Correlation of sensitizing capacity and T-cell recognition within the Bet v 1 family

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Background: Bet v 1 is the main sensitizing allergen in birch pollen. Like many other major allergens, it contains an immunodominant T cell–activating region (Bet v $1_{142-156}$). Api g 1, the Bet v 1 homolog in celery, lacks the ability to sensitize and is devoid of major T-cell epitopes.
Objective: We analyzed the T-cell epitopes of Mal d 1, the nonsensitizing Bet v 1 homolog in apple, and assessed possible differences in uptake and antigen processing of Bet v 1, Api g 1, and Mal d 1.
Methods: For epitope mapping, Mal d 1–specific T-cell lines were stimulated with overlapping synthetic 12-mer peptides. The surface binding, internalization, and intracellular degradation of Bet v 1, Api g 1, and Mal d 1 by antigen-presenting cells were compared by using flow cytometry. All proteins were digested with endolysosomal extracts, and the resulting peptides were identified by means of mass spectrometry. The binding of Bet v $1_{142-156}$ and the homologous region in Mal d 1 by HLA class II molecules was analyzed *in silico*.
Results: Like Api g 1, Mal d 1 lacked dominant T-cell epitopes. The degree of surface binding and the kinetics of uptake and endolysosomal degradation of Bet v 1, Api g 1, and Mal d 1 were comparable. Endolysosomal degradation of Bet v 1 and Mal d 1 resulted in very similar fragments. The Bet v $1_{142-156}$ and Mal d $1_{141-155}$ regions showed no striking difference in their binding affinities to the most frequent HLA-DR alleles.
Conclusion: The sensitizing activity of different Bet v 1 homologs correlates with the presence of immunodominant T-cell epitopes. However, the presence of Bet v $1_{142-156}$ is not conferred by differential antigen processing. (J Allergy Clin Immunol 2015;136:151-8.)

Key words: *Allergic sensitization, Bet v 1, birch pollen–associated food allergy, immunodominant T-cell epitope, molecular allergology*

In patients with IgE-mediated allergy, the full manifestation of symptoms is preceded by a sensitization phase in which antigen-presenting cells (APCs) take up allergens and degrade them in endosomes/lysosomes. The generated peptides are then transferred into exocytic vesicles, where they bind to HLA class II molecules. HLA/peptide complexes are transported to the plasma membrane, where they can be recognized by T cells through their T-cell receptor.[1] In atopic subjects the cytokine milieu during presentation favors the development of naive T cells into T_H2 cells,[2-4] which in turn produce cytokines that lead to immunoglobulin class-switching in B cells and the production of allergen-specific IgE.[5] The latter is bound to high-affinity receptors on effector cells, so that on each subsequent contact, sensitized subjects can have allergic symptoms to the respective allergens.

One of the most common causes of IgE-mediated allergy in Northern and Central Europe and North America is birch pollen. Its main sensitizing allergen is Bet v 1, to which 93% of patients with birch pollen allergy have specific IgE.[6] Homologs of Bet v 1 have been identified in a wide range of foods[7]; they show sequence similarities of 50% to 80% and share a tertiary structure called the Bet v 1 fold.[8] With the exception of Dau c 1 from carrot[9,10] and Cor a 1 from hazelnut,[11,12] Bet v 1–related food allergens are considered to be unable to initiate sensitization in atopic subjects. However, because of IgE cross-reactivity, they cause immediate allergic symptoms in more than 70% of Bet v 1–sensitized patients.[6]

It is still not known which properties make a protein allergenic. For Bet v 1[13] and other major allergens, such as Der p 1 and Der p 2 from house dust mite,[14,15] Ves v 5 from wasp venom,[16] Art v 1 from mugwort,[17] Amb a 1 from ragweed,[18] Hev b 6.01 from latex,[19] Cry j 1 and Cry j 2 from Japanese cedar,[20] Pru p 3 from peach,[21-23] Fel d 1 from cat,[24] or Equ c 1 from horse,[25] it has been shown that they contain 1 or more immunodominant T-cell epitopes recognized by more than 50% of allergic patients. However, the nonsensitizing Bet v 1 homolog from celery, Api g 1, is devoid of frequently recognized T-cell epitopes.[26] Hence the presence of immunodominant T cell–activating regions might be an intrinsic feature of sensitizing allergens.

To further elucidate this hypothesis, we analyzed the T-cell epitopes of Mal d 1, another highly homologous but nonsensitizing Bet v 1–related protein in apple.[27] To assess whether the presence or absence of an immunodominant T cell–activating region depends on antigen processing and presentation, we used recombinant Bet

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Abbreviations used
aa: Amino acid
APC: Antigen-presenting cell
DC: Dendritic cell
DOL: Degree of labeling
FITC: Fluorescein isothiocyanate
mDC: Myeloid dendritic cell
mdDC: Monocyte-derived dendritic cell
nsLTP: Nonspecific lipid transfer protein
pDC: Plasmacytoid dendritic cell
TCL: T-cell line

v 1, Api g 1, and Mal d 1 labeled with different fluorescent dyes. We studied their internalization by different APCs from human blood. Furthermore, we followed these proteins through the endocytic pathway in monocyte-derived dendritic cells (mdDCs) and analyzed their degradation both in mdDCs and by endolysosomal extracts derived from mdDCs. Finally, we compared the binding affinities of T-cell epitopes derived from Bet v 1 and Mal d 1 to MHC class II molecules *in silico*.

