Native Art v 1 and recombinant Art v 1 are able to induce humoral and T cell–mediated in vitro and in vivo responses in mugwort allergy

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Background: Mugwort pollen is an important allergen source in hay fever and pollen-related food allergy. Little is known about the clinical relevance of the major mugwort allergen Art v 1 and its importance in allergy.
Objective: In this study we aimed to investigate the allergenicity of mugwort extract compared with the allergenicity of native (n)Art v 1 and recombinant (r)Art v 1, one major allergen of mugwort, in vivo and in vitro.
Methods: Thirty-two patients allergic to mugwort and 10 control subjects were investigated by means of skin prick and nasal provocation testing with different concentrations of mugwort extract, nArt v 1, and rArt v 1. nArt v 1 was purified from aqueous mugwort extract, and rArt v 1 was cloned, expressed in *Escherichia coli*, and then purified. The in vitro allergenicity was measured by means of ImmunoCAP, ELISA, ELISA-inhibition experiments, and T-cell proliferation assays.
Results: nArt v 1 and rArt v 1 were able to elicit positive in vivo and in vitro reactions. The IgE-binding capacity, as determined by means of ELISA, was slightly higher for nArt v 1 than for rArt v 1, and both allergens were able to induce T-cell proliferation in sensitized patients. However, rArt v 1 elicited a reduced response in skin and nasal provocation tests compared with nArt v 1. Compared with mugwort extract, both nArt v 1 and rArt v 1 showed lower sensitivity in patients with mugwort allergy in vivo.
Conclusions: Art v 1, either in its native or recombinant form, is able to induce allergic reactions in patients with mugwort allergy. rArt v 1 induced comparable humoral and cell-mediated responses in vitro but showed reduced in vivo allergenicity compared with biochemically purified nArt v 1. (J Allergy Clin Immunol 2003;111:1328-36.)

Key words: *Allergy, Art v 1, hay fever, IgE, nasal provocation test, mugwort pollen, recombinant allergens, skin prick test, T cell*

Abbreviations used
MALDI-TOF: Matrix-assisted laser desorption/ionization-time of flight
NPT: Nasal provocation test
SPT: Skin prick test

Pollens from the species mugwort *(Artemisia vulgaris)* are an important cause of hay fever during late summer and fall. Mugwort pollen is abundant in Northern and central parts of Europe. Botanic relationship and cross-reacting specific IgE with other plants of the family Asteraceae, such as ragweed *(Ambrosia artemisiifolia)*, can lead to clinically significant reactions in other geographic areas, such as Northern America.[1] Asteraceae, also called Compositae, can also cause occupational disease among workers in the flowers industry.[2,3] Furthermore, cross-reactive structures shared between mugwort pollen and several important foods, including celery, can lead to various forms of food allergy, such as the celery-mugwort-spices syndrome.[4-7]

Despite its importance as an allergen source, little is known about the allergenic proteins present in mugwort. Various allergens with molecular weights of 10, 14, 20, 28, 46, and 60 kd have been detected by means of SDS-PAGE[1]; however, the biochemical nature of these proteins is still unknown. Thus far, Art v 2[8] and Art v 3[9] represent the best characterized allergens from mugwort pollen, sharing potential homology with pathogenesis-related proteins and lipid transfer proteins, respectively, as shown by using N-terminal amino acid sequencing. Additional studies on the basis of poly(L-proline)-affinity purification and IgE cross-inhibition experiments demonstrated the presence of profilin as an allergenic component in mugwort pollen.[10] Although the major allergen Art v 1 has recently been cloned,[11] crystallization would be necessary to reveal the complete molecular structure of this and any of the other mugwort pollen allergens.

In contrast to natural allergens present in extracts, recombinant allergens produced as single allergenic molecules in heterologous hosts can be purified and standardized in terms of concentration and IgE reactivity. Problems related to contamination of the preparation with IgE-binding natural components,[12] as mentioned

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above, leading to variability in allergen amount can be circumvented. Moreover, the same defined allergen preparation can be used for both in vitro and in vivo tests, allowing a direct comparison of the diagnostic results obtained with different methods.[13] However, differences in the molecular structure of recombinant allergens caused by the lack of posttranscriptional modifications and folding might influence the allergenicity of the recombinant protein molecules and therefore need to be carefully controlled. Art v 1, a major allergen of mugwort pollen, is a modular glycoprotein with a defensin-like and a hydroxyproline-rich domain able to induce strong T-cell responses in PBMCs of individuals with mugwort allergy.[11] However, nothing is known about the clinical relevance of this important mugwort allergen.

In this study we report a comparison of the allergic reactivity of mugwort extract, biochemically purified native (n)Art v 1, and recombinant (r)Art v 1 with regard to their capacity to bind specific IgE, to induce T-cell response, and to elicit skin prick test (SPT) and nasal provocation responses in 32 individuals with mugwort allergy.

