

Antigen presentation of the immunodominant T-cell epitope of the major mugwort pollen allergen, Art v 1, is associated with the expression of HLA-DRB1*01

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Background: Mugwort pollen allergens are the main cause of pollinosis in late summer in Europe. Ninety-five percent of patients allergic to mugwort are sensitized to the major allergen Art v 1. In contrast to other common pollen allergens that contain multiple T-cell epitopes, Art v 1 contains only 1 immunodominant T-cell epitope (Art v 1₂₅₋₃₆).

Objective: To characterize the minimal epitope of Art v 1₂₅₋₃₆ and to investigate a possible association of Art v 1 reactivity with HLA class II phenotypes.

Methods: Art v 1-specific T-cell lines and clones were established from 51 patients with clinically defined mugwort pollen allergy and IgE specific for Art v 1. To define minimal epitopes and binding sites within Art v 1₂₅₋₃₆, truncated and single-substitution analog peptides were used for T-cell stimulation. To study HLA restriction, monoclonal anti-HLA antibodies and antigen-presenting cells with defined HLA-DRB and -DQB1 alleles were used. HLA typing of patients with allergy was performed by hybridization with sequence-specific oligonucleotides, PCR, and nucleotide sequencing.

Results: In 96% of the patients, a cellular response to Art v 1₂₅₋₃₆ was obtained, and a core region of 5 to 10 amino acids containing 3 to 5 amino acids essential for T-cell reactivity was defined. The frequency of HLA-DRB1*01 in patients recognizing Art v 1₂₅₋₃₆ was significantly increased as compared with healthy controls (69% vs 21%; odds ratio, 8.45; $P < 10^{-6}$), and HLA-DRB1*01 was identified as the main restriction element for the presentation of the immunodominant epitope. **Conclusions:** Allergy to Art v 1 is characterized by a uniform T-cell response. The disease is apparently associated with the HLA-DR1 phenotype. Therefore, mugwort pollinosis is an ideal candidate for a peptide-based immunotherapy. (J Allergy Clin Immunol 2005;115:399-404.)

Key words: Type I allergy, mugwort pollinosis, Art v 1, HLA association, HLA-DRB1*01, peptide immunotherapy

Abbreviations used

APC: Antigen-presenting cell
nArt v 1: Natural Art v 1
OR: Odds ratio
rArt v 1: Recombinant Art v 1
TCL: T cell lines
TCC: T-cell clones
TCR: T-cell receptor

In Europe, pollen from mugwort (*Artemisia vulgaris*) is an important cause of hay fever in late summer or autumn that affects 10% to 14% of patients with pollinosis.¹ Mugwort, a widespread weed belonging to the Asteraceae plant family, is predominantly found in the temperate and humid zones of the Northern hemisphere and along the Mediterranean basin.² Significant clinical reactions upon exposure to other pollen allergens—for example, ragweed (*Ambrosia artemisiifolia*)—can be observed because of cross-reactivity to the mugwort-specific IgE. Cross-reactivity between mugwort and food allergens also leads to various forms of food allergy, such as the so-called celery-mugwort-spices syndrome.³⁻⁶

Mugwort pollen contains various allergens with molecular weights of 10, 14, 20, 28, 46, and 60 kD,⁷ but so far only 2—Art v 1 and Art v 4—have been characterized at a molecular level. Among patients allergic to mugwort, 36% are sensitized to Art v 4, a 14-kD protein that has been defined as mugwort profilin.⁸ Up to 95% of patients allergic to mugwort recognize the major allergen in mugwort pollen, Art v 1, a glycoprotein with an apparent molecular weight of 24 to 28 kD.⁹ Structurally, Art v 1 is a modular molecule that consists of 2 domains: a compact globular “head” at the cysteine-rich defensin-like N-terminus (amino acids 1-55) and a hydroxyproline-rich C-terminal extended “tail”-like domain (amino acids 51-108). Natural Art v 1 (nArt v 1) also contains 40% carbohydrates, which consist of plant O-glycans containing galactose and arabinose that are partly involved in the formation of IgE-binding epitopes of nArt v 1.^{9,10} The carbohydrate moiety of Art v 1 plays no obvious role in T-cell activation, because T cells recognize linear peptides that arise during antigen processing in the context of