METHODS

Patients and allergens

Twelve patients with birch pollen allergy had typical case histories, positive skin prick test responses to birch pollen extract (ALK-Abelló, Hørsholm, Denmark), and birch pollen–specific IgE levels of greater than 3.5 kU_A/L (ImmunoCAP; Thermo Fisher Scientific, Uppsala, Sweden). Patients with birch pollen allergy with associated apple allergy additionally reported oral allergy syndrome to apple. Bet v 1– and Mal d 1–specific IgE levels were determined by using ImmunoCAP. HLA-DRB and HLA-DQB1 typing was performed with a commercial sequence-specific oligonucleotide typing kit (Histo Spot SSO HLA-DRB and HLA-DQB1 typing kit; BAG Healthcare, Lich Germany). Samples with only a single detectable HLA-DRB1 or HLA-DQB1 allele were also typed by using sequence-specific primers (All Set SSP DRB and DQB1 low resolution; Olerup, Vienna, Austria). High-resolution typing was performed by means of nucleotide sequencing (SeCore Invitrogen, Life Technology, Brown Deer, Wis).

Three included nonallergic donors had no case history of early spring pollinosis and no Bet v 1–specific IgE. The study was approved by the local ethics committee. Donors provided written informed consent.

Recombinant Bet v 1.0101 (Bet v 1), Api g 1.0101 (Api g 1), and Mal d 1.0108 (Mal d 1) were purchased from Biomay (Vienna, Austria). These proteins were produced in *Escherichia coli* and contained less than 20 EU LPS/mg of protein. Their IgE binding was demonstrated in ELISAs and immunoblots (data not shown). Proteins were conjugated to pHrodo succinimidyl ester, fluorescein isothiocyanate (FITC)–succinimidyl ester, and Alexa Fluor 488–succinimidyl ester (all from Invitrogen, Carlsbad, Calif), according to the manufacturer's instructions.

The degree of labeling (DOL; moles of dye per mole of protein) was determined by using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, Del) and the following equation:

$$\mathrm{DOL} = \frac{Abs_{\lambda\ \max\ Dye}}{\varepsilon_{Dye} \times protein\ concentration\ (\mathrm{M})}$$

(λ_{max} pHrodo = 560 nm, λ_{max} Alexa Fluor 488 = 494 nm, ε_{pHrodo} = 65,000 $cm^{-1}M^{-1}$, $\varepsilon_{Alexa488}$ = 71,000 $cm^{-1}Mol^{-1}$).

The DOLs for pHrodo and for Alexa Fluor 488 of Api g 1 were only about a third of the DOLs of Bet v 1 and Mal d 1, probably because of the lower number of lysine residues in its primary structure.

Cell preparation

PBMCs were isolated from peripheral blood by using Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. Monocytes were isolated from PBMCs by using immunomagnetic cell sorting (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany), resulting in greater than 95% $CD14^+$ cells. mdDCs and microsomes thereof were generated, as described previously.[28-30]

Epitope mapping of Mal d 1–specific T-cell lines

Mal d 1–specific T-cell lines (TCLs) were generated from PBMCs (1.5×10^6 per well) by using 10 μg/mL Mal d 1, as previously described.[31] Cultures without Mal d 1 served as controls. After 5 days, suboptimal doses of human rIL-2 (10 U/mL; Boehringer, Mannheim, Germany) were added. At day 7, T-cell blasts were harvested by using density gradient centrifugation and expanded with irradiated PBMCs and IL-2. Ten days after the last feeding, TCLs were stimulated in duplicates with 1×10^5 irradiated (60 Gy) autologous PBMCs plus either Bet v 1, Mal d 1 (5 μg/mL), or 49 overlapping synthetic 12-mer peptides (each 5 μg/mL; Intavis, Köln, Germany), representing the complete amino acid (aa) sequence of Mal d 1. The latex allergen Hev b 3 served as a negative control. Incorporation of tritiated thymidine was measured after 48 hours. Stimulation indices were calculated as the ratio between counts per minute obtained in cultures with T cells plus autologous PBMCs plus peptide and counts per minute obtained in cultures containing T cells and PBMCs alone. A stimulation index of 2.5 was defined as positive T-cell proliferation.

Flow cytometry

The following mAbs were used: CD14–peridinin-chlorophyll-protein, CD19-allophycocyanin (BD Biosciences, San Jose, Calif), CD1c-allophycocyanin, CD141-allophycocyanin, and CD303-phycoerythrin (Miltenyi Biotec). Isotype controls were used to detect nonspecific binding. Flow cytometry was performed with a FACSCanto II (BD Biosciences) and analyzed with FACSDiva software (BD Biosciences) and FlowJo software (TreeStar, Ashland, Ore).

