

Allergens, IgE, mediators, inflammatory mechanisms

Identification of common allergenic structures in mugwort and ragweed pollen

Reinhold Hirschwahr, MD,^a Claudia Heppner, MSc,^a Susanne Spitzauer, MD,^b
Wolfgang R. Sperr, MD,^c Peter Valent, MD,^c Uwe Berger,^d Friedrich Horak, MD,^d
Siegfried Jäger, PhD,^d Dietrich Kraft, MD,^a and Rudolf Valenta, MD^a
Vienna, Austria

Background: Despite the rare occurrence of ragweed in Middle Europe, a surprisingly high number of patients allergic to mugwort, a frequently encountered weed, display IgE reactivity against ragweed pollen allergens.

Objective: The aim of this study was to investigate whether the high prevalence of IgE reactivity against ragweed in patients allergic to mugwort is caused by the presence of common allergenic determinants. We also sought to characterize any cross-reactive allergens.

Methods: Common allergenic structures in mugwort and ragweed pollen were characterized by qualitative IgE immunoblot inhibition experiments performed with natural allergen extracts and recombinant allergens. The degree of cross-reactivity was estimated by quantitative CAP-FEIA competitions. The clinical significance of cross-reactive IgE antibodies was studied with histamine release experiments and nasal provocation tests.

Results: Mugwort and ragweed RAST values were significantly correlated in a population of 82 Austrian patients allergic to mugwort. IgE antibodies cross-reacted with allergens of comparable molecular weight that were present in both extracts. By using recombinant birch profilin and specific antisera for IgE inhibition experiments, profilin was identified as one of the cross-reactive components in mugwort and ragweed pollen. Preincubation of sera from patients allergic to mugwort with mugwort extract inhibited IgE binding to ragweed pollen extract greater than 80%. Mugwort and ragweed pollen extract induced comparable histamine release and reduction of nasal air flow in a patient with IgE reactivity against the major mugwort allergen Art v 1.

Conclusion: In addition to profilin, mugwort and ragweed pollen contain a number of cross-reactive allergens, among them the major mugwort allergen Art v 1. Cross-reactive IgE antibodies can lead to clinically significant allergic reactions. (J Allergy Clin Immunol 1998;101:196-206.)

Key words: Weed allergy, mugwort (*Artemisia vulgaris*), ragweed (*Ambrosia elatior*), IgE cross-reactivity, profilin, histamine release, nasal provocation

Pollen derived from anemophilous weeds, especially from the species mugwort (*Artemisia vulgaris*) and short ragweed (*Ambrosia artemisiifolia*) of the Family Asteraceae (Compositae), comprise more than 20,000 species. These pollen species are considered to be important elicitors of type I allergy during late summer and fall.¹ With a prevalence of about 50% in atopic individuals, ragweed pollen represents one of the most potent elicitors of type I allergy in Northern America.² A much lower prevalence of weed-pollen allergy, in particular sensitization to ragweed and mugwort (20%), among atopic individuals is reported for Central Europe (Germany).³ The distribution area of certain weeds (mugwort and ragweed) in Europe is highly divergent. Mugwort is abundant throughout the continent from Scandinavia to the Mediterranean. It is, however, lacking in the southern Mediterranean, and there is a gradient of frequent occurrence in the East to less abundance in the West. Ragweed occurs mainly in two distinct areas, Eastern Europe (Hungary) and the Rhone valley in France.⁴⁻⁷

Despite the rare distribution of ragweed in Germany and Austria and the improbability that allergic individuals become sensitized against ragweed, a high prevalence of positive ragweed RAST results is described for these areas.³ One explanation for the high prevalence of serologic positivity to ragweed could be that patients produce IgE antibodies that cross-react with antigens (i.e., common allergenic structures) shared with a more frequently occurring weed species (e.g., mugwort). The term "common allergenic structures" refers to antigens that are recognized by cross-reactive IgE antibodies in different allergen sources. IgE recognition of such common allergenic structures will lead to allergic reactions (e.g., allergic rhinitis, conjunctivitis, and asthma) in sensitized individuals after contact with each of the allergen-containing sources. Structural and immunologic similarities among relevant tree pollens, grass pollens, weeds, and food allergens have already been demonstrated to represent the molecular basis for a number of clinically well-described allergy syndromes.⁸⁻¹⁸

From ^aInstitute of General and Experimental Pathology, ^bInstitute of Medical and Chemical Laboratory Diagnostics, ^cthe Department of Internal Medicine I, Division of Hematology, and ^dthe Department of Otorhinolaryngology, AKH, University of Vienna, Vienna.

Supported by grant S06703 of the Austrian Science Foundation and by a grant from Pharmacia-Upjohn, Uppsala, Sweden.

Received for publication Jan. 14, 1997; revised Aug. 12, 1997; accepted for publication Aug. 19, 1997.

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

Copyright © 1998 by Mosby, Inc.

0091-6749/98 \$5.00 + 0 1/1/85505

Abbreviation used

SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Cross-reactive structures were studied in mugwort and ragweed pollen by using qualitative (immunoblot) and quantitative (CAP-FEIA) IgE inhibition experiments, antibodies raised against defined allergens (profilin), and recombinant allergens (Bet v 1¹⁹ and Bet v 2⁸), and the degree of shared IgE epitopes was determined. The clinical significance of IgE antibodies cross-reacting with mugwort and ragweed pollen allergens was studied with basophil release experiments and nasal provocation tests.

METHODS

Sera from allergic patients and control subjects

Patients allergic to mugwort were characterized by case history and skin testing. Sera were characterized by RASTs and testing with recombinant birch and timothy grass pollen allergens as described previously.^{20,21} Sera from 82 patients allergic to mugwort with a RAST class of 2 or more were analyzed by CAP (Pharmacia, Uppsala, Sweden) measurements for IgE reactivity to ragweed pollen, birch pollen, mite, and cat hair/dander extracts.

CAP-FEIA measurements and statistical analysis

Sera from 82 patients allergic to mugwort with a RAST class of 2 or more were analyzed for the presence of IgE antibodies specific for ragweed pollen, birch pollen, cat, and mite allergens by CAP measurements. The mean values of the RAST levels were determined and compared. In addition, the RAST classes demonstrated by the different extracts were correlated as described previously²² by using the Statistical Package for Social Sciences program packages (SPSS Inc., Chicago, Ill.) for PC version 6.1.3. Because the data were of a classificatory type converted to an ordinal scale, the Pearson Product Moment Correlation could not be used. Instead, parametric free coefficients (Kendall's coefficient of rank correlation and Spearman's rank correlation coefficient) were used.

