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

Beatrice Jahn-Schmid, PhD,[a] Gottfried F. Fischer, MD,[b] Barbara Bohle, PhD,[a] Ingrid Faé,[b] Gabriele Gadermaier, MSc,[c] Azra Dedic, MSc,[c] Fatima Ferreira, PhD,[c] and Christof Ebner, MD[a] *Vienna and Salzburg, Austria*

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 $1_{25\text{-}36}$).
Objective: To characterize the minimal epitope of Art v $1_{25\text{-}36}$ 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 $1_{25\text{-}36}$, 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 $1_{25\text{-}36}$ 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 $1_{25\text{-}36}$ 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.
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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

From [a]the Department of Pathophysiology and [b]Department of Blood Group Serology, Medical University of Vienna; and [c]the Department of Immunology and Allergology, University of Salzburg.
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Reprint requests: Beatrice Jahn-Schmid, PhD, Department of Pathophysiology, Waehringer Guertel 18-20, AKH-3Q, A-1090 Wien, Austria. E-mail: beatrice.jahn-schmid@meduniwien.ac.at.
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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 $1_{25\text{-}36}$).[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 [^{3}H]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 sequence[9] (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 $1_{25\text{-}36}$ 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 $1_{25\text{-}36}$ (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 $1_{25\text{-}36}$–specific TCLs and TCCs were stimulated with 2.5 μg/mL Art v $1_{25\text{-}36}$ 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 the 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, Bromborrough, United Kingdom). Samples with only a single detectable DRB1 allele were also typed by using sequence-specific primers (All Set SSP DR low resolution;