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MHC molecules on antigen-presenting cells (APCs). Accordingly, nArt v 1 and recombinant (r)Art v 1 have been shown to induce comparable responses in PBMCs and oligoclonal and monoclonal T-cell cultures *in vitro*.^{10,11} In line with the cellular response to many other respiratory allergens, the T-cell response to Art v 1 is dominated by a T_H2-like cytokine profile.^{11,12} However, in contrast to other allergens, which typically contain multiple T-cell epitopes,¹³ Art v 1 harbors only 1 immunodominant epitope (Art v 1₂₅₋₃₆).¹¹

Since 1911, the only causative treatment of type I allergy is specific immunotherapy.¹⁴ The efficacy of conventional allergen-specific immunotherapy with complete allergen extracts is well documented for patients with allergic rhinitis/conjunctivitis.¹⁴ This treatment consists of repeated injections of increasing amounts of complete allergen extract and, hence, bears the risk of IgE-mediated anaphylactic side effects. Molecules with retained T-cell determinants but devoid of IgE reactivity have been considered as safe alternatives to conventional immunotherapy.¹⁵ In this respect, one strategy is the use of small T cell-reactive peptides incapable of cross-linking cell-bound IgE. Allergen-derived peptides have been used to successfully treat individuals allergic to cats or bee venom.¹⁶⁻²² Vaccination with peptides apparently relies on mechanisms similar to those of conventional specific immunotherapy; this leads to the tolerization of allergens according to immune deviation and suppressive mechanisms.^{23,24} However, a major limitation in the clinical use of peptides is the interindividual diversity of T-cell epitopes of allergens, primarily resulting from the polymorphism of HLA class II molecules. Thus, peptide immunotherapy seems more feasible in allergies with a clear-cut HLA association.

In this study, a possible association of Art v 1 reactivity with HLA class II phenotypes was investigated in 51 patients, and immune reactivity to the immunodominant region of Art v 1 was characterized in detail.

METHODS

Characterization of patients allergic to mugwort

Fifty-one patients with mugwort pollen allergy (27 female and 24 male; mean age, 34.5 years; age range, 12-65 years) were included in this study. The diagnosis of mugwort allergy was based on typical clinical history, ie, recurrent rhinitis/conjunctivitis during late summer and positive skin prick tests (wheal diameter >5 mm) to mugwort pollen extract (ALK, Horsholm, Denmark). The CAP-RAST test for mugwort pollen (w6; Pharmacia Diagnostics, Uppsala, Sweden) was positive for all patients (mean kU/L, 22.8; RAST class mean, 3.5). In total, 7 patients were monosensitized for mugwort. Sensitization to Art v 1 was assessed by detection of IgE in patients' sera reactive with mugwort extract, nArt v 1, and rArt v 1 by using immunoblotting as previously described.¹¹ In addition, IgE reactivity was tested by ELISA. Total IgE levels varied from 16 to >2000 kIU/L (median, 195 kIU/L). All patients were white Austrians.

Allergens

The nArt v 1 was purified from mugwort extract by cation exchange and size-exclusion chromatography and was characterized as

previously described.⁹ The rArt v 1 was obtained from Biomay (Vienna, Austria).

Art v 1-specific IgE ELISA

The nArt v 1 or rArt v 1 was coated onto ELISA plates (Maxisorb; Nunc, Roskilde, Denmark) in carbonate buffer (pH 9.6) at a concentration of 1 µg/mL overnight at 4°C. After blocking with 2% dry milk in Tris-buffered saline 0.5%/Tween 20, patients' sera diluted 1:4 in 1% BSA in Tris-buffered saline 0.5%/Tween 20 were incubated overnight at 4°C. Bound IgE was detected by mouse anti-human IgE conjugated with alkaline phosphatase (Pharmingen, BD Biosciences, Mountain View, Calif) and para-nitrophenylphosphate as the substrate. The cutoff value was defined as the mean + 5 SDs of 5 control sera from individuals not allergic to mugwort.