Surface binding, endocytosis, and degradation of labeled proteins

Alexa Fluor 488–labeled proteins (400 ng/mL) were incubated with freshly isolated PBMCs (2×10^6) for 1 hour at 4°C, washed, transferred to 37°C for another 20 hours, and labeled with cell type–specific surface markers to assess protein uptake. mdDCs (1×10^6) were incubated with Alexa Fluor 488–labeled proteins (400 ng/mL) for 3 hours at 4°C, washed, and fixed in 2% paraformaldehyde to assess surface binding. mdDCs (2×10^5) were incubated with pHrodo-labeled proteins (3 μg/mL) for 1 hour at 4°C, washed, and further incubated at 37°C in fresh medium and analyzed at the indicated time points to determine endocytosis. mdDCs (1×10^6) were incubated with FITC-labeled proteins (15 μg/mL) and incubated for 3 hours at 37°C, washed, and further incubated at 37°C for the indicated time periods to assess intracellular degradation. The percentage of antigen degradation is represented as the ratio of $FITC^+$ cells at the indicated time to $FITC^+$ cells at 0 hours.

Degradome assays

Allergens (5 μg each) were digested with microsomal enzymes (7 μg) isolated from mdDCs, as described previously.[28] Reactions were stopped at indicated time points by using heat denaturation and analyzed by means of mass spectrometry with an ESI-QTOF mass spectrometer fitted with a capillary rpHPLC (Waters, Milford, Mass).

HLA-DR–binding predictions

MHC class II–binding predictions were performed with the homologous 15-mer peptides Bet v $1_{142\text{-}156}$ (TLLRAVESYLLAHSD) and Mal d $1_{141\text{-}155}$ (GLFKLIESYLKDHPD) on August 20, 2014, by using the Immune Epitope Database analysis resource Consensus tool (http://tools.immuneepitope.org/mhcii/).[32,33] Percentile ranks are generated by comparing the peptide's score with 15 million random 15-mers from SWISSPROT database; a small percentile rank indicates high affinity.

TABLE I. Characteristics of patients with birch pollen allergy and associated apple allergy

	IgE specific for:		HLA phenotype			
Patient no.	rBet v 1 (kU_A/L)	rMal d 1 (kU_A/L)	DRB1*		DRB3-DRB5	DQ
1	4.7	3.5	07:01	11:01	3, 4	B1*02, *03
2	18.0	4.0	09:01	11:01	3, 4	B1*03
3	5.9	4.5	03:01	15:01	3, 5	B1*06, *02
4	3.9	1.2	07:01	11:01	3, 4	B1*02, *03
5	82.5	22.1	11:01	11:04	3	B1*03
6	34.7	16.1	11:04	13:01	3	B1*06, *03
7	80.2	19.6	01:01	11:01	3	B1*05, *03
8	12.2	6.2	07:01	13:01	3, 4	B1*06, *02
9	8.2	1.5	01:01	13:01	3	B1*03, *05
10	7.5	1.7	07:01	13:01	3, 4	B1*02, *06
11	22.3	3.5	07:01	14:05	3, 4	B1*05, *02
12	3.0	0.82	07:01	15:01	4, 5	B1*02, *06

Statistics

Data were analyzed with IBM SPSS 16.0 software (SPSS, Chicago, Ill). Data from different patients were not normally distributed (Shapiro-Wilk test) and therefore were presented as medians and interquartile ranges. Statistical differences were determined by using the Wilcoxon signed-rank test and considered statistically significant at a P value of less than .05. Data from duplicate and triplicate experiments are shown as means with SDs.