METHODS

Patients

Thirty-two patients with mugwort pollen allergy (17 female and 15 male patients; mean age, 23.4 years; age range, 16-43 years) and 10 control subjects (7 female and 3 male subjects; mean age, 26.2 years; age range, 21-41 years) were included in the study. The diagnosis of mugwort allergy was based on a typical clinical history of recurrent rhinitis during the late pollen season (July-August), a positive SPT response to commercially available mugwort extracts (ALK-Abello), and increased specific IgE serum levels to mugwort pollen, as determined by using the Pharmacia CAP System (w6, Pharmacia). Control subjects included 5 healthy persons and 5 allergic individuals sensitized to different allergenic sources, such as house dust mites, dog dander, and molds unrelated to mugwort, as demonstrated by means of negative SPT responses and a lack of mugwort-specific serum IgE in the ImmunoCAP w6 assay. All individuals enrolled in the study had stable lung functions.

During the study, patients were not allowed to use either antihistamines or topical or systemic steroids for at least 1 week before the investigation. Patients with other nasal diseases, such as chronic allergic or nonallergic rhinitis and atopic dermatitis, were excluded from the study.

The study was approved by the Ethical Committee of the University of Zurich. All patients and control subjects provided oral and written informed consent.

Purification of nArt v 1

nArt v 1 was purified from aqueous extract of mugwort pollen by means of cationic exchange chromatography with a CM Sepharose CL-6B (Pharmacia) column of 1.5 × 20 cm equilibrated with 20 mmol/L sodium phosphate buffer (pH 6.8). After washing, bound protein was eluted with a linear gradient to 0.3 mol/L NaCl and collected in 3-mL fractions. Samples showing absorbance at 280 nm were analyzed by means of SDS-PAGE, and those containing nArt v 1 were pooled and dialyzed against PBS (pH 6.8). After concentration to 5 mL, the protein solution was separated by means of size-exclusion chromatography on a 2.5 × 120–cm Sephacryl S-100 HR column (Pharmacia). Fractions containing nArt v 1 were pooled, dialyzed against H_2O, and placed in aliquots in 1-mg portions. Aliquots were lyophilized and stored at –20°C until use.

Cloning, expression, and purification of rArt v 1

The cDNA encoding mature Art v 1 (accession no. AX016334) was amplified by means of RT-PCR in a standard reaction with the following primers: Art33-Nde1, 5′-GAGAGACATATGGCTG-GTTCAAAGTTGTGTGA-3′; Art33-Mlu1, 5′-GAGAGAACG-CGTTTAGTGAGTGGACGGAGGAGG-3′ (*Nde*1 and *Mul*1 restriction sites underlined). The PCR amplification product was digested with *Nde*1 and *Mlu*I and unidirectionally ligated into a modified *Nde*1/*Mlu*1-restricted, dephosphorylated pMW172 vector.[14] The ligation mixture was transformed into electrocompetent *Escherichia coli* BL21(DE3) cells (Stratagene), and selected single clones were grown in liquid culture to verify the nucleotide sequence according to the Dye Terminator Cycle Sequencing protocol (Applied Biosystems). For the production of rArt v 1, a single transformant harboring the correct construct was inoculated to 1 L of LB medium (Amp 100 µg/mL) overnight. This culture was used to inoculate a 10-L fermentor (Bioflow 3000, Edison) containing the same medium. Culture conditions, induction of recombinant protein, harvesting, and purification of rArt v 1 were performed as described. Fractions containing pure rArt v 1 were concentrated, dialyzed against 1 mmol/L sodium phosphate buffer (pH 6.8), and placed in aliquots in 1-mg portions. For long-term storage, samples were lyophilized and stored at –20°C.

Deglycosilation of nArt v 1

nArt v 1 represents a glycosilated protein, as shown by means of periodic acid–Schiff staining of blots after SDS-PAGE.[11] The native protein was incubated under reducing conditions with 2 deglycosilating enzymes to investigate whether carbohydrates influence the various in vitro and in vivo reactivities of nArt v 1. N-Glycosidase F (New England Biolabs), also known as PNGase F, is an amidase that cleaves between the innermost GlcNAc and asparagine residues of high-mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins. Endo H_f (New England Biolabs) is a recombinant fusion protein of endoglycosidase H and maltose-binding protein. Endo H_f cleaves the chitobiose core of high-mannose and some hybrid oligosaccharides from N-linked glycoproteins.[15] nArt v 1 samples were treated with Endo H_f and PNGase F under reducing conditions and analyzed by means of 10% SDS-PAGE.

Determination of total and specific serum IgE levels

Total serum IgE levels were determined in all individuals by using the CAP System (Pharmacia). Specific serum IgE levels to mugwort (w6) were determined for all patients and control subjects by using the ImmunoCAP system, as prescribed by the manufacturer (Pharmacia). In addition, an ELISA was established to determine specific IgE levels to nArt v 1 and rArt v 1, as previously described.[16] Briefly, Maxisorp polystyrene microtiter plates (Nunc) were coated overnight at 4°C with rArt v 1 or nArt v 1 at 10 µg/mL in PBS, washed, and blocked. After several washing steps, sera were diluted 2-fold throughout the plate and incubated for 2 hours at room temperature. IgE binding was detected by means of incubation with mAb TN-142 mouse anti-human IgE mAb (provided by Ch. Heusser, Novartis) and visualized with alkaline phosphatase–conjugated goat-anti mouse IgG H+L chain, as described in detail elsewhere.[17]