Characterization of Art v 1-specific T cells

Art v 1-induced proliferative responses of freshly isolated PBMCs were determined as described.¹¹ Art v 1-specific T-cell lines (TCLs) and T-cell clones (TCCs) were established from PBMCs by using purified nArt v 1 or rArt v 1 as the initial stimulus, and the specificity of T-cell cultures was assessed in proliferation assays by using [³H]thymidine uptake as described.¹¹ For PBMCs and TCLs, which showed different degrees of background proliferation because of autoreactivity, a stimulation index >3 (ratio between the counts per minute obtained in cultures containing T cells plus autologous APCs plus antigen and the counts per minute obtained in cultures containing T cells and APCs alone) or 10,000 disintegrations per minute, respectively were considered as cutoffs for antigen specificity. For TCCs, a stimulation index >10 was considered positive. The expression of T-cell receptor (TCR) Vβ families in TCCs was determined by RT-PCR with Vα- and Vβ-specific primers as described.¹¹

Epitope characterization

Proliferation of 5×10^4 T cells of TCLs or TCCs was tested with a panel of 33 overlapping dodecapeptides (Mimotopes/Biotrend, Köln, Germany) synthesized according to the Art v 1 amino acid sequence⁹ (GenBank Accession No. AF493943) in the presence of 1×10^5 autologous irradiated APCs after 48 hours. Peptides were used at a previously determined optimum concentration of 5 µg/mL. The minimal epitope of Art v 1₂₅₋₃₆ was analyzed by using a set of analogous peptides 6 to 11 amino acids long and truncated at the N- or C-terminus, as listed in Fig 1. In addition, a set of variant peptides with single alanine substitutions of each amino acid within Art v 1₂₅₋₃₆ (or glycine at the position occupied by alanine in the native protein, as listed Fig 2B) was used to determine the amino acids essential for T-cell reactivity.

HLA restriction

Art v 1₂₅₋₃₆-specific TCLs and TCCs were stimulated with 2.5 µg/mL Art v 1₂₅₋₃₆ plus 2.5×10^4 irradiated, autologous APCs in the presence of blocking antibodies. Anti-HLA-DP (B7/21; IgG1), -DQ (SK10; IgG1), and -DR (L243; IgG2a) (Becton Dickinson, Franklin Lakes, NJ) or isotype-matched control antibodies were used in a final concentration of 10 µg/mL in these proliferation assays. To test antigen presentation by HLA-DRB1*01, a homozygous EBV cell line with the HLA phenotype DRB1*01, DQB1*05 was used for antigen presentation.

HLA typing and statistical analysis

HLA-DRB and -DQB1 typing was performed with a commercial sequence-specific oligonucleotide typing kit (RELI SSO HLA-DRB and -DQB1 typing kit; Dynal, Broomborough, United Kingdom). Samples with only a single detectable DRB1 allele were also typed by using sequence-specific primers (All Set SSP DR low resolution;

	TCC						TCL										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
KCIEWEKAQHGA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIEWEKAQHGA	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
IEWEKAQHGA	-	+	+	-	-	+	+	-	+	-	+	-	+	+	+	+	+
EWKAQHGA	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+
WEKAQHGA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EKAQHGA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KAQHGA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KCIEWE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KCIEWEK	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KCIEWEKA	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
KCIEWEKAQ	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+
KCIEWEKAQH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
KCIEWEKAQHG	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

FIG 1. Proliferation of Art v 1₂₅₋₃₆-specific TCCs (n = 6) and TCLs (n = 11) derived from 17 representative individuals in response to Art v 1₂₅₋₃₆ (first line) and truncated peptides in the presence of autologous APCs. Proliferation was assessed after 48 hours by [³H]thymidine uptake. +, Proliferation similar to the stimulation with Art v 1₂₅₋₃₆; -, no proliferation.

Dynal). High-resolution typing was performed by nucleotide sequencing (BigDye Terminator Cycle Sequencing Kit; ABI, Foster City, Calif).