RESULTS

T-cell epitopes of Mal d 1

Allergen-specific IgE levels and HLA phenotypes of the 12 patients with birch pollen allergy and associated apple allergy are summarized in Table I. Mal d 1–specific TCLs generated from these patients were stimulated with overlapping 12-mer peptides representing the primary structure of Mal d 1 (Fig 1). Similar to Api g 1,[26] we could not identify any regions that were recognized by more than 33% of TCLs. Four patients recognized the peptide Mal d $1_{1\text{-}12}$, and 3 patients recognized the peptides Mal d $1_{67\text{-}78}$, Mal $d_{73\text{-}84}$, and Mal d $1_{97\text{-}108}$. The peptides Mal d $1_{4\text{-}15}$, Mal d $1_{25\text{-}36}$, Mal d $1_{34\text{-}45}$, Mal d $1_{37\text{-}48}$, Mal d $1_{49\text{-}60}$, Mal d $1_{52\text{-}63}$, Mal d $1_{58\text{-}69}$, Mal d $1_{61\text{-}72}$, Mal d $1_{70\text{-}81}$, Mal d $1_{76\text{-}87}$, Mal d $1_{142\text{-}153}$, and Mal d $1_{145\text{-}157}$ were recognized by 2 patients each. Seventeen peptides stimulated a proliferative response in only 1 patient (Mal d $1_{7\text{-}18}$, Mal d $1_{13\text{-}24}$, Mal d $1_{22\text{-}33}$, Mal d $1_{28\text{-}39}$, Mal d $1_{31\text{-}42}$, Mal d $1_{46\text{-}57}$, Mal d $1_{55\text{-}66}$, Mal d $1_{82\text{-}93}$, Mal d $1_{88\text{-}99}$, Mal d $1_{91\text{-}102}$, Mal d $1_{94\text{-}105}$, Mal d $1_{109\text{-}120}$, Mal d $1_{112\text{-}123}$, Mal d $1_{118\text{-}129}$, Mal d $1_{124\text{-}135}$, Mal d $1_{127\text{-}138}$, and Mal d $1_{139\text{-}150}$). The peptides Mal d $1_{10\text{-}21}$, Mal d $1_{16\text{-}27}$, Mal d $1_{19\text{-}30}$, Mal d $1_{40\text{-}51}$, Mal d $1_{43\text{-}54}$, Mal d $1_{64\text{-}75}$, Mal d $1_{79\text{-}90}$, Mal d $1_{85\text{-}96}$, Mal d $1_{100\text{-}111}$, Mal d $1_{103\text{-}114}$, Mal d $1_{106\text{-}117}$, Mal d $1_{115\text{-}126}$, Mal d $1_{121\text{-}132}$, Mal d $1_{130\text{-}141}$, Mal d $1_{133\text{-}144}$, and Mal d $1_{136\text{-}147}$ did not induce proliferation in any of the TCLs. Except for patients 2, 3, 9, and 10, all subjects recognized more than 1 peptide.

Uptake of Bet v 1, Api g 1, and Mal d 1 by different types of APCs

First, we analyzed the uptake of recombinant proteins labeled with Alexa Fluor 488 by different types of APCs in PBMCs from patients with birch pollen allergy with associated food allergies and nonallergic donors. Compared with Api g 1 and Mal d 1, uptake of Bet v 1 was enhanced in monocytes ($CD14^+$), plasmacytoid dendritic cells ($CD14^-CD303^+$), and the 2 types of myeloid dendritic cells (mDCs; mDC1, $CD1c^+CD19^-$; mDC2, $CD14^-CD141^+$)[34] in patients with birch pollen allergy (Fig 2, *A*). However, this increase could be caused by allergen-specific IgE in the sera from allergic patients because no difference in uptake of the 3 allergens was detected in nonallergic subjects (Fig 2, *B*).

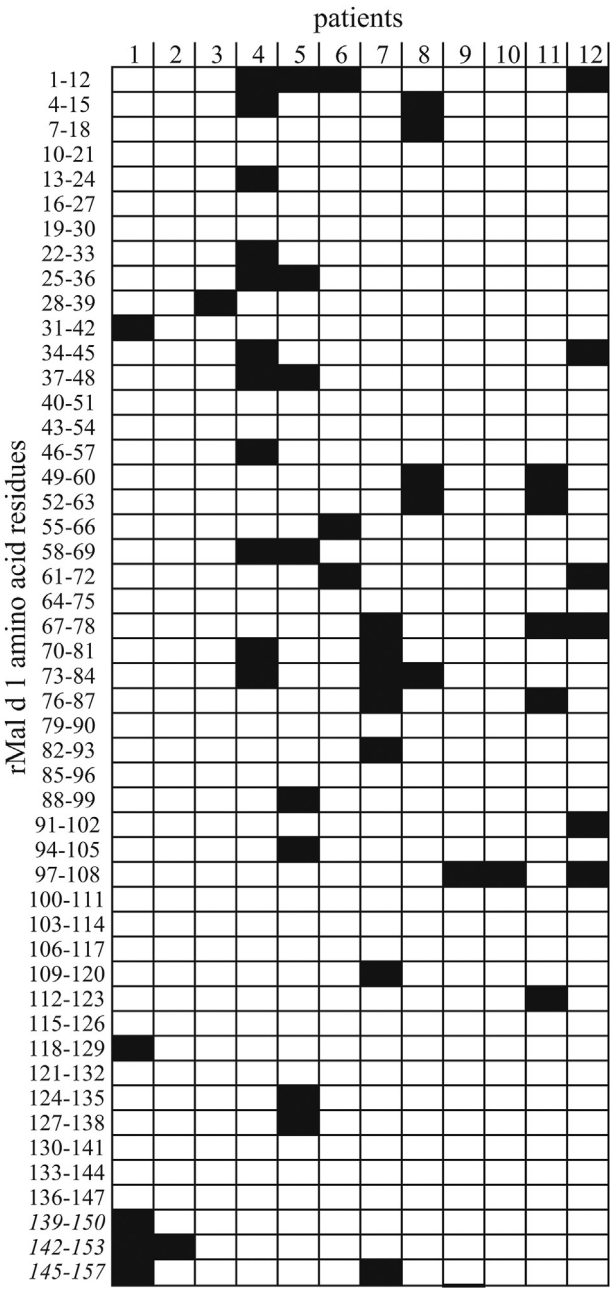

FIG 1. T-cell epitope mapping of Mal d 1. Proliferation of Mal d 1–specific TCLs from 12 different patients to 49 overlapping peptides spanning the aa sequence of Mal d 1 is shown. Peptides inducing a stimulation index of greater than 2.5 are denoted in black. Peptides matching the major T-cell epitope Bet v $1_{142\text{-}156}$ are denoted in italics.