Allergen extracts, purified allergens, and antisera

Mugwort (*Artemisia vulgaris*) and ragweed (*Ambrosia artemisiifolia*) pollen that was more than 95% pure as determined by light microscopy was purchased from Allergon, Valinge, Sweden. Aqueous extracts were prepared as described by Vrtala et al.²³ and stored after lyophilization at -20°C until use. Protein concentrations were determined according to the method of Bradford²⁴ and estimation of Coomassie Blue-stained sodium dodecylsulfate-polyacrylamide gel.²⁵ Recombinant birch profilin was expressed in *Escherichia coli* DE3 (BL21) and purified by poly(L-proline)-affinity chromatography as described by Susani et al.²⁶ Purified recombinant Bet v 1 expressed in *E. coli* DE3 (BL21) as a nonfusion protein was obtained from BIOMAY (Linz, Austria). Rabbit antisera raised against purified plant profilin, celery profilin-RP 1, recombinant birch profilin (RP2), and recombinant tobacco profilin (RP5) are described elsewhere.^{10,27,28}

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

Mugwort and ragweed pollen extracts were separated by 12.5% SDS-PAGE.²⁵ Protein gels were stained with Coomassie

brilliant blue or blotted onto nitrocellulose (Schleicher & Schuell, Dassel, Germany). For a preparative sheet, 100 µg protein extract/cm gel were separated in a 12.5% SDS-PAGE and blotted onto nitrocellulose according to the method of Towbin et al.²⁹ For the analysis of protein samples under nonreducing conditions, β-mercaptoethanol was omitted from the sample buffer. Nitrocellulose strips were blocked and washed in buffer A (50 mmol/L sodium phosphate [pH 7.5] containing 0.5% wt/vol bovine serum albumin, 0.5% vol/vol Tween 20, and 0.05% wt/vol NaN₃). Sera from allergic patients or control subjects were diluted 1:5 and rabbit antisera were diluted 1:1000 in buffer A before incubation with the blots. Bound human IgE antibodies were detected with Iodine 125-labeled anti-human IgE antibodies (RAST; Pharmacia, Uppsala, Sweden).⁹ Bound rabbit antibodies were detected with a ¹²⁵I-labeled donkey anti-rabbit Ig antiserum (Amersham, Buckinghamshire, U.K.). Blots were exposed to Kodak XOMAT films with intensifying screens (Kodak, Heidelberg, Germany) at -70°C.

Qualitative IgE immunoblot inhibition experiments

For the identification of common allergens in mugwort and ragweed pollen, qualitative IgE immunoblot inhibition experiments were done. Sera from patients allergic to mugwort were diluted 1:5 in buffer A and preincubated with 100 µg of mugwort or ragweed pollen extract and, for control purposes, 100 µg dog albumin (Sigma, St. Louis) per milliliter serum dilution or without addition of other proteins overnight at 4°C. Recombinant allergens rBet v 1 and rBet v 2 (birch profilin) were used for preadsorption at a concentration of 10 µg/ml serum dilution. Preadsorbed serum dilutions were then used for the detection of nitrocellulose-blotted mugwort or ragweed pollen extract as described above.

Quantitative IgE inhibition experiments

To estimate the degree of cross-reactivity between mugwort and ragweed, quantitative CAP-FEIA inhibition experiments were performed. Sera from five representative patients allergic to mugwort were preincubated with mugwort pollen extract (50 µg), ragweed pollen extract (50 µg), and, for control purposes, an equal volume of isotonic sodium chloride solution at 4°C overnight. The amount of mugwort and ragweed pollen-specific IgE antibodies was then determined by CAP measurements.

Basophil histamine release assay

Granulocytes were isolated by dextran sedimentation from the heparinized blood of a volunteer allergic to mugwort pollen without profilin-specific IgE antibodies.³⁰ Cells were incubated with different concentrations of mugwort pollen extract, ragweed pollen extract, or anti-human IgE antibodies. Histamine released into the cell-free supernatants was measured by radioimmunoassay (Immunotech, Marseille, France). Total histamine release was determined in cell lysates after freeze-thawing. Results were obtained as mean values from triplicate determinations and were expressed as percentages of total histamine release.³¹

Nasal provocation tests

The biologic effects of mugwort and ragweed allergen preparations were compared by active anterior rhinomanometry in two patients allergic to mugwort without profilin-specific IgE

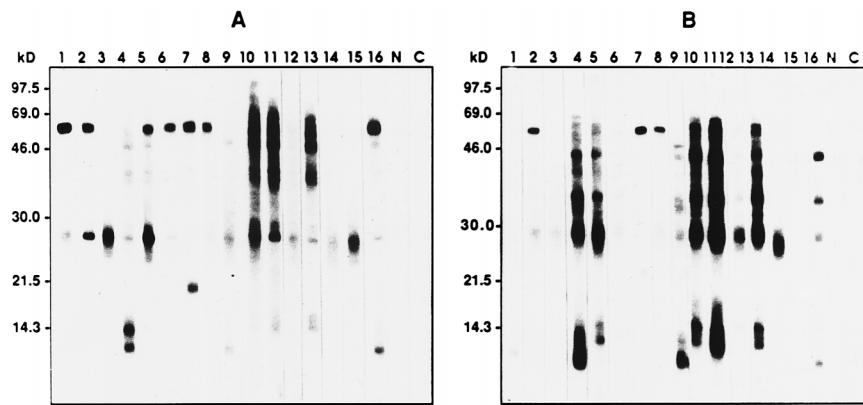


FIG. 1. Comparison of the IgE-binding components detected by sera from individuals allergic to mugwort pollen in nitrocellulose-blotted mugwort and ragweed pollen extracts. Nitrocellulose strips containing blotted mugwort pollen extract (**A**) and ragweed pollen extract (**B**) were incubated with serum IgE from individuals allergic to mugwort (*lanes 1 through 16*), serum from a nonallergic individual (*lane N*), and buffer (*lane C*). Molecular weights are displayed on right side of each panel.