For statistical analysis, the frequency of DRB1 alleles was compared in patients (n = 51) and healthy controls²⁵ (n = 355) in 2 × 2 contingency tables. Odds ratios (ORs), confidence intervals (CIs), and significance values were calculated with the SAS 8.2 software (SAS, Cary, NC). The probability that the differences in the distribution of HLA alleles in the 2 groups were random was calculated by using the Fisher exact test. The probability values obtained were multiplied by the number of alleles tested (n = 13) according to the Bonferroni correction and are indicated as *P*_{corr} in Table I.

RESULTS

Immunodominance of Art v 1₂₅₋₃₆ in T-cell responses

All 51 patients included in this study had clinically defined mugwort pollen allergy and possessed serum IgE specific for nArt v 1 and rArt v 1, as determined by immunoblot and ELISA (not shown). In addition, nArt v 1 and rArt v 1 induced proliferation in PBMCs, oligoclonal TCLs, or TCCs derived from all patients. Similar proliferative responses were obtained in TCLs stimulated with nArt v 1, rArt v 1, and Art v 1₂₅₋₃₆ (mean ± SD: 41,066 ± 27,849 cpm, 44,104 ± 30,626 cpm, and 46,136 ± 30,626 cpm, respectively). Epitope mapping by stimulating TCLs or TCCs with synthetic dodecapeptides revealed that 49 (96%) of 51 patients harbored T cells specific for the immunodominant epitope Art v 1₂₅₋₃₆. For 39 (76%) of 51 individuals, Art v 1₂₅₋₃₆ was the exclusive epitope recognized. In 10 patients, 1 or 2 additional T-cell epitopes besides Art v 1₂₅₋₃₆ were obtained at residues Art v 1₁₋₁₈, Art v 1₄₃₋₅₄, or Art v 1₇₃₋₈₄. In 1 patient each, Art v 1₁₋₁₈ and Art v 1₄₃₋₅₄ represented the sole T-cell epitope.

Determination of the core epitope of Art v 1₂₅₋₃₆

To define the core region within the dominant T-cell epitope Art v 1₂₅₋₃₆, the truncated peptides listed in Fig 1 were used in proliferation assays. T-cell responses to these

A.

TCC No.	Patient No. 5					
	24	44	9	40	38	43
TCR V family α:β	18;7	19;3	9;5.2	1;20	5;11	nt
KCIEWEKAQHGA	+	+	+	+	+	+
CIEWEKAQHGA	-	-	+	+	+	+
IEWEKAQHGA	-	+	+	+	+	-
EWKAQHGA	-	-	+	-	-	-
WEKAQHGA	-	-	-	-	-	-
EKAQHGA	-	-	-	-	-	-
KAQHGA	-	-	-	-	-	-
KCIEWE	-	-	-	-	-	-
KCIEWEK	-	-	-	-	-	-
KCIEWEKA	-	+	+	+	-	-
KCIEWEKAQ	+	+	+	+	-	-
KCIEWEKAQH	+	+	+	+	+	+
KCIEWEKAQHG	+	+	+	+	+	+

B.

KCIEWEKAQHGA	+	+	+	+	+	+
ACIEWEKAQHGA	+	+	+	+	+	+
KAIEWEKAQHGA	-	-	+	+	+	+
KCAIEWEKAQHGA	-	+	+	+	-	-
KCIAIEWEKAQHGA	-	-	-	-	-	-
KCI EAEKAQHGA	-	-	-	-	-	-
KCIEWAKAQHGA	+	-	-	-	-	-
KCIEWEAAQHGA	+	-	-	+	-	-
KCIEWEKGQHGA	+	+	+	+	+	+
KCIEWEKAHGA	+	+	+	+	+	+
KCIEWEKAQAGA	+	+	+	+	+	-
KCIEWEKAQHAA	+	+	+	+	+	+
KCIEWEKAQHGG	+	+	+	+	+	+