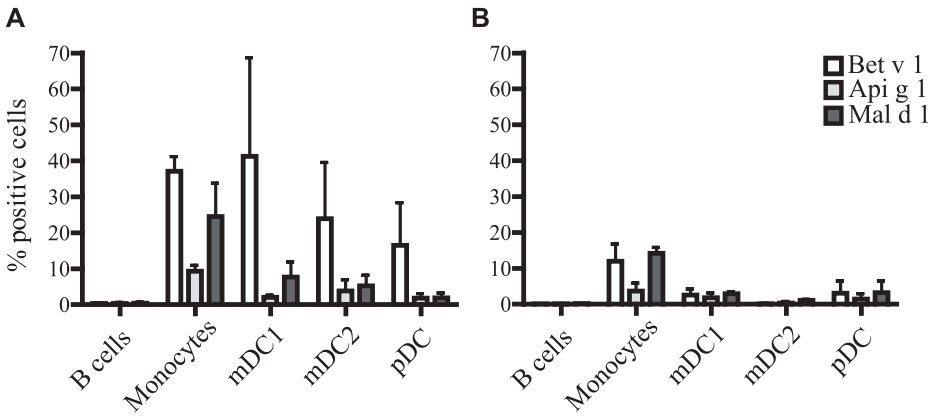

FIG 2. Uptake of allergens by APCs in human blood. PBMCs from donors with birch pollen allergy with associated food allergies **(A)** or nonallergic donors **(B)** were incubated with Alexa Fluor 488–labeled Bet v 1, Api g 1, or Mal d 1 for 1 hour at 4°C; washed; and further incubated at 37°C for 20 hours. The percentage of Alexa Fluor 488–positive cell populations was determined by using flow cytometry (n = 3, mean ± SD). *pDC*, Plasmacytoid dendritic cells.

Uptake and intracellular degradation of Bet v 1, Api g 1, and Mal d 1 by mdDCs

To perform a more detailed analysis of the endocytosis and subsequent processing of the different members of the Bet v 1 family, we used immature mdDCs, which can be obtained in higher numbers than dendritic cells from peripheral blood. mdDCs were incubated with Alexa Fluor 488–labeled allergens for 3 hours at 4°C for prevention of endocytosis to study the surface binding of the allergens. Bet v 1 was bound by a slightly higher percentage of mdDCs than Api g 1 and Mal d 1 (Fig 3, *A*); however, the number of positive cells was generally extremely low. To monitor the internalization of the allergens by mdDCs, we labeled the proteins with pHrodo, which starts to emit bright fluorescent light only when it reaches the acidic environment of endosomes. We followed the protein uptake for up to 24 hours and found no difference in internalization kinetics (Fig 3, *B*). To assess intracellular degradation, we used FITC-conjugated allergens and performed a pulse-chase experiment in which we first loaded the cells with the allergens for 3 hours at 37°C and subsequently monitored the disappearance of fluorescence. No differences in the degradation kinetics of the proteins were found (Fig 3, *C*).

Endolysosomal processing of Bet v 1, Api g 1, and Mal d 1

Bet v 1, Api g 1, and Mal d 1 were incubated with microsomal proteases isolated from mdDCs from allergic patients to learn more about the peptides generated by means of endolysosomal processing of the different allergens. At defined time intervals (1, 3, 6, 12, 24, 36, and 48 hours) of lysosomal degradation, proteolytic fragments of all allergens were identified by using tandem mass spectrometry (Fig 4). Peptide clusters relating to the regions Bet v $1_{1\text{-}22}$, Bet v $1_{23\text{-}36}$, Bet v $1_{83\text{-}102}$, and Bet v $1_{146\text{-}157}$ appeared after 1 hour of proteolysis of either food allergen. After 3 hours, additional clusters related to Bet v $1_{33\text{-}55}$, Bet v $1_{56\text{-}65}$, and Bet v $1_{103\text{-}115}$ were detected. In contrast, fragments matching Bet v $1_{66\text{-}80}$ were rarely detected in either food allergen. Peptide clusters in the region Bet v $1_{116\text{-}127}$ appeared earlier on degradation of both food allergens than of Bet v 1. Fragments corresponding to Bet v $1_{104\text{-}115}$ appeared in Api g 1 but not in Mal d 1. Together, these data indicated a highly similar endolysosomal processing of Bet v 1 and its dietary homologs. In the immunodominant T cell–activating region Bet v $1_{142\text{-}156}$, early fragments were found for Bet v 1 (Bet v $1_{146\text{-}157}$) and Mal d 1 (Mal d $1_{145\text{-}158}$), whereas they were missing for Api g 1. As previously demonstrated for Bet v 1,[28] all proteolytic peptide clusters of the food allergens covered T cell–activating regions identified by using epitope mapping (Fig 4).