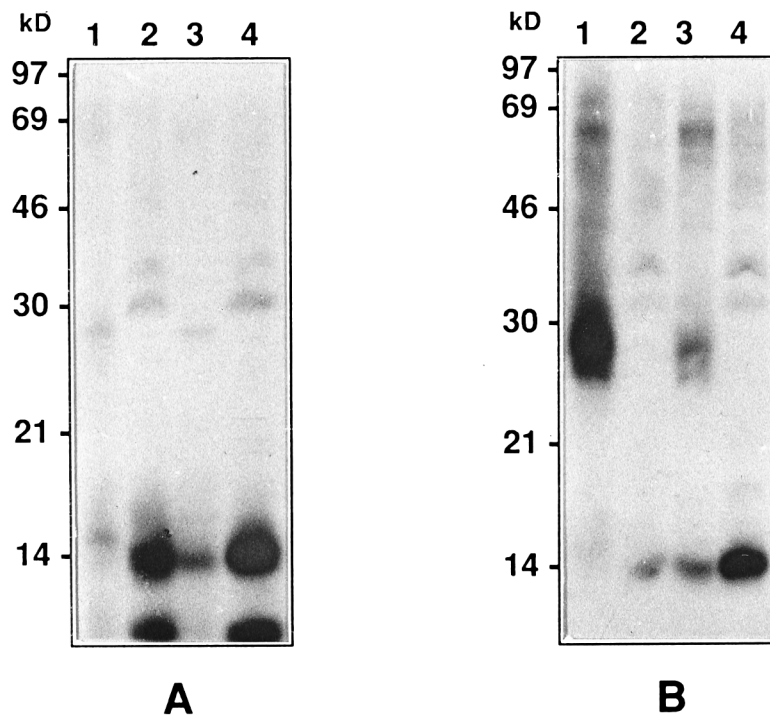


FIG. 2. Effect of reducing conditions on the pattern of IgE-binding components in mugwort and ragweed pollen extracts. Mugwort and ragweed pollen extracts were separated by an analytical SDS-PAGE under reducing and nonreducing conditions (*lane 1*: mugwort, nonreduced; *lane 2*: ragweed, nonreduced; *lane 3*: mugwort, reduced; *lane 4*: ragweed, reduced) and blotted onto nitrocellulose sheets. Identically prepared sheets were incubated with serum IgE from two different patients allergic to mugwort (**A** and **B**), and bound IgE was detected with ^{125}I -labeled anti-human IgE antibodies.

and two healthy volunteers as described by Horak et al.³² Individuals were challenged in a first session with isotonic sodium chloride solution and mugwort allergen extract in increasing concentrations (1 to 100 $\mu\text{g}/\text{ml}$). After 4 weeks, the same volunteers were challenged again first with sodium chloride and then with ragweed pollen extract.

RESULTS

IgE reactivity to mugwort pollen extract and ragweed pollen extract is significantly correlated

We correlated the mugwort RAST classes with those of ragweed, birch, cat, and mite allergens with Kendall's

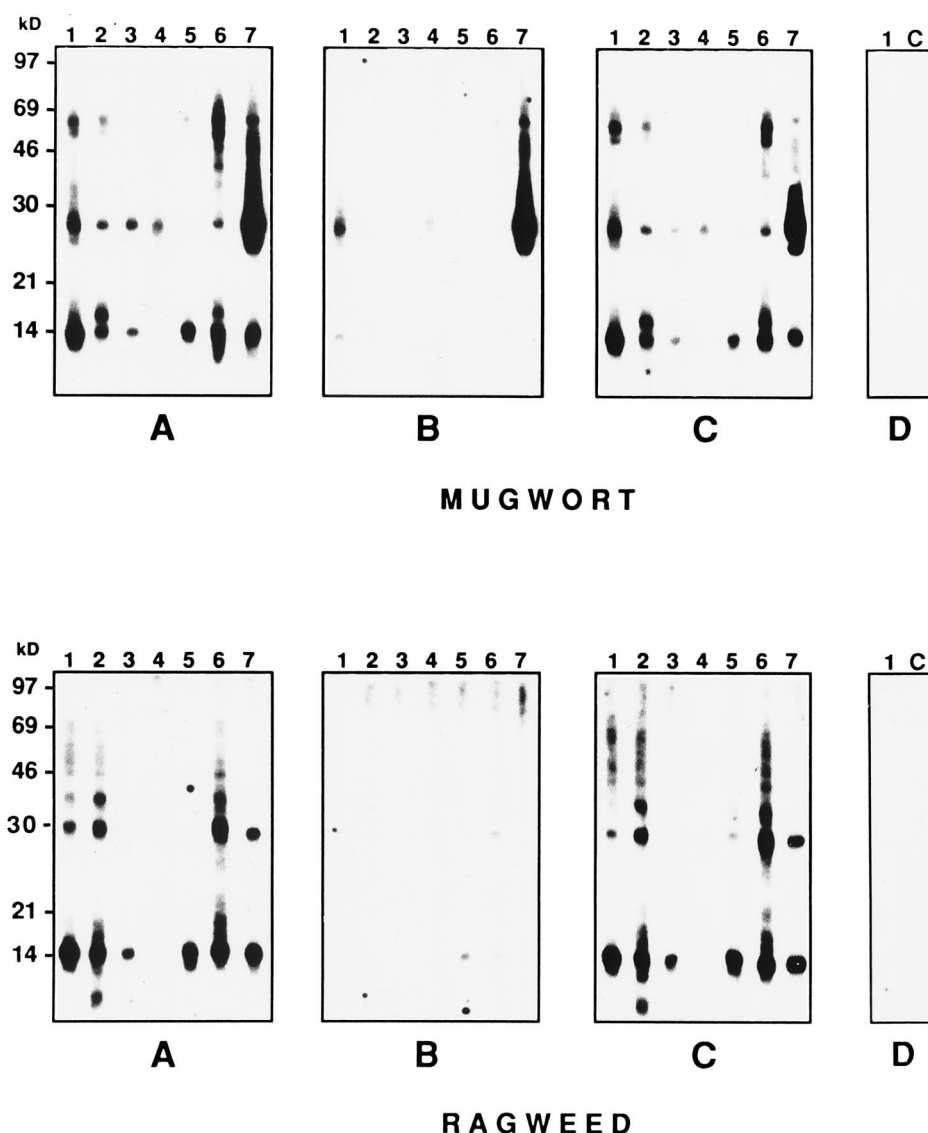


FIG. 3. IgE immunoblot inhibition experiment. Sera from seven patients allergic to mugwort (*lanes 1 through 7 in blocks A through C*) were probed with nitrocellulose-blotted mugwort pollen extract (*top*) and ragweed pollen extract (*bottom*). In *block D*, serum from a nonallergic individual (*lane 1*) and buffer without addition of serum (*lane C*) was used. Sera from patients allergic to mugwort were preincubated with ragweed pollen extract (*block B, top*), mugwort pollen extract (*block B, bottom*), and dog albumin (*block C, top and bottom*). Sera in *block A* (*top and bottom*) were not preadsorbed.

coefficient of rank correlation and Spearman's rank correlation coefficient with similar outcomes using sera from 82 patients allergic to mugwort with a mugwort RAST class of 2 or more. The mugwort RAST values were significantly correlated only with the ragweed results (significant correlation: $p < 0.001$), whereas no significant correlation was found with birch, cat, and mite results.

