with a high degree of sequence similarity and identity (Fig 3).^{18,19} We observed a decreasing Mal d 1 reactivity in the time course in several TCCs, some of which even lacked proliferation in response to this allergen after some cycles of specific stimulation during the expansion process; however, these TCCs remained reactive to Bet v 1 (Table I). These results argue in favor of BP as the dominant allergen in this cross-reactivity. Analysis of the reactivity of Mal d 1-induced TCCs revealed that the majority of these clones were cross-reactive with Bet v 1 and could be attributed to the T_{H2} subset. Interestingly, none of the Mal d 1-specific, noncross-reactive clones were T_{H2} -like (Table I). Approximately one half of Bet v 1-induced TCCs were reactive with Mal d 1. Again, the majority of these TCCs produced high amounts of IL-4 in response to stimulation.

Epitope mapping of Mal d 1-induced, Bet v 1-reactive TCCs revealed 2 sequence parts harboring cross-reactive T-cell epitopes. Mapping of the Bet v 1-induced, Mal d 1-reactive TCCs revealed 5 cross-reactive T-cell epitopes. Evaluating all cross-reactive TCCs, a distinct preference for an epitope located on the C-terminus (in the area Bet v 1 139-156; Fig 3) could be observed. By testing these birch- and apple-specific TCCs with highly purified peptides synthesized according to the corresponding region on the rMal d 1 molecule, cross-reactivity could be confirmed (Fig 4). This (highly conserved)^{34,35} part of Bet v 1 has been described as an