FIG 2. Proliferation of 6 TCCs with different TCRs derived from patient 5 in response to stimulation with peptides. **A**, Proliferation induced by Art v 1₂₅₋₃₆ (first line) or truncated peptides. **B**, Proliferation in response to the Art v 1₂₅₋₃₆ peptide (first line) and derivatives containing single amino acid substitutions. +, Proliferation similar to the stimulation with Art v 1₂₅₋₃₆; -, no proliferation; nt, not tested.

peptides were assessed in Art v 1₂₅₋₃₆-specific TCCs derived from 6 different donors and in Art v 1₂₅₋₃₆-reactive TCLs derived from 11 more patients. The results

TABLE I. Frequency of HLA-DRB1 alleles in individuals allergic to mugwort sensitized to Art v 1 compared with healthy individuals

HLA-DRB1*	Patients (n)†	Controls (n)‡	Odds ratio	95% Confidence interval	P _{corr} §
01	35	73	8.45	4.25-17.19	1.95 × 10 ⁻⁷
03	8	73	0.72	0.28-1.64	NS
04	5	72	0.43	0.16-1.11	NS
07	8	84	0.60	0.27-1.33	NS
08	0	21			NS
09	0	6			NS
10	0	4			NS
11	9	88	0.65	0.30-1.39	NS
12	2	13	1.07	0.23-4.49	NS
13	4	93	0.24	0.08-0.68	.0338
14	3	29	0.70	0.21-2.40	NS
15	11	88	0.83	0.41-1.70	NS
16	8	21	2.95	1.24-7.47	NS

NS, Not significant.

†The total number of patients allergic to mugwort was 51.

‡A total of 355 healthy, unrelated Viennese blood donors (mean age, 28.8 ± 9.6 years) served as controls.

§Probability corrected according to Bonferroni.

are shown in Fig 1. The minimal epitope was heterogeneous in length, varying from 5 to 10 amino acids within Art v 1₂₅₋₃₄ (KCIEWEKAQH). Art v 1₂₈₋₃₂ (EWEKA) represented the central region shared by all minimal epitopes. The most common minimal epitope, found in 8 of 17 individuals, was 8 amino acids long and encompassed Art v 1₂₇₋₃₄ (IEWEKAQH). Testing different TCCs with varying TCRs derived from a single patient revealed again a variable extension of the core region (Fig 2A). These TCCs expressed different TCR V α and V β families.

Critical residues in the core epitope

Experiments were performed to identify the residues within Art v 1₂₅₋₃₆ that are critical for T-cell activation. In total, 9 TCCs from 2 donors and TCLs from 11 different donors were stimulated with a set of peptide analogs of Art v 1₂₅₋₃₆ containing single substitutions of each amino acid. For the TCCs tested, the most frequent amino acids indispensable for T-cell reactivity were E₂₈ in 9, W₂₉ in 9, E₃₀ in 7, and K₃₁ in 6 instances; for the TCLs tested, these were E₂₈ in 9, W₂₉ in 10, E₃₀ in 6, and K₃₁ in 10 cases (data not shown). Six representative TCCs derived from 1 patient expressing different TCRs are shown in Fig 2B. The number of critical residues that led to a substantial or complete loss of T-cell proliferation ranged from 3 to 6 amino acids, and these were mostly located within Art v 1₂₆₋₃₁ (CIEWEK). Fig 3 illustrates the position of essential amino acids in the minimal epitope.

HLA association with HLA-DRB1*01

We determined the distribution of HLA-DRB and -DQB1 phenotypes in the 51 white patients included in this study. The comparison of HLA-DR expression in individuals allergic to mugwort and sensitized to Art v 1 with a healthy Austrian control population revealed a significant increase in the HLA-DRB1*01 phenotype in our patient population (69% vs 21%; OR, 8.45; 95% CI,

4.25-17.19; Table I). Among the other phenotypes, the distribution of the DRB1*13 allele seemed to be significantly different in patients and control populations (8% vs 26%; OR, 0.24; 95% CI, 0.08-0.68).

The 7 patients monosensitized to mugwort pollen showed an even higher preference for HLA-DRB1*01: 6 (88%) of 7 displayed this phenotype. Two of the patients were homozygous. The DRB1*01-negative patient possessed the HLA-DRB1*16, *03 genotype. It is interesting to note that the 2 patients whose T cells did not react with Art v 1₂₅₋₃₆ did not possess HLA-DRB1*01 alleles but had the DRB1*13, *07 and DRB1*15, *03 genotypes.