IgE antibodies from patients allergic to mugwort detect proteins of comparable molecular weight in mugwort and ragweed pollen extracts

Sera from 16 representative individuals allergic to mugwort were probed for IgE reactivity with nitrocellulose-

blotted mugwort and ragweed pollen allergens. Fig. 1 shows that allergens of comparable molecular weight are detected in a comparable intensity in both extracts. Thirteen of the sixteen mugwort-positive sera also displayed IgE reactivity to nitrocellulose-blotted ragweed pollen extract. Among the prominent IgE-binding bands, allergens of approximately 60 kD, 46 kD, and 28 to 30 kD were detected in mugwort and ragweed pollen extract by most of the sera. Fewer sera reacted with 20 kD, 14 kD, 10 kD, and less than 10 kD components. Certain sera bound also to allergens of different molecular weights in both extracts (e.g., serum 4 strongly reacted with proteins between 60 and 28 kD in ragweed, whereas binding was observed to a

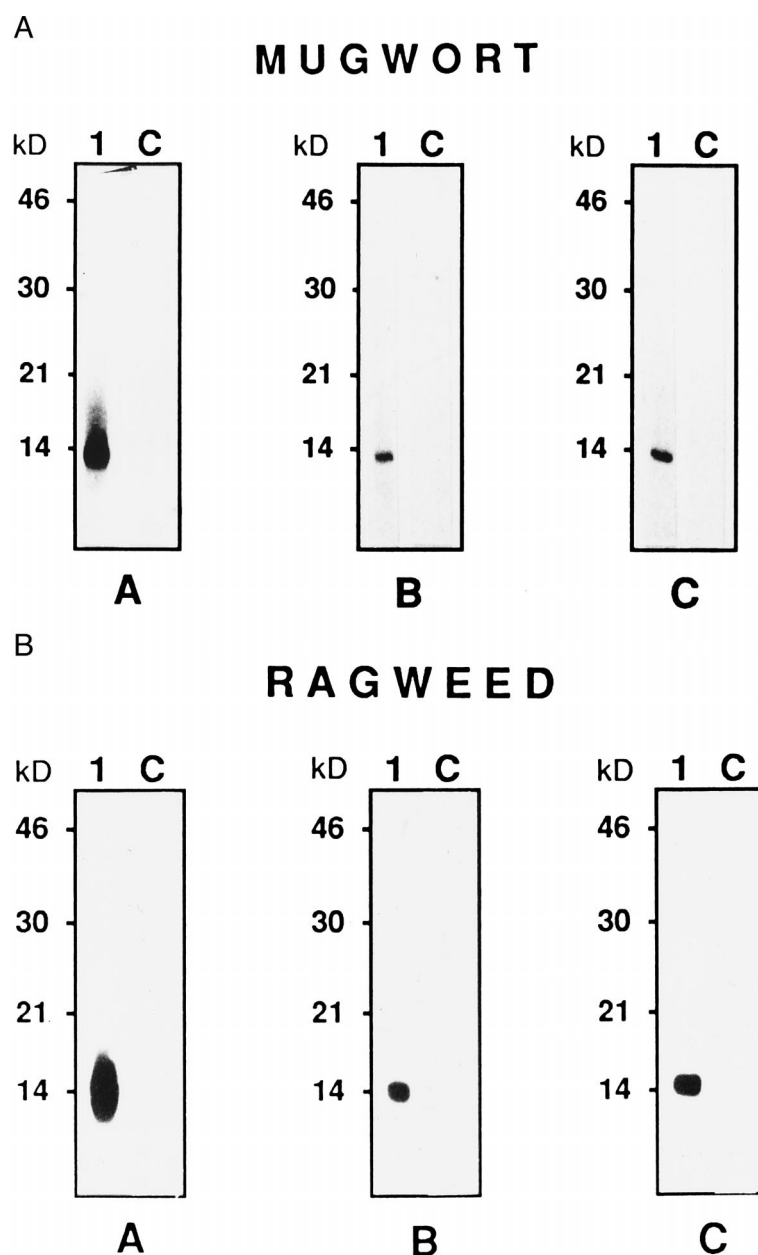


FIG. 4. Detection of profilin as a cross-reactive 14 kD allergen in mugwort and ragweed pollen extract. Nitrocellulose strips containing mugwort pollen extract (**A**) and ragweed pollen extract (**B**) were probed with three different rabbit anti-profilin antisera (**A**, **B**, and **C**) in lane 1. Lane **C** represents buffer control without addition of anti-serum.

28 kD band in mugwort) or failed to react with a comparable component (e.g., sera 1 and 6 strongly reacted with the 60 kD component in mugwort but failed to react with a similar protein in ragweed).

Major allergens from mugwort and ragweed are sensitive to reducing agents

The complementary DNA (cDNA) and deduced amino acid sequence of the major ragweed allergen Amb a 1 has been determined,³³ and a number of

homologous plant proteins and allergens have been meanwhile described.³⁴ Amb a 1 represents an acidic glycoprotein that can undergo cleavage into two chains (α and β) of approximately 26 and 12 kD, respectively. Limited data are available regarding the major mugwort allergens Art v 1 and Art v 2.³⁵⁻³⁷ Like Amb a 1, Art v 2 represents an acidic glycoprotein that is composed of two subunits linked by disulfide bridges. However, no sequence homology between these allergens has been reported so far.