Inhibition assays

For ImmunoCAP inhibitions, serum of a patient highly sensitized to mugwort (specific IgE w6 CAP class 4, 49.6 kU/L) was diluted to a value of 1 kU/L specific IgE with PBS. This diluted serum was

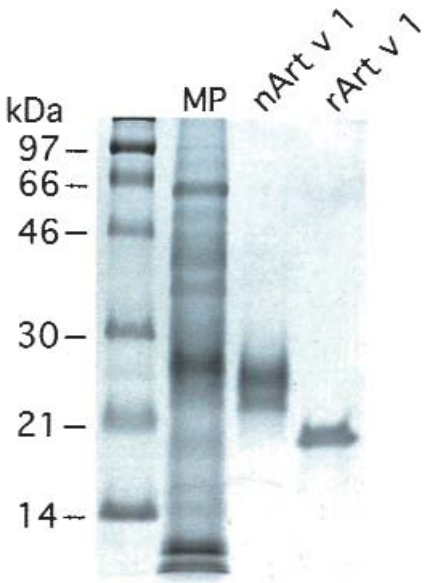

FIG 1. SDS-PAGE of mugwort pollen extract *(MP)*, purified nArt v 1, and rArt v 1 followed by Coomassie staining.

incubated with serially increasing doses of nArt v 1, rArt v 1, and BSA overnight at 4°C. Binding of mugwort-specific serum IgE to ImmunoCAPs was measured as prescribed by the manufacturer.

For ELISA inhibition, coating and blocking were performed as for the standard ELISA with rArt v 1. By using a separate 96-well plate (Costar), nArt v 1 and BSA were serially diluted in blocking buffer and incubated with 1:10 diluted patient sera for 2 hours at 37°C. The preincubated sera from 3 mugwort-sensitized patients were transferred to the coated plates and incubated for 2 hours at 37°C. Residual IgE-binding capacity was determined as described above. The inhibition was calculated from the adsorbancy of serial dilutions containing BSA in the fluid phase.

Proliferative response of PBMCs

PBMCs were isolated from heparinized peripheral venous blood of 5 individuals with mugwort allergy by means of Ficoll density gradient centrifugation, washed 3 times, and resuspended in RPMI 1640 supplemented with 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 50 µg/mL 2-mercaptoethanol, 1% minimal essential medium, nonessential amino acids and vitamins, 100 µg/mL streptomycin, 100 U/mL penicillin (all from Life technologies), and 10% heat-inactivated FCS (Sera-Lab). Samples of 5×10^5 cells were stimulated with 5 different concentrations (0.01, 0.1, 1.0, 10.0, and 100 µg/mL) of nArt v 1 or rArt v 1 in quadriplicate for 4 and 6 days. PHA was used as a positive control, and BSA served as a negative control. Proliferation was measured as incorporation of titrated thymidine (DuPont-NEN) during the final 8 hours of culture. A stimulation index of greater than 3 was considered positive.

SPTs with mugwort extract, nArt v 1, and rArt v 1

SPTs were performed with commercial mugwort pollen extract (ALK), the major allergen of mugwort in its native form (nArt v 1), and rArt v 1 produced in *E coli*. The individual proteins were diluted in NaCl 0.9% in concentrations of 1, 10, and 100 µg/mL. Sodium chloride 0.9% and histamine hydrochloride 0.01% (ALK) served as negative and positive controls.[18] All skin tests were performed in duplicate on the volar forearm and applied in 2 opposite directions with Stallerpointe devices. Twenty microliters of allergen solution at each concentration was used to perform in vivo titrations. A wheal diameter of 3 mm or greater surrounded by erythema was considered positive. The surface of the wheal was calculated according to the following formula:

$$[(D_1 + D_2/2)]^2,$$

where D_1 and D_2 represent the mutual perpendicular diameters of the wheal measured in millimeters.[18,19]

Nasal provocation tests

Nasal provocation tests (NPTs) were performed in 17 patients with placebo (sterile NaCl 0.9%), and the allergens were used in the same concentrations as for the SPTs. NaCl 0.9% solution was applied as a negative control first. Thereafter, the patients received increasing doses of allergen (1, 10, and 100 µg/mL) or placebo at 15-minute intervals. Allergen solutions were administered into one nostril by using a metered pump delivering 15 µL per puff. Evaluations were performed 15 minutes after each provocation to determine objective and subjective parameters. Objective parameters were the number of sneezes and the reduction in nasal airflow determined by means of active anterior rhinomanometry (Rhinotest 2000, Allergopharma) and rhinoscopy.[20] As subjective parameters, sneezing, nasal secretion, nasal obstipation, and symptoms in other organs (eg, itching of ears or eyes) were recorded. For each of the subjective parameters, 0 to 2 points could be achieved after administration of each of the 3 allergen concentrations. The points achieved after testing all 3 allergen concentrations were added to yield the total score. The test result was regarded as positive and the test was stopped when rhinomanometric flow was decreased by more than 30% and a symptom score of more than 3 points was achieved.

Statistical analysis

Statistical analysis was performed by using the Mann-Whitney *U* test. A *P* value of less than .05 was considered significant. Correlation coefficients were determined by means of linear regression with the Spearman rank test.