Art v 1₂₅₋₃₆ presentation by HLA-DRB1*01

To further define the restriction elements involved in the presentation of Art v 1₂₅₋₃₆, inhibition experiments were performed. Eight TCLs from different donors and 5 TCCs from 4 different donors were stimulated with either the complete Art v 1 molecule or the Art v 1₂₅₋₃₆ peptide in the presence of antibodies specific for HLA-DR, -DQ, or -DP molecules. Although HLA-DR-specific antibodies inhibited the proliferation in all instances, HLA-DQ and -DP antibodies did not modulate the proliferation (data not shown).

As a next step, patients expressing an HLA-DRB1*01 molecule together with a non-HLA-DRB1*01 molecule were stimulated with peptides. Either autologous APCs were used or an EBV cell line homozygous for HLA-DRB1*01 was used as APCs. Both types of APCs induced similar T-cell proliferation (Fig 4). This confirms that the HLA-DRB1*01 molecule is actually capable of presenting the immunodominant epitope Art v 1₂₅₋₃₆.

DISCUSSION

Mugwort pollen allergy is frequently encountered in Central Europe, and sensitization to the major allergen Art

TCC No.	Art v 1 ₂₅₋₃₆
24	K C I E W E K A Q H G A
44	K C I E W E K A Q H G A
9	K C I E W E K A Q H G A
40	K C I E W E K A Q H G A
38	K C I E W E K A Q H G A
43	K C I E W E K A Q H G A

FIG 3. Illustration of clone-specific stimulation requirements. The minimal epitope (boxed sequence) and critical amino acids (gray) within the amino acid sequence of the immunodominant epitope Art v 1₂₅₋₃₆ are shown.

v 1 is observed in the vast majority of patients. Individuals allergic to mugwort who display IgE against Art v 1 also respond to the immunodominant peptide Art v 1₂₅₋₃₆ with their T cells and a typical TH2 response.¹¹ In this study, the minimal epitope and essential amino acids within Art v 1₂₅₋₃₆ were characterized, and their restriction by HLA-DR was demonstrated. The T-cell response to Art v 1 was clearly associated with the HLA-DRB1*01 phenotype, and DRB1*01 was shown to be capable of presenting Art v 1₂₅₋₃₆.

In 96% of our patients with cellular reactivity to Art v 1, T cells obtained from peripheral blood were found to be reactive with Art v 1₂₅₋₃₆ *in vitro*. The fact that Art v 1₂₅₋₃₆ represents the single predominant T-cell epitope is not due to a restricted use of TCR V α /V β families¹¹ (Fig 2), as observed, eg, in Amb a 5–specific T cells.²⁶ Another possibility explaining the predominance of the Art v 1₂₅₋₃₆ T-cell epitope is a preferable presentation of this peptide by HLA class II molecules. We therefore characterized the HLA class II molecules involved in peptide presentation. Inhibition experiments with HLA-DR–, -DQ–, and -DP–specific monoclonal antibodies indicated that only HLA class II molecules of the DR type present Art v 1₂₅₋₃₆ to T cells and TCCs.

Analyses of the HLA-DRB1* alleles in the patient group revealed a highly skewed distribution. Thirty-five (69%) of 51 patients possessed the HLA-DRB1*01 allele, in contrast to 22% of the control population. This difference was highly statistically significant ($P < 10^{-6}$), and the OR was 8.45, with a 95% CI of 4.25 to 17.19, indicating that within a probability of 95%, an HLA-DRB1*01–positive individual is 4 to 17 times more likely to become allergic to Art v 1 than an individual expressing other HLA-DR phenotypes. Moreover, a weak negative association was observed between DRB1*13 and Art v 1 reactivity.