HLA class II binding affinity of Bet v $1_{142\text{-}156}$ and Mal d $1_{141\text{-}155}$

Fig 4 shows that very similar peptide clusters were generated by means of endolysosomal processing in the C-termini of Bet v 1 and Mal d 1. Although Mal d $1_{141\text{-}155}$ shows an aa similarity of 60% with the immunodominant region Bet v $1_{142\text{-}156}$, it was recognized by less than 25% of the studied patients (Fig 1). Differential loading of these peptides into HLA class II molecules might be a reason for this observation. Thus we compared the binding affinities of these peptides with those of the most frequent HLA-DR alleles in European white subjects (HLA-DRB1*01:01, *03:01, *04:01, *07:01, *11:01, *13:01, and *15:01)[35,36] because Bet v $1_{142\text{-}156}$ has been shown to be HLA-DR restricted[37] and to bind promiscuously to several HLA-DR alleles.[38] Apart from some variations in the binding to individual alleles, we found no general difference (Table II). Bet v $1_{142\text{-}156}$ had a lower percentile rank for HLA-DRB1*03:01, *07:01, *13:01, and *15:01, whereas Mal d $1_{141\text{-}155}$ had a lower percentile rank for HLA-DRB1*01:01, *04:01, and *11:01.

DISCUSSION

Major allergens from various sources contain immunodominant T cell–activating regions[13-25] recognized by allergen-specific $CD4^+$ T lymphocytes from more than 50% of the respective allergic subjects. Because T lymphocytes play a major role in the initiation of allergic disorders, the distinct T-cell recognition of allergens might account for their sensitizing activity. Indeed, by comparing T-cell epitope mappings from highly homologous

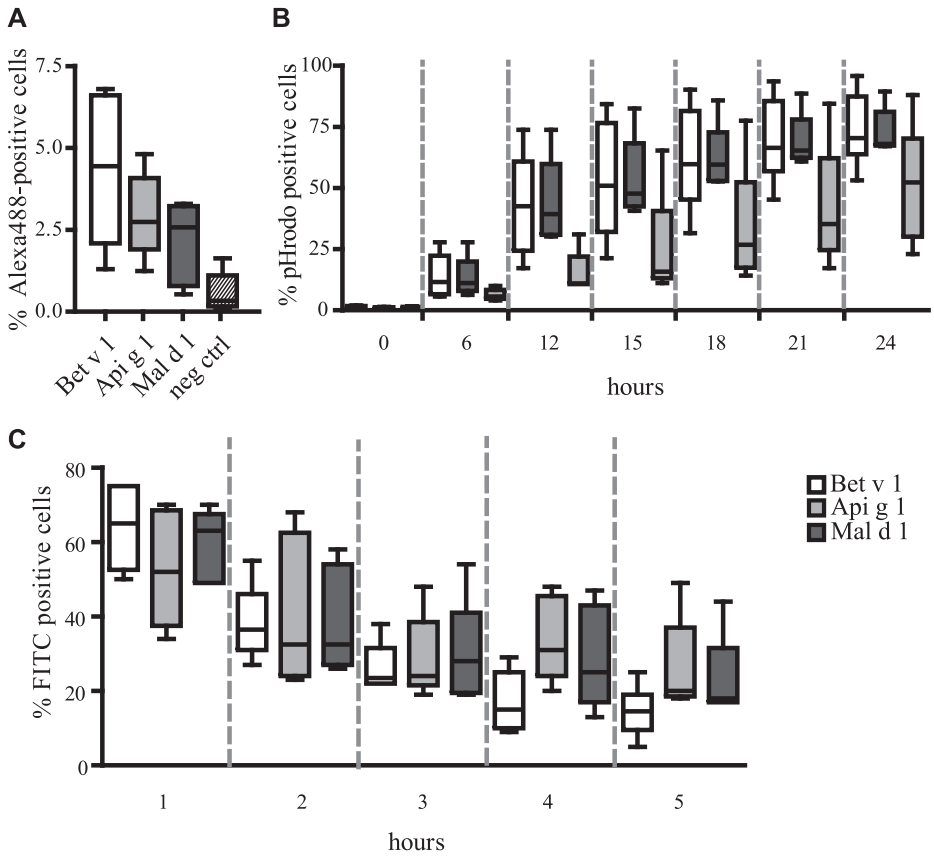

FIG 3. Bet v 1, Api g 1, and Mal d 1 in the endocytic pathway. Surface binding **(A)**, endocytosis **(B)**, and intracellular degradation **(C)** by mdDCs incubated with Bet v 1, Api g 1, or Mal d 1 conjugated to different fluorophores are shown. Fig 3, *A*, Alexa Fluor 488–positive cells after 1 hour at 4°C (n = 6). Fig 3, *B*, pHrodo-positive cells after 1 hour at 4°C and shift to 37°C for indicated periods of time (n = 5). Fig 3, *C*, Relative percentage of $FITC^+$ cells at indicated time points: cells after 3 hours at 37°C (time point 0) were set to 100% (n = 5).