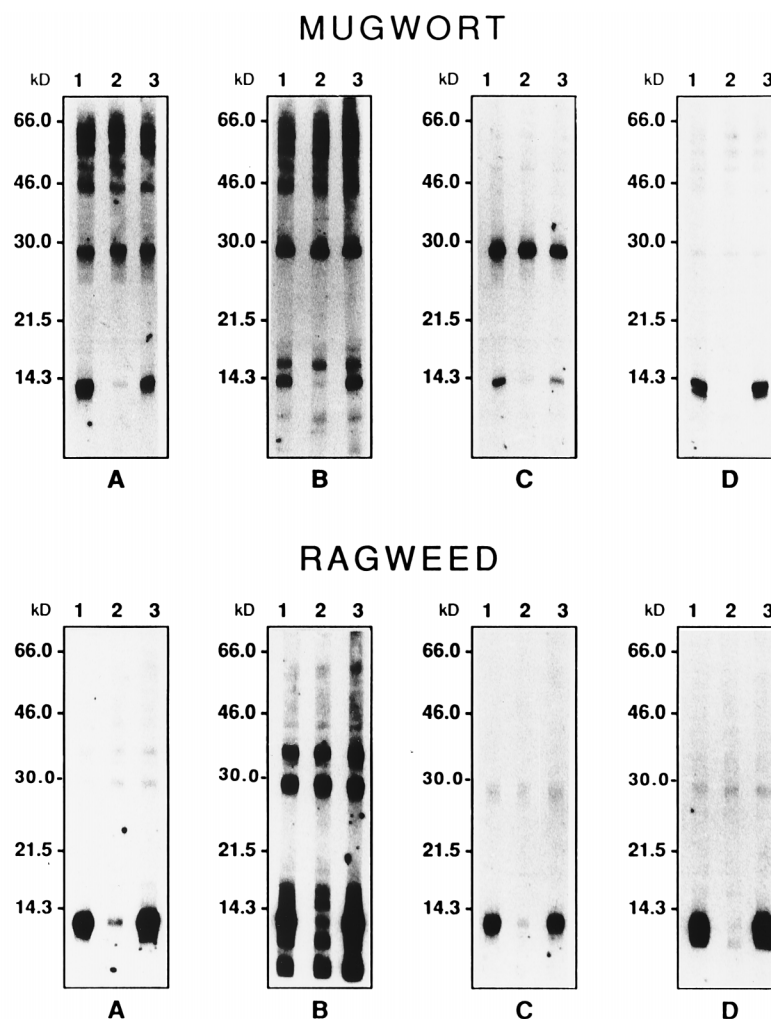


FIG. 5. Identification of profilin as a cross-reactive 14 kD allergen in mugwort and ragweed pollen extract by inhibition with recombinant birch profilin. Sera from four patients allergic to mugwort (*blocks A, B, C, and D*) were probed for IgE reactivity with nitrocellulose-blotted mugwort pollen extract (*top*) and ragweed pollen extract (*bottom*). In *lane 1* sera were not preadsorbed, in *lane 2* sera were preincubated with recombinant birch profilin (Bet v 2), and in *lane 3* sera were preincubated with recombinant Bet v 1. Bound IgE was detected with ¹²⁵I-labeled anti-human IgE antibodies.

In an attempt to find common biochemical properties of mugwort and ragweed allergens, we compared the influence of reducing agents on the IgE binding profile of two patients allergic to mugwort with nitrocellulose-blotted mugwort and ragweed pollen extracts (Fig. 2). Although the low molecular weight allergens (10 to 14 kD) in both extracts were not sensitive to reducing conditions (Fig. 2, *A*), a remarkable change in the IgE-binding pattern was observed when a serum reacting with the high molecular weight allergens (28 to 60 kD) was tested (Fig. 2, *B*). The addition of reducing agents (β -mercaptoethanol) led to a strong reduction of IgE binding to the high molecular weight allergens and increased binding in the low molecular weight range (10 to 14 kD), presumably caused by cleavage of the larger components.

Mugwort and ragweed pollen extract contain cross-reactive allergens of comparable molecular weights

The presence of cross-reactive allergens in mugwort and ragweed pollen extract was investigated by IgE immunoblot inhibition experiments (Fig. 3). Mugwort pollen extract almost completely inhibited IgE binding of seven sera to nitrocellulose-blotted ragweed pollen extract. Binding to the high molecular weight ragweed allergens (60 kD, 46 kD, and 28 kD), as well as to the low molecular weight components (14 kD, 10 kD, and <10 kD), was abolished. A similar degree of inhibition of IgE binding to mugwort allergens was observed after preincubation with ragweed extract. Only sera 1 and 7 showed binding to the high molecular weight mugwort allergens

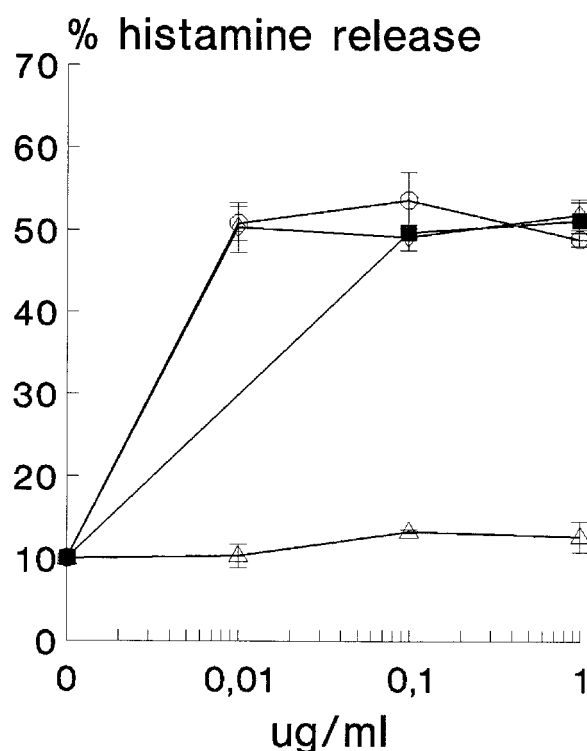


FIG. 6. Basophil histamine release. Granulocytes from an individual allergic to mugwort with profilin-specific IgE were incubated with increasing doses of mugwort pollen extract (open circles), ragweed pollen extract (open diamonds), recombinant birch profilin (open triangles), and anti-human IgE antibodies (filled squares). Percentage of histamine release shown on y-axis reflects means of triplicate determinations. Error bars are displayed.

after preincubation with ragweed pollen extract. Preincubation of the sera with a comparable amount of control protein (dog albumin: Fig. 3, block 3) did not significantly affect IgE binding to mugwort and ragweed pollen allergens.