In the past, type I hypersensitivity to certain allergens such as Fel d 1, Alt a 1, or bee venom has been associated with certain HLA class II phenotypes; however, the OR was less than 4.^{27,28} None of these publications included

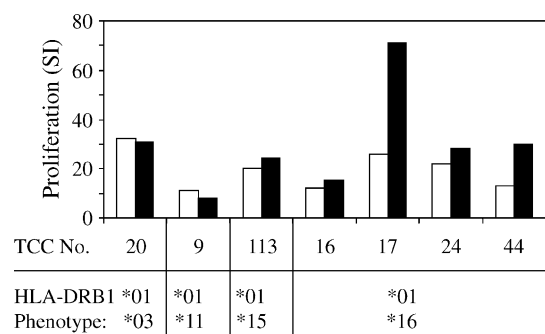


FIG 4. Presentation of Art v 1₂₅₋₃₆ by HLA-DRB1*01 in 7 representative TCCs derived from 4 different individuals. Proliferation of Art v 1₂₅₋₃₆–specific TCCs in response to Art v 1₂₅₋₃₆ in the presence of autologous APCs (open bar) and an EBV cell line homozygous for HLA-DRB1*01 (filled bar) is shown. HLA-DRB1 phenotypes are listed.

an analysis at the T-cell epitope level. Associations of HLA types with any of the various allergies studied have not been confirmed. By contrast, Amb a 5, a 5-kd minor allergen in the pollen of short ragweed, is significantly associated with HLA-DRB1*15, because 98% of patients with allergy who are sensitized to Amb a 5 carry this phenotype. In the case of Amb a 5, the DR molecule has been shown to be functional in the restriction of Amb a 5–specific T cells.²⁹ Obviously, the small size of the molecule does not allow for a variety of T-cell epitopes, thus explaining the restricted reaction. We also demonstrated with a homozygous EBV cell line that HLA-DRB1*01 acts as an antigen-presenting molecule for the immunodominant epitope of Art v 1 (Fig 4). Approximately one third of our patients who reacted with Art v 1 were not positive for HLA-DRB1*01. Therefore, it cannot be excluded that other DR molecules are also able to present Art v 1₂₅₋₃₆ on the basis of “promiscuous” binding, as has been demonstrated for other peptides in the past.³⁰⁻³³

To define critical amino acid residues in the immunodominant peptide involved in the activation of T cells, we performed cell stimulation experiments with truncated peptides and analogous peptides containing single mutations. Our observations suggested 3 to 6 critical residues in the Art v 1₂₅₋₃₆ peptide (Fig 3), which include W₂₉. It is conceivable that this residue is involved in the binding of the peptide to the HLA molecule, because the amino acids essential for high-affinity binding of peptide to HLA-DRB1*01 are hydrophobic and located near the amino-terminus, with aromatic amino acids superior to the aliphatic side chains.^{31,34}

The T-cell response to Art v 1 is primarily restricted to a single epitope and is clearly associated with HLA-DRB1*01 expression. Therefore, mugwort pollen allergy would be a suitable model for monitoring immunotherapy peptide-specific T-cell responses. In addition, mugwort allergy is an ideal candidate for peptide immunotherapy. Peptide immunotherapy²³ has been shown to have potential in the treatment of certain allergies such as bee venom or cat allergy.^{16,21,22,24} However, the principle

limitation of success in case of many other allergies is the diversity of the cellular responses and HLA polymorphism. In mugwort pollen allergy, Art v 1₂₅₋₃₆ would be a suitable source for peptide immunotherapy because (1) it is recognized by 96% of Art v 1-reactive patients, so most patients would be covered by this single peptide; (2) it does not contain remaining IgE-binding epitopes, because preincubation of Art v 1-specific sera did not affect IgE binding to Art v 1 in an ELISA (not shown); and (3) the epitope is common to all known isoforms of Art v 1. Moreover, the T-cell response to nArt v 1 and the presently used recombinant isoform of rArt v 1, Art v 1₂₅₋₃₆, accounted for similar T-cell responses *in vitro*.

In conclusion, we characterized Art v 1 as a unique major allergen with a uniform cellular response and a clear association with the HLA-DRB1*01 phenotype. These features strongly suggest an application of peptides in specific immunotherapy of mugwort allergy.

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