members of the Bet v 1 family, we found an association between their sensitizing activity and the existence of dominant T-cell epitopes. Bet v 1, the major sensitizing protein in the Bet v 1 family, contains the region Bet v $1_{142\text{-}156}$, which is recognized by 63% of patients with birch pollen allergy.[13] Cor a 1 in hazelnut contains the region Cor a $1_{142\text{-}153}$, which is recognized by 47% of patients with birch pollen allergy and concomitant hazelnut allergy.[39] In addition to its cross-reactivity with the major birch pollen allergen,[39] Cor a 1 has been reported to induce IgE production independently of Bet v 1 in a low number of children with hazelnut allergy.[12] Conversely, the allergic response to Api g 1 is restricted to previous sensitization to Bet v 1, and no T-cell epitope in the celery allergen was recognized by more than 33% of the studied subjects.[26] Similarly, the allergic response to Mal d 1, the most frequent trigger for birch pollen–related food allergy,[6] predominantly results from humoral and cellular cross-reactivity with Bet v 1.[27] In this work we mapped the T-cell epitopes of Mal d 1. Although Mal d 1 shows an overall sequence similarity of 71% with Bet v 1, none of the 24 T-cell epitopes found was recognized by more than 33% of the patients under investigation (Fig 1).

Along these lines, we have obtained very similar results for homologous members of another family of relevant plant-food allergens (ie, nonspecific lipid transfer proteins [nsLTPs]). Studying the cellular and humoral response to Pru p 3 and Cor a 8, the respective nsLTPs from peach and hazelnut, revealed that Pru p 3 was the original cause for sensitization to Cor a 8 in a group of Italian patients with allergies to both foods.[40] For Pru p 3, immunodominant T-cell epitopes located within aa 12 to 27, aa 34 to 48, and aa 57 to 80 were described,[21-23] whereas Cor a 8 contained none.[40] Together, the identification of major T-cell epitopes solely in sensitizing members of the Bet v 1 and nsLTP families indicates that distinct T-cell recognition of an allergen might determine its sensitizing activity.

But why do highly homologous members from the same protein family behave so differently in their T-cell reactivity? Differences in antigen processing might partly answer this question. Indeed, we previously found that Pru p 3 was more stable to endolysosomal processing than Cor a 8,[40] which is characteristic for immunogenic proteins.[41] Here we compared the behavior of Bet v 1, Api g 1, and Mal d 1 in the major steps of antigen processing, namely uptake, endolysosomal degradation, and peptide loading to common HLA class II molecules. We could not identify obvious differences of the proteins in any of these processes. These findings imply that in contrast to nsLTP, antigen processing plays no role for the sensitizing activity of members of the Bet v 1 family. However, we have used mdDCs as APCs. Regarding the different routes of exposure to the different allergens (ie, inhalant for Bet v 1 and gastrointestinal for its dietary homologs), we cannot exclude potential differences in processing by