Identification of profilin as one of the cross-reactive allergens in mugwort pollen and ragweed pollen

The results of the IgE immunoblot inhibition experiments identified the higher molecular weight (28 to 60 kd) mugwort and ragweed allergens (presumably Art v 1, Art v 2, Amb a 1, and Amb a 2) as cross-reactive components. Among the low molecular weight mugwort allergens, profilin was identified.⁹ Using three different rabbit antisera raised against different purified plant profilins, we could identify profilin at 14 kd as a cross-reactive component in mugwort (Fig. 4, A) and ragweed pollen extract (Fig. 4, B). By using purified recombinant birch profilin for IgE inhibition experiments, profilin could be identified as one of the cross-reactive allergens in mugwort and ragweed pollen extract (Fig. 5). Preincubation of four sera (Fig. 5, blocks A through D) from individuals allergic to mugwort pollen with recombinant birch profilin selectively inhibited IgE binding to profilin

in mugwort and ragweed pollen at 14 kd (Fig. 5, lanes 2). Although the major birch pollen allergen Bet v 1 shares IgE epitopes with a homologous allergen present in celery (Api g 1³⁸) and is known to be an important cross-reactive allergen in the birch-mugwort-celery syndrome, recombinant Bet v 1 did not inhibit IgE binding to any of the mugwort pollen allergens (Fig. 5, lanes 3).

Mugwort and ragweed pollen allergens share a high degree of cross-reacting IgE epitopes

To quantify the extent of IgE cross-reactivity between mugwort and ragweed pollen allergens, CAP inhibition experiments were performed. Five sera with a RAST class of 2 or more to mugwort and a RAST class of 3 or more to ragweed were preadsorbed with nondenatured mugwort or ragweed pollen extract before quantitative determination of mugwort- and ragweed-specific IgE. Mugwort pollen extract inhibited IgE binding to ragweed in all patients to a degree greater than 80%, and no less than 80% of IgE binding to mugwort was observed after preincubation with ragweed pollen extract. As in the immunoblot inhibition experiments, mugwort pollen allergens seemed to harbor more IgE epitopes than ragweed, which is most likely due to the fact that Austrian patients were initially sensitized against mugwort pollen allergens.

Mugwort and ragweed pollen extract induce comparable histamine release

The biologic significance of serum IgE antibodies cross-reacting with mugwort and ragweed pollen allergens other than profilin was evaluated by histamine release in a donor who was born in Vienna and had not left Austria except for occasional traveling. Granulocytes from this patient, who had no profilin-specific IgE, were incubated with increasing concentrations of mugwort and ragweed pollen extract, recombinant birch profilin, and anti-human IgE antibodies (positive control) (Fig. 6). Mugwort and ragweed pollen extract induced comparable dose-dependent histamine releases, whereas birch profilin was inactive. The histamine release experiments confirm the biologic significance of the cross-reactivity of serum IgE with the 28 kd and 10 kd components present in mugwort and ragweed pollen extracts. Neither mugwort nor ragweed pollen extract induced significant histamine release from basophils of a nonallergic individual, indicating a lack of toxicity of the protein preparations (data not shown).

Comparable reduction of nasal flow rate by mugwort and ragweed pollen extract

To further substantiate the clinical relevance of cross-reactivity of serum IgE with mugwort and ragweed pollen extract, nasal provocation experiments were done in volunteers allergic to mugwort pollen and nonallergic control subjects. Mugwort and ragweed pollen extracts were tested for their capacity to reduce total nasal flow by active anterior rhinomanometry. Two patients without profilin-specific IgE and two control subjects were

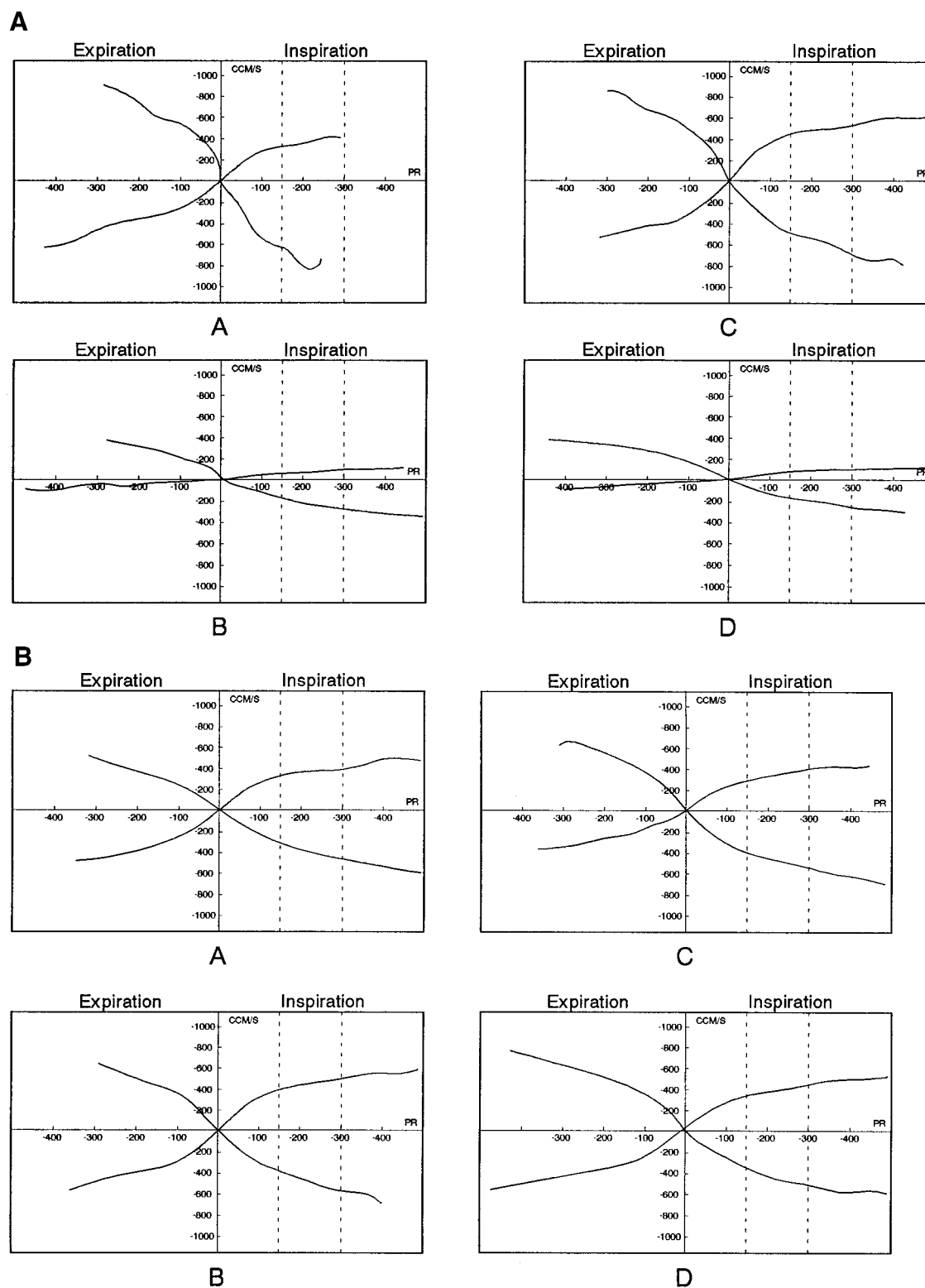


FIG. 7. Measurement of nasal air flow after allergen provocation. Nasal air flow in a volunteer allergic to mugwort pollen (**A**) and a nonallergic individual (**B**) was measured after application of isotonic sodium chloride solution (*blocks A and C*) and after application of mugwort pollen extract (*block B*) and ragweed pollen extract (*block D*). Nasal flow was recorded in cubic centimeters per second displayed on the y-axis at the pressure shown on the x-axis during expiration and inspiration. CCM/S, Cubic centimeters per second; PR, pressure in pascals.

challenged first with mugwort pollen extract and, after 4 weeks, with ragweed pollen extract. Fig. 7, *A* shows the effect of mugwort and ragweed pollen extract challenge in one of the patients. Mugwort and ragweed pollen induced significant nasal obstruction at a comparable dose (i.e., a protein concentration of 10 µg/ml). The extracts had no effect on nasal air flow in nonallergic volunteers as shown for one person in Fig. 7, *B*. These experiments support the contention that serum IgE, which cross-reacts with mugwort and ragweed, can cause clinical symptoms on contact with both allergen sources.

DISCUSSION

Weed pollens are important elicitors of type I allergy in late summer and fall. Ragweed represents one of the most prominent allergenic weeds in America, and the relevant ragweed allergens have been studied in great detail by immunochemistry³⁹⁻⁴² and cDNA cloning techniques.⁴³⁻⁵⁰ While ragweed occurs only rarely in Europe, mainly in the Rhone valley in France and Eastern Europe, mugwort represents a frequently encountered allergenic weed there. Despite the rare occurrence of ragweed in Europe and the minimal chance that patients can become sensitized, a surprisingly high prevalence of ragweed allergy, mostly associated with mugwort sensitization, has been reported. In this study we confirm the significant association of positive IgE reactivity to ragweed pollen allergens in a population of patients allergic to mugwort from Middle Europe (Austria) and demonstrate that this association is due to common allergenic structures in both weeds, which are recognized by cross-reactive IgE antibodies.

Analysis of a population of 82 Austrian patients allergic to mugwort showed that IgE antibody reactivity to mugwort and ragweed is highly correlated. IgE immunoblotting revealed that sera from patients allergic to mugwort bound to allergens of comparable molecular weights (60 kd, 28 to 46 kd, 20 kd, 14 kd, 10 kd, and <10 kd) in both mugwort and ragweed pollen extracts. Qualitative IgE immunoblot inhibitions identified profilin, a highly conserved 14 kd actin-binding protein as a cross-reactive allergen. Food allergens homologous to the major birch pollen allergen Bet v 1 (e.g., Api g 1,³⁸ the major celery allergen) were described as targets for cross-reactive IgE antibodies in birch-mugwort-celery syndrome.⁵¹ IgE inhibition experiments performed with recombinant Bet v 1 did not, however, provide any evidence for the presence of a Bet v 1-homologous allergen in mugwort or ragweed pollen. In addition to profilin, components of 60 kd and 28 to 46 kd (presumably representing the major mugwort allergens Art v 1 and Art v 2) were identified as cross-reactive allergens. Because of the close botanical relationship of mugwort and ragweed, it would not be too surprising if the major mugwort allergens Art v 1 and Art v 2 (60 kd and 28 to 46 kd, respectively) would represent proteins homologous to the major ragweed allergens Amb a 1 and Amb a 2. The Amb a 1 and Amb a 2 allergen family is indeed highly homologous to a number of other plant allergens, among them the major cypress allergens Cry j 1⁵² and

Cha o 1⁵³ and plant proteins from divergent species (maize⁵⁴ and tomato³⁴). There is also evidence that the major ragweed allergen family is immunologically related to group 4 grass pollen allergens.¹⁷

Although the expression of fully IgE-reactive recombinant Amb a 1 in heterologous expression systems (*E. coli*) has progressed, no functional recombinant ragweed allergens were available for IgE competition experiments. We could, however, show that the high molecular weight cross-reactive mugwort and ragweed allergens are both sensitive to reducing agents (i.e., 2-mercaptoethanol), a phenomenon described for Art v 2.³⁷

To further study the significance of the presence of cross-reactive structures in mugwort and ragweed pollen, quantitative inhibition experiments were done with nondenatured allergens used as competitors. The results of the CAP inhibitions confirmed the immunoblot experiments and, moreover, revealed a remarkably high degree of conserved IgE epitopes on mugwort and ragweed allergens. Preincubation of sera with either extract inhibited IgE binding by at least 80%, indicating that most epitopes are conserved between both weeds. It was interesting to note that according to the immunoblot inhibitions and the CAP competitions, mugwort allergens seemed to carry more IgE epitopes when compared with ragweed. Although it is possible that certain Austrian patients experienced contact with ragweed during traveling, this observation would rather fit the assumption that because of the relative absence of ragweed in Middle Europe, the Austrian patients were sensitized primarily against mugwort. IgE reactivity would then represent the consequence of the induction of cross-reactive IgE antibodies by mugwort or other more prevalent weeds.

The clinical relevance of IgE cross-reactivity could be substantiated by histamine release experiments and nasal provocation tests. It could be shown that mugwort and ragweed pollen extract induced comparable histamine releases and nasal obstructions in patients allergic to mugwort. We believe that the identification of cross-reactive structures in mugwort and ragweed might be useful for the following reasons. First, the demonstration of cross-reactive structures in different allergen sources may contribute to our understanding of clinically relevant cross-reactivity phenomena. Second, the identification of the most relevant cross-reactive structures will provide basic information for the production of these components as recombinant allergens for a more sophisticated and component-based diagnosis of type I allergy (i.e., allow for the establishment of the individual IgE reactivity profile, allergogram). Finally, perhaps it will allow for the development of candidate molecules for a component-based form of immunotherapy.