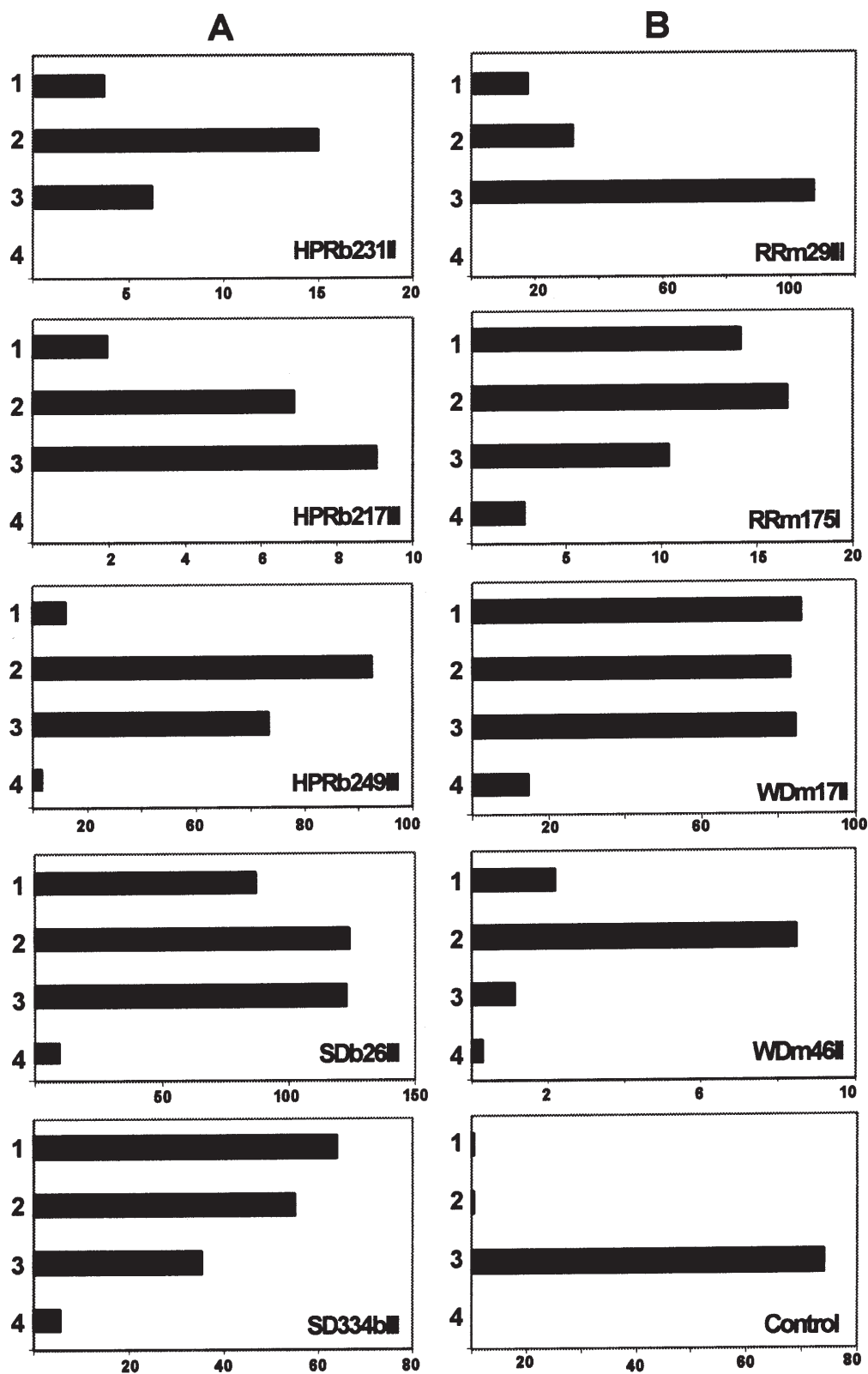


FIG 4. Crossreactivity of Bet v 1- and Mal d 1-induced TCCs at the level of epitope recognition. Five TCCs produced with Bet v 1 (**A**) and 4 TCCs produced with rMal d 1 (**B**) as initial stimulus were tested with peptides synthesized according to the respective amino acid sequences. 1, peptide GLFKLIESYLKEHQD (Mal d 1 141-155); 2, MGETLLRAVESYLLAHS (Bet v 1 139-156); 3, recombinant human IL-2; 4, medium. Values on the ordinate are $\text{cpm} \times 10^3$. Control, TCC not specific for Bet v 1 or Mal d 1 does not proliferate in response to the peptides.

important T-cell epitope in allergic and nonallergic individuals.^{31,32} We were able to isolate TCCs with the same epitope specificity from 1 individual using apple or birch pollen allergen as stimulus (eg, TCC WDb514II [Bet v 1–derived, noncross-reactive epitope 142-156; Table I] and TCC WDM17II [Mal d 1–derived, cross-reactive epitope 142-156; Table II]). This donor obviously harbored TCCs specific for epitope Bet v 1 142-156, which displayed degenerate recognition of the Bet v 1– and the Mal d 1–derived fragment, and TCCs expressing a T-cell receptor, which bound to residues present only on the Bet v 1–derived fragment. This reflects the characteristic polyclonal recognition of the allergen and even that of 1 epitope within an allergen.³⁶

Our results demonstrate that allergens from BPRF are not only recognized by BP-induced IgE antibodies but possess the capacity to activate Bet v 1–specific T lymphocytes. This T_H activation (in long-lived, allergen-specific lymphocytes)³⁷ possibly provides the help for the maintenance of the IgE production outside the pollen season. On the other hand, assuming that the modulation of allergen-specific T_H is the operative mechanism in specific immunotherapy,^{38,39} BP desensitization should also lead to an amelioration of the allergy to apple.

From clinical observations and from published studies, it seems probable that the inhalant sensitization subsequently leads to food hypersensitivity. However, our findings provide a new aspect concerning the question of whether Bet v 1–related food allergens could play a role during the sensitization process. It cannot be excluded that food allergens prime T cells, which then also react with pollen proteins. It is commonly believed that immaturity of the mucosal immune system accounts for allergic sensitization in childhood.⁴⁰ As a matter of fact, apples, carrots, and other vegetables containing Bet v 1 homologues do represent the earliest foreign protein sources in infant nutrition. On the other hand, these allergens do not represent common food allergens in childhood, implicating that tolerance induction to BPRF does occur. Considering the observed T-cell cross-reactivity, a situation of systemic tolerance (oral tolerance), which is incompatible with allergy, should be the consequence.⁴¹ Therefore perhaps a breakdown of the oral tolerance induced against cross-reactive BPRF antigens may account for the sensitization to pollen allergens. Recently, we demonstrated that the peptide Bet v 1 139-152 represents the immunodominant T-cell epitope in Balb/c mice experimentally sensitized to BP.⁴² Moreover, immunization of this mouse strain with rBet v 1 leads to apple allergy with positive skin test responses and high levels of specific IgE to rMal d 1 (unpublished data). A model of inhalant allergic sensitization to BP⁴³ in orally tolerized mice (with BPRF) might be suited to examine our hypothesis.

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