RESULTS

nArt v 1–specific serum IgE from mugwort-sensitized individuals recognizes epitopes on rArt v 1

Fig 1 shows the SDS-PAGE analysis of the reagents used in this work. nArt v 1 appears as a double-band allergen with an apparent molecular weight of 24 to 28 kd and rArt v 1 as a single-band allergen with an apparent molecular weight in the range of 18 kd. The molecular mass of rArt v 1, as determined by using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry analysis, corresponds to 10,802 d, which is in agreement with the calculated value of 10,800 d on the basis of the cDNA sequence.[11] nArt v 1 generates 2 series of peaks, with molecular weights ranging from 12,916 to 13,451 d and from 14,053 and 16,313 d in MALDI-TOF spectra corresponding to the double-band pattern observed in SDS-PAGE.[11] The heterogeneous molecular weight of nArt v 1 is due to different degrees of glycosilation of hydroxyprolines in the C-terminal domain of the protein.[11]

The quantitative levels of specific serum IgE against mugwort extract, as determined by means of ImmunoCAP, are shown in Table I. Relative specific serum IgE

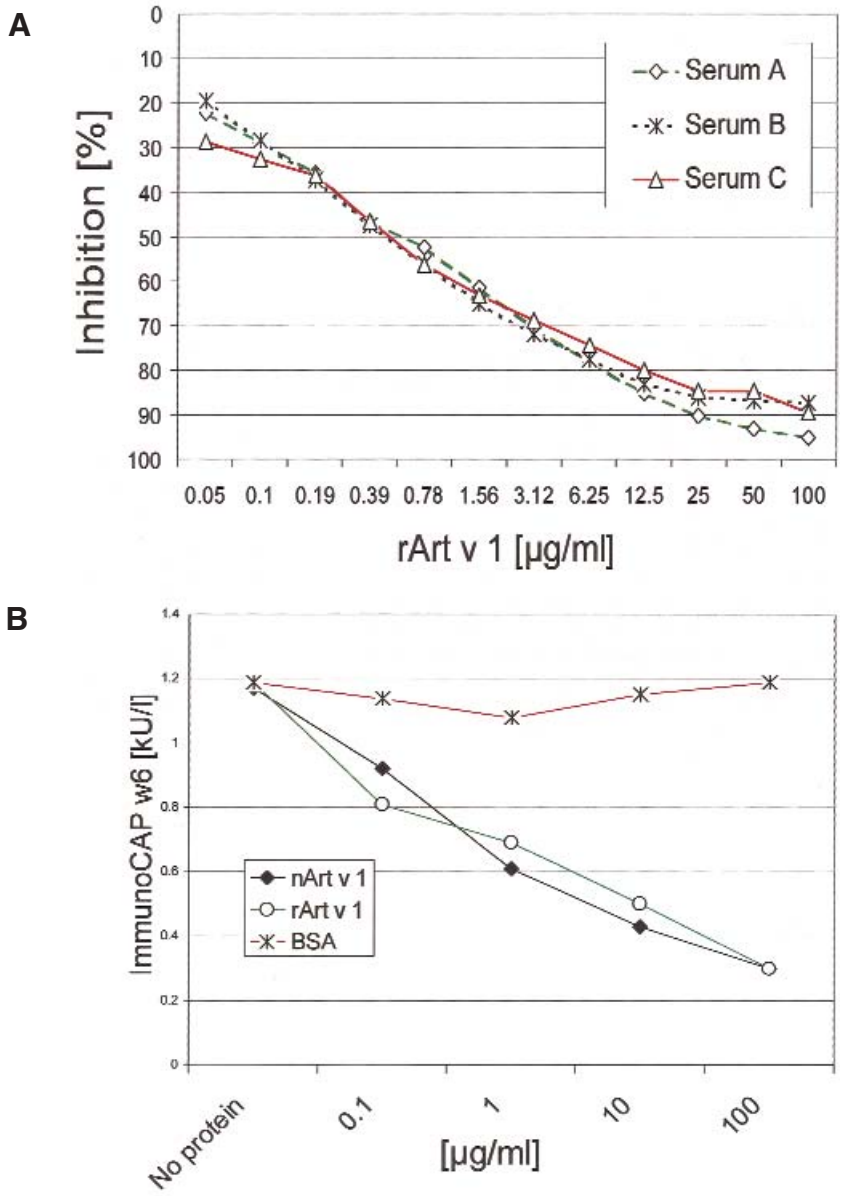

FIG 2. A, Inhibition of IgE binding to solid phase–coated nArt v 1 by rArt v 1 in the fluid phase. Sera from 3 patients were preincubated with different amounts of rArt v 1, samples were transferred to nArt v 1-coated wells, and residual IgE binding was analyzed by means of ELISA. **B,** Inhibition of CAP in sera with mugwort-specific IgE after preincubation with nArt v 1 and rArt v 1 is demonstrated in **C**.

levels against nArt v 1 and rArt v 1, as determined by means of ELISA, showed a significant correlation, with generally lower levels of specific IgE binding to rArt v 1 ($R = 0.976607$, $P < .0015$). There was also a significant statistical difference ($P < .05$) between mean optical density values for nArt v 1 (0.349 ± 0.118 [SEM]; range, 0.015-2.943) and rArt v 1 (0.270 ± 0.116 [SEM]; range, 0.013-2.974).

rArt v 1 in the fluid phase was also able to inhibit IgE binding to solid phase–coated nArt v 1 up to a maximum inhibition of 95% (Fig 2, *A*). nArt v 1 ($R = 0.734$, $P < .001$) and rArt v 1 ($R = 0.554$, $P < .002$) IgE levels, as determined by means of ELISA, correlated fairly well with the quantitative ImmunoCAP values of serum IgE raised against mugwort extract, indicating that Art v 1 corresponds to a major mugwort allergen.

This indication is corroborated by the results presented in Fig 2, *B*, showing that nArt v 1 and rArt v 1 are able to inhibit most of the IgE-binding capacity of serum to mugwort extract in a RAST inhibition assay and confirming the dominant role of Art v 1 as an IgE-binding component in mugwort extract.

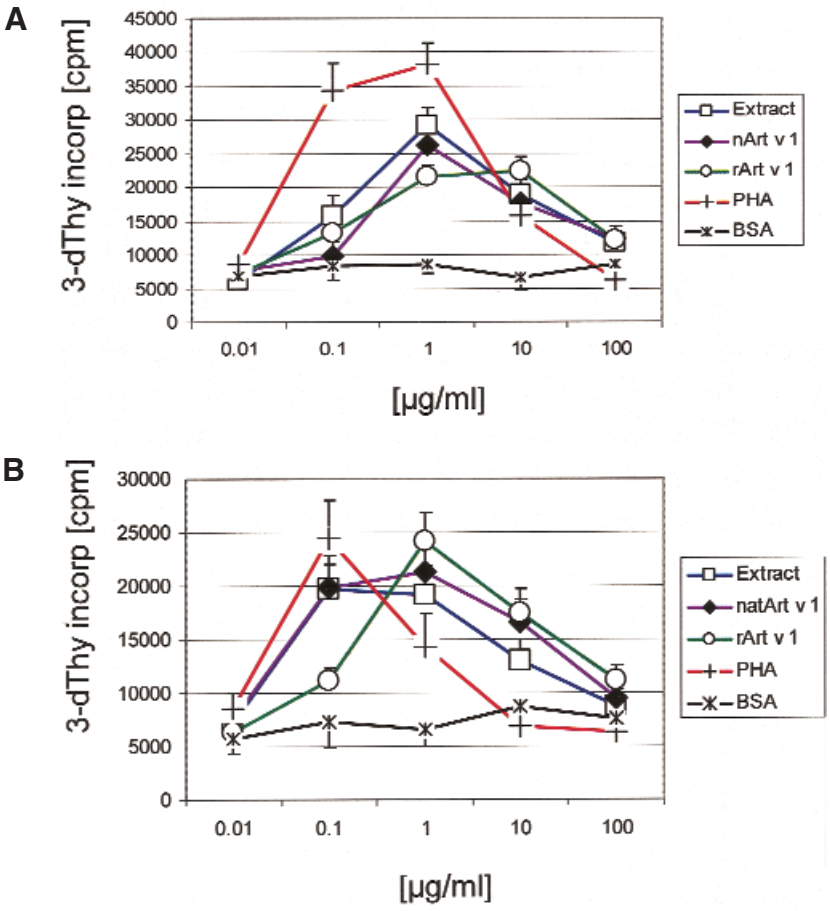

FIG 3. PBMC proliferation with various concentrations of mugwort, nArt v 1, and rArt v 1 after 4 days **(A)** and 6 days **(B)** is shown. PHA served as a positive control, and BSA served as a negative control.

Art v 1 induces T-cell proliferation in patients with mugwort allergy

Both allergens alone were able to induce T-cell proliferation in mugwort-sensitized patients. The proliferative responses of PBMCs to nArt v 1 and rArt v 1 were qualitatively and quantitatively comparable (Fig 3) and showed an optimal antigen concentration in the range of 1 to 10 µg/mL. A decreased stimulation was seen at higher doses.

Stimulation indexes varied from 3 to 6.5 between individual patients. However, there were differences in the incubation period needed to induce lymphocyte proliferation. Although mugwort extract and nArt v 1 induced maximal proliferation after 4 days, rArt v 1 achieved the highest stimulation after 6 days.

rArt v 1 elicits lower SPT reactivity than nArt v 1 and mugwort extract

All patients reacting to the natural mugwort extract in skin tests also had positive SPT responses to 100 µg of nArt v 1 or rArt v 1. There was a significant correlation among all 3 allergen sources in terms of skin reactivity, especially between the 2 forms of Art v 1 ($R = 0.917$, $P < .001$) (Fig 4, *A*). The reactivity to the natural extract was highest, followed by nArt v 1 and rArt v 1. There was a significantly decreased reactivity to the rArt v 1 allergen compared with nArt v 1 ($P < .05$) with regard to the size of the wheals induced. At a concentration of 1 and 10 µg/mL of the native and recombinant form of Art v 1, skin test reactivity was markedly reduced. The number of positive SPT responses with nArt v 1 and rArt v 1 at 100 µg/mL were comparable (34 vs 33, Table I), whereas at 10 µg/mL, the number of positive SPT responses decreased to 26 of 34 with nArt v 1 and 21 of 34 with rArt v 1 (Fig 4, *A*). Six patients had a positive SPT response already at 1 µg of nArt v 1 and 4 patients had a positive SPT response at 1 µg of rArt v 1. No positive reaction was observed in unrelated allergic or healthy control subjects, either to the extract or to the proteins (data not shown).

Reactivity in NPTs is lower with rArt v 1 than with nArt v 1

Most patients only reacted with a fully positive response when a nasal challenge of 100 µg of the allergen was applied (Fig 5, *B*). Only 2 of 17 patients reacted to 10 µg of rArt v 1 applied into the nostrils, and 4 of 17 reacted to the same concentration of nArt v 1. No reac-

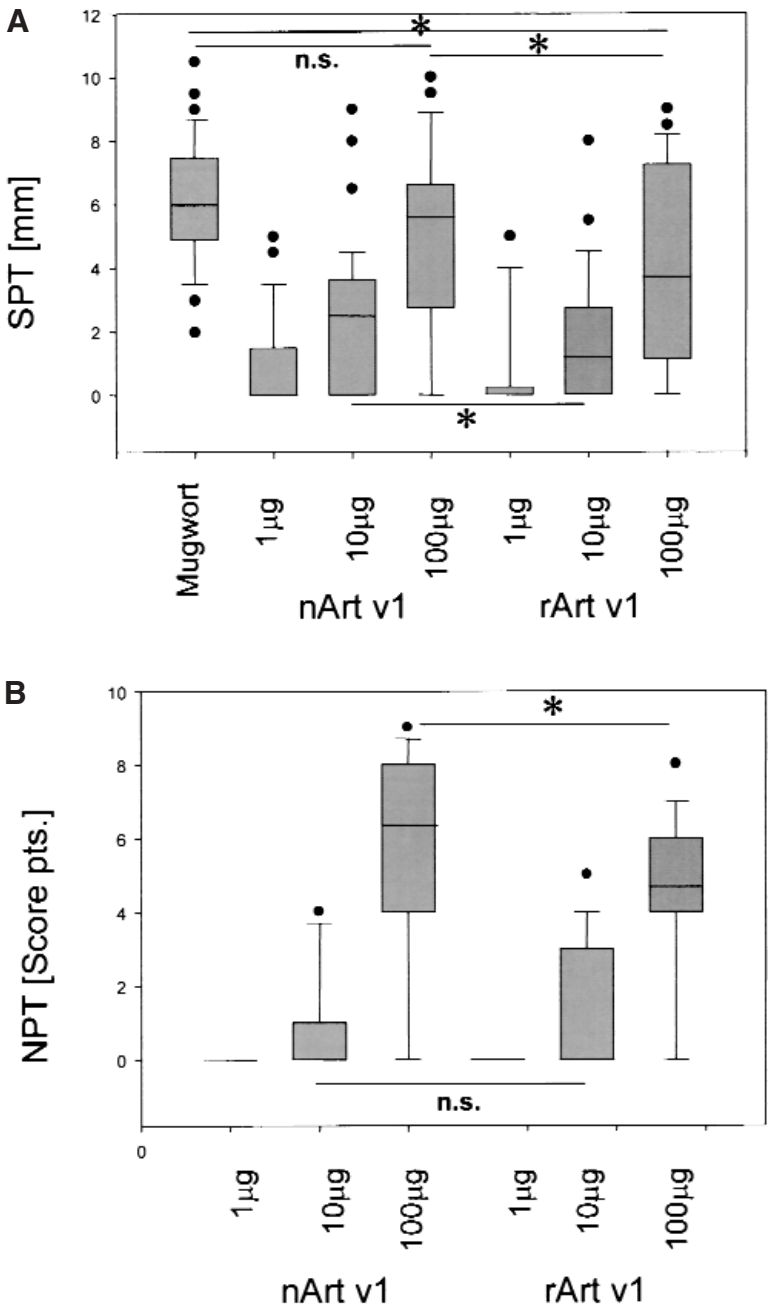

FIG 4. A, SPT at a concentration of 100 µg of allergen per milliliter. Results are given as means of D1+D2/2 of the wheal. SPT results with natural mugwort extract and 3 different concentrations of the individual nArt v 1 and rArt v 1 are shown. *Difference significant at $P < .05$. *n.s.,* Not significant. **B,** The mean reponse to nArt v 1 and rArt v 1 in NPTs is shown. Points reflect the various symptoms observed by the investigator. Only positive test reactions, including a decrease of nasal flow of greater than 30%, are shown.

tion was observed with 1 µg/mL. In general, the response to rArt v 1 was lower than that to nArt v 1. There was also a significant difference between the threshold levels to elicit a positive nasal challenge response with rArt v 1 (Fig 4, *B*). In 3 control individuals NPT responses with both forms of Art v 1 were negative.

No adverse side effects were observed in either SPTs or NPTs.

Skin test responses reflect the nasal reactivity of Art v 1 more than serum IgE levels

There was a significant correlation for the in vivo reactivity by SPTs and NPTs for nArt v 1 and rArt v 1 (Fig 5). There was also a weak but statistically significant correlation between serum IgE levels and SPT responses for

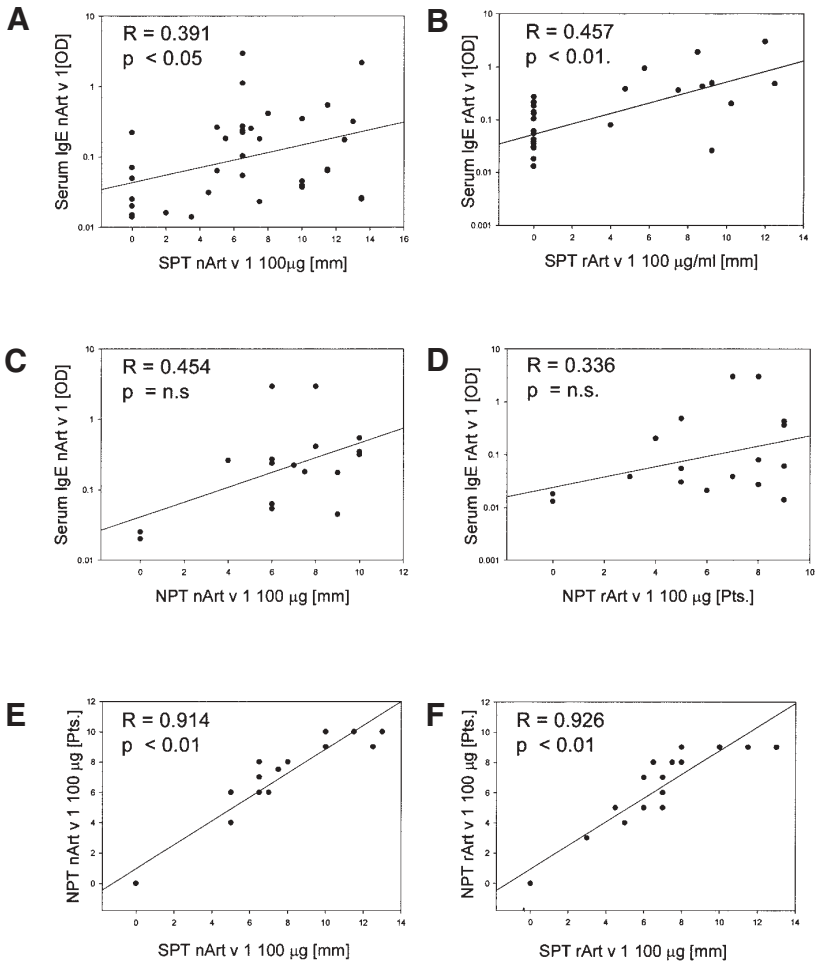

FIG 5. The interdependence of SPT responses and serum IgE levels to nArt v 1 **(A)** and rArt v 1 **(B)** is shown. In **C** (nArt v 1) and **D** (rArt v 1) the relationship between response in NPTs and serum IgE levels analyzed by means of ELISA are shown. In **E** (nArt v 1) and **F** (rArt v 1) the correlation between SPT responses and nasal reactivity are shown.

nArt v 1 and rArt v 1. NPT responses and serum IgE levels did not correlate for both allergens.

DISCUSSION

Mugwort represents an allergenic pollen species frequently encountered in central Europe. Thus far, it was not known to what degree Art v 1, a major mugwort allergen, was responsible for allergic reactions caused by *Artemisia vulgaris* because studies in vivo with the single allergen are still lacking. Earlier in vitro studies demonstrated the IgE-binding capacity of an allergen of 60 kd termed Art v 1 by means of immunoblotting.[21] Moreover, an additional 47-kd protein isolated from mugwort pollen able to elicit positive SPT responses in 70% of the individuals with mugwort allergy and termed Art v I has been reported.[8] Because no sequence information became available, these 2 proteins are no longer designated Art v 1 according to the rules of the IUIS allergen nomenclature subcommittee.[22] In the present study we investigated the clinical relevance of the officially recognized Art v 1 allergen, a glycoprotein encoded by an open-reading frame spanning 108 amino acid residues that has been recently cloned and biochemically characterized.[11] We demonstrate that Art v 1 as a single allergen is capable not only of inducing IgE binding but also T-cell proliferation in mugwort-sensitized patients. T-cell proliferation is dose dependent. In higher doses possibly toxic effects of Art v 1 might overweight the stimulation induced by Art v 1. By means of nArt v 1 and rArt v 1 challenges, we could induce positive SPT and allergic reactions by means of NPTs in patients showing detectable Art v 1–specific IgE in serum. The sensitivity of rArt v 1 was lower, and the amount of allergen needed to elicit symptoms was higher than for the natural protein. However, both forms of Art v 1 could be used to detect patients with mugwort allergy, as shown by means of positive SPT responses and detection of Art v 1–specific IgE by means of ELISA. The strong inhibition of specific IgE binding in ImmunoCAP by both single allergens supports the observation that Art v 1 is responsible to a high degree for the IgE-binding property of mugwort extracts.

TABLE I. In vivo and in vitro pattern of sensitization to mugwort, nArt v 1, and rArt v 1

Patient no.	History	Additional sensitization	SPT (mm)			CAP w6: mugwort (kU/L)
			Mugwort pollen	nArt v 1 (100 μg/mL)	rArt v 1 (100 μg/mL)	
1	RC	G	8.5	12.25	10.25	5.21
2	RC	G,T	6	5.75	5.5	3.45
3	RC	G,T	5.5	7.25	6.75	1.83
4	RC	G,T,M	6.5	5	2.5	4.09
5	RC	G	7.5	6.75	5.50	2.92
6	RC	G,T	12.5	14.75	13.75	7.83
7	RC	T	9.25	15.5	9	4.06
8	RC	None	6.5	8.5	7.75	3.62
9	RC	G	5.75	6.25	6.5	1.31
10	RC	G	5.75	5.5	5.00	0.44
11	RC	G,T	6.5	6.25	7.25	3.65
12	RC	G	4.25	10	13.5	4.85
13	RC	G,C	8.25	9.25	3	5.33
14	RC	None	8.75	8.25	2.75	9.17
15	RC	G,T,A,M	14.5	13.25	12.25	49.5
16	RC	G,T	6.75	8.5	2.5	3.45
17	RCA	G,T	12.5	13.25	11.75	3.49
18	RCA	None	7.0	7.75	8.25	4.06
19	RCA	G,T	5.5	6	7.75	1.19
20	RCA	G	5.25	4	4.5	4.41
21	RCA	G	12.25	11.5	12.25	3.25
22	RCA	G,T,C	10.75	12.25	9.5	0.72
23	RC,FA	G, C	6	5.75	6.75	0.91
24	RC,FA	G,T,C	6.25	7.25	5	4.89
25	RC,FA	G,T,M,C	3.75	4.25	4.75	0.5
26	RC,FA	G,C	8.25	6	5	2.43
27	RC,FA	G,T,C	6.75	5.5	6.25	2.89
28	RC,FA	G,C	10.75	12.5	11.5	2.46
29	RC,FA	C	9.75	11.5	10.75	7.01
30	RC,FA	C	3.75	3.25	4.25	0.37
31	RC,FA	C	12.5	8.25	4.25	2.13
32	RC,FA	G	12.5	9.75	10	0.47
33	Control	G	0	0	0	<0.35
34	Control	G	0	0	0	<0.35
36	Control	G	0	0	0	<0.35
37	Control	G,T	0	0	0	<0.35
38	Control	G,T	0	0	0	<0.35
39	Healthy	None	0	0	0	<0.35
40	Healthy	"	0	0	0	<0.35
41	Healthy	"	0	0	0	<0.35
42	Healthy	"	0	0	0	<0.35
43	Healthy	"	0	0	0	<0.35

RC, Rhinoconjunctivitis; *G,* grass pollen; *T,* tree pollen; *M,* molds; *C,* celery; *A,* animal dander; *RCA,* rhinoconjunctivitis + asthma; *FA,* food allergy.

There were substantial differences between the recombinant and naturally purified forms of Art v 1 in relation to the molecular weight. These differences can mostly be attributed to glycosylation present in the native allergen,[11] which is absent in recombinant proteins produced in *E coli*. Carbohydrate groups are able to bind specific IgE and might be responsible for cross-reactivity between different patients.[23,24] In this study rArt v 1 showed a lower reactivity compared with nArt v 1 in vivo, whereas the IgE-binding capacity and cellular immune responses were similar. Unfortunately, it was not possible to directly assess the contribution of glycosilation to the allergenicity of nArt v 1 because the native protein was resistant to treatment with Endo H_f and PNGase F. This negative result can be explained by the presence of a new type of plant *o*-glycan,[11] which was not known at the time when the experiments were performed.

There is evidence that posttranslational modifications, such as glycosilations, are important for IgE binding. Comparing the major allergen of Bermuda grass, Cyn d 1, the IgE-binding sites were only recognized by patient sera if Cyn d 1 was produced in yeast but not if Cyn d 1 was produced in *E coli*.[25] On the other hand, the enzymatic and immunologic properties of recombinant bee venom allergen phospholipase A2 were identical to those of the native glycosilated enzyme isolated from bee

venom, indicating that posttranslational modification does not play a relevant role in this special case.[26,27] In our study the in vitro reactivity to both rArt v 1 and nArt v 1 was similar, whereas the amount of rArt v 1 required to elicit positive SPT and NPT responses was significantly higher than for its natural counterpart.

Skin tests did reflect the allergenicity of the single allergens as evoked by NPTs the best. A similar observation was found in a recent study performed with recombinant allergens of various grass and birch pollens.[28] On the other hand, SPT responses did correlate with specific serum IgE levels, as shown already by means of intradermal skin test titration with recombinant allergens by means of endpoint titration.[26,29] Nevertheless, skin tests reflect the clinically relevant sensitization to a higher degree than in vitro findings. There was no significant difference in this aspect between nArt v 1 or rArt v 1.

In conclusion, our study demonstrates the importance of Art v 1 as a clinically relevant inhalant allergen for mugwort-sensitized patients and shows the high specificity of diagnostic procedures with single allergens. However, rArt v 1 shows less reactivity than nArt v 1 in vivo, probably because of structural differences between the 2 proteins. Because T-cell reactivity is conserved, rArt v 1 can be useful for future application in specific immunotherapy.

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