REFERENCES

1. Frenz DA, Palmer MA, Hokanson JM, Scamehorn RT. Seasonal characteristics of ragweed pollen dispersal in the United States. *Ann Allergy Asthma Immunol* 1995;75:417-22.
2. Boulet LP, Turcotte H, Laprise C, Lavertu C, Bedard PM, Lavoie A,

- et al. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy* 1997;27:52-9.
3. Treudler R, Kitay M, Rudolph R, Kunkel G. Association of ragweed allergy and mugwort sensitization in patients with pollinosis in Berlin. *Allergo J* 1995;5:267-71.
4. Charpin J, Surinyach R. Atlas of European allergenic pollens. London: Gower Medical Publishing; 1974.
5. Mezei G, Jaraine KM, Medzihradsky Z, Cserhati E. Seasonal allergic rhinitis and pollen count (a 5-year survey in Budapest). *Orv Hetil* 1995;136:1721-4.
6. Endre L. Increasing frequency of childhood asthma in Hungary and other countries, and the possible role of air pollution. *Int Rev Allergol Clin Immunol* 1996;2:74-9.
7. Dechamp C, Le Gal M, Deviller P. Prevalence of ragweed hayfever in the south and east of the greater Lyon region in 1993. *Allerg Immunol Paris* 1995;320:323-5.
8. Valenta R, Duchêne M, Pettenburger K, Sillaber C, Valent P, Bettelheim P, et al. Identification of profilin as a novel allergen: IgE autoreactivity in sensitized individuals. *Science* 1991;253:557-60.
9. Valenta R, Duchêne M, Ebner C, Valent P, Sillaber C, Deviller P, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377-85.
10. Vallier P, Dechamp C, Valenta R, Vial O, Deviller P. Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification as a profilin. *Clin Exp Allergy* 1992;22:774-82.
11. van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a crossreactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 1992;98:97-104.
12. Breiteneder H, Ferreira F, Reikerstorfer A, Duchêne M, Valenta R, Hoffmann-Sommergruber K, et al. cDNA cloning and expression in *Escherichia coli* of *Aln g I*, the major allergen in pollen of alder (*Alnus glutinosa*). *J Allergy Clin Immunol* 1992;90:909-17.
13. Larsen JN, Stroman P, Ipsen H. PCR based cloning and sequencing of isogenes encoding the tree pollen major allergen *Car b I* from *Carpinus betulus*, hornbeam. *Mol Immunol* 1992;29:703-11.
14. Breiteneder H, Ferreira F, Hoffmann-Sommergruber K, Ebner C, Breitenbach M, Kraft D, et al. Four recombinant isoforms of *Cor a I*, the major allergen of hazel pollen, show different IgE-binding properties. *Eur J Biochem* 1993;212:355-62.
15. Ebner C, Hirschwehr R, Bauer L, Breiteneder H, Valenta R, Ebner H, et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;95:962-9.
16. Valenta R, Kraft D. Type I allergic reactions to plant-derived food: a consequence of primary sensitization to pollen allergens. *J Allergy Clin Immunol* 1996;97:893-5.
17. Fischer S, Grote M, Fahlbusch B, Müller WD, Kraft D, Valenta R. Characterization of Phi p 4, a major timothy grass (*Phleum pratense*) pollen allergen. *J Allergy Clin Immunol* 1996;233:484-9.
18. Heiss S, Fischer S, Müller WD, Weber B, Hirschwehr R, Spitzauer S, et al. Identification of a 60 kD cross-reactive allergen in pollen and plant-derived food. *J Allergy Clin Immunol* 1996;98:938-47.
19. Breiteneder H, Pettenburger K, Bito A, Valenta R, Kraft D, Rumpold H, et al. The gene coding for the major birch pollen allergen *Bet v I*, is highly homologous to a pea disease response gene. *EMBO J* 1989;8:1935-8.
20. Valenta R, Duchêne M, Vrtala S, Birkner T, Ebner C, Hirschwehr R, et al. Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. *J Allergy Clin Immunol* 1991;88:889-94.
21. Valenta R, Vrtala S, Ebner C, Kraft D, Scheiner O. Diagnosis of grass pollen allergy with recombinant timothy grass (*Phleum pratense*) pollen allergens. *Int Arch Allergy Immunol* 1992;97:287-94.
22. Henrysson S. Elementarstatistik. Stockholm: Almqvist & Wiksell & Gebers; 1957.
23. Vrtala S, Grote M, Duchêne M, van Ree R, Kraft D, Scheiner O, et al. Properties of tree and grass pollen allergens; reinvestigation of the linkage between solubility and allergenicity. *Int Arch Allergy Immunol* 1993;102:160-9.
24. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. *Anal Biochem* 1976;72:248-4.
25. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
26. Susani M, Jertschin P, Dolecek C, Sperr WR, Valent P, Ebner C, et al. High level expression of birch pollen profilin (Bet v 2) in *Escherichia coli*: purification and characterization of the recombinant allergen. *Biochem Biophys Res Commun* 1995;215:250-63.
27. Valenta R, Ferreira F, Grote M, Swoboda I, Vrtala S, Duchêne M, et al. Profilins constitute a novel family of actin-binding proteins in higher plants. *J Biol Chem* 1993;268:22777-81.
28. Mittermann I, Swoboda I, Pierson E, Eller N, Kraft D, Valenta R, et al. Molecular cloning and characterization of profilin from tobacco (*Nicotiana tabacum*): increased profilin expression during pollen maturation. *Plant Mol Biol* 1995;27:137-46.
29. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979;76:4350-4.
30. Valent P, Besemer J, Muhm M, Majdic O, Lechner K, Bettelheim P. Interleukin 3 activates human blood basophils via high affinity binding sites. *Proc Natl Acad Sci USA* 1989;86:5542-6.
31. Valenta R, Sperr WR, Ferreira F, Valent P, Sillaber C, Tejkl M, et al. Induction of specific histamine release from basophils with purified natural and recombinant birch pollen allergens. *J Allergy Clin Immunol* 1993;91:88-97.
32. Horak F, Toth J, Jäger S, Berger U. Effects of H1-receptor antagonists on nasal obstruction in atopic patients. *Allergy* 1993;48:226-9.
33. Rafnar T, Griffith IJ, Kuo M, Bond JF, Rogers BL, Klapper DG. Cloning of Amb a 1 (antigen E), the major allergen family of short ragweed pollen. *J Biol Chem* 1991;266:12291-36.
34. Valenta R, Steinberger P, Duchêne M, Kraft D. Immunological and structural similarities among allergens: prerequisite for a specific and component-based therapy of allergy. *Immunol Cell Biol* 1996;74:187-94.
35. Nilsen BM, Smestad B. Isolation and characterization of a glycoprotein allergen, Art v II, from pollen of mugwort. *Mol Immunol* 1990;