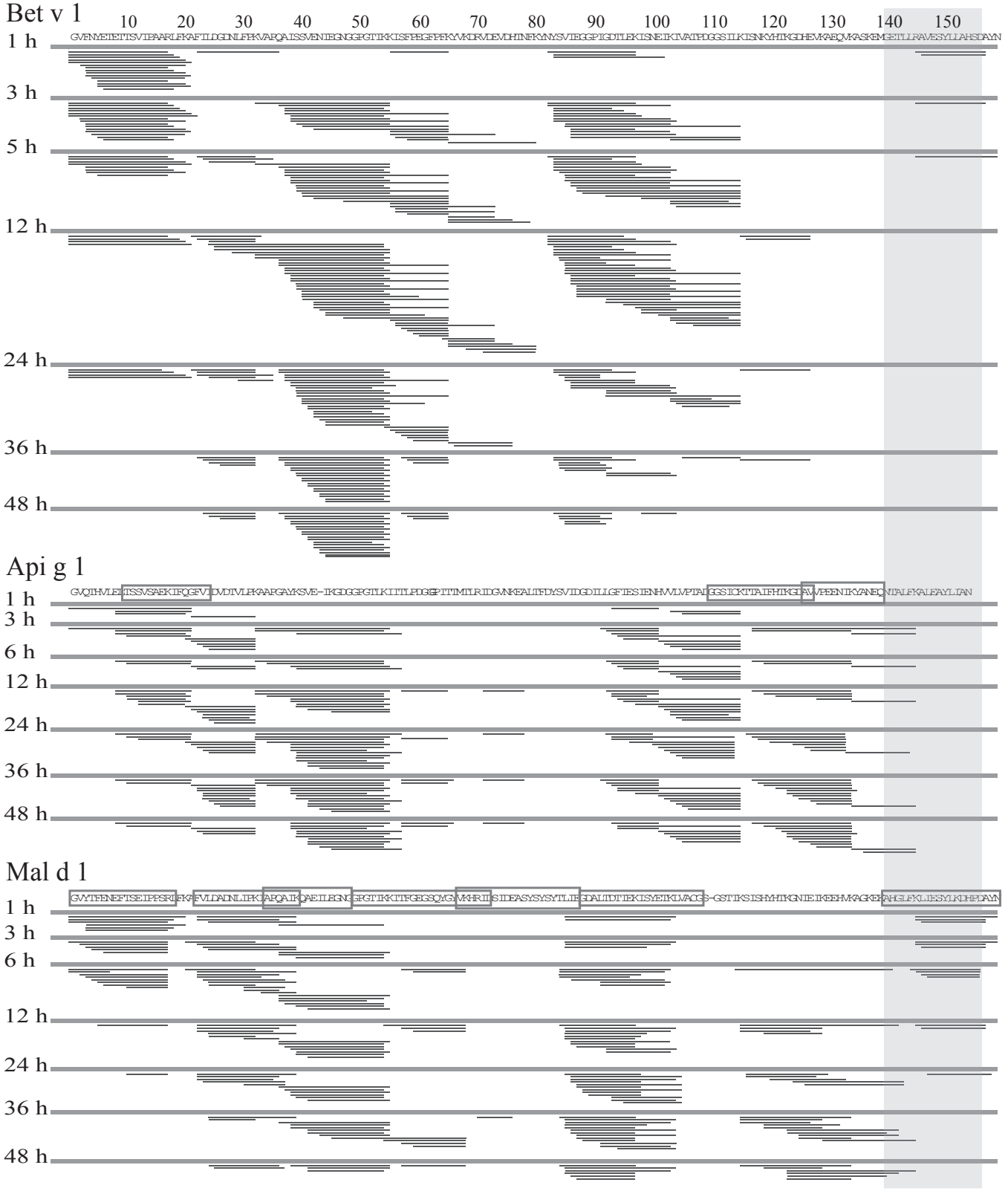

FIG 4. Endolysosomal processing of Bet v 1, Api g 1, and Mal d 1. Peptide sequences derived from Bet v 1, Api g 1, and Mal d 1 incubated with microsomal fractions from mdDCs for 1, 3, 6, 12, 24, 36, and 48 hours are shown. Each *line* represents a unique peptide found. The immunodominant T cell–activating region Bet v $1_{142\text{-}156}$ is denoted in *gray*, and T-cell epitopes in Api g 1 and Mal d 1 are framed in the respective aa sequence.

TABLE II. Percentile ranks of binding to HLA-DR alleles

aa Region	Sequence	Percentile ranks of binding to HLA-DR B1*						
		01:01	03:01	04:01	07:01	11:01	13:01	15:01
Bet v $1_{142-156}$	TLLRAVESYLLAHSD	6.71	**6.68**	10.51	**9.14**	8.05	**5.02**	**0.4**
Mal d $1_{141-155}$	GLF*KL*/*E*SYLKD**H**P**D**	**3.49**	11.53	**1.49**	19.43	**1.49**	15.71	2.87

Percentile ranks as calculated by using the Immune Epitope Database analysis resource consensus tool. The lower percentile rank for each HLA-DR allele representing higher binding affinity is shown in boldface. Identical aa residues are shown in boldface, and similar aa residues are shown in italics and underlined.

APCs located in the respective target organs, namely the lung and gastrointestinal tract.

By means of direct comparison of the highly homologous proteins Bet v 1 and Mal d 1 in the major steps of antigen processing, we found no inherent characteristic of the pollen allergen explaining the existence of the dominant T cell–activating region Bet v $1_{142-156}$. The fact that the very similar region Mal d $1_{141-155}$ contained no relevant T-cell epitopes could not be explained by a bias of HLA phenotypes in our study cohort because several Mal d $1_{141-155}$–negative patients expressed HLA alleles predicted to bind this peptide with high affinity. However, using pure recombinant allergens, we omitted other intrinsic features associated with allergenicity, such as lipid binding and activation of Toll-like receptors or glycosylation and binding to C-type lectins, which might affect uptake and endolysosomal degradation. Only recently, the natural ligand for Bet v 1, quercetin-3-O-sophoroside, was identified.[42] Bet v 1 ligand interaction generally enhances the volume of the hydrophobic pocket and thereby alters the protein surface.[43,44] Whether such structural changes influence the uptake and processing of Bet v 1 is currently not known. Because quercetin is abundant in apples, it might also bind to Mal d 1. Apart from that, Bet v 1 and Mal d 1 might pass differently through the nasal and oral mucosa, respectively. Therefore our future studies will also address their passage through the respective epithelial barriers.

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Key message

- **Sensitizing allergens contain dominant T cell–reactive regions, whereas nonsensitizing allergens do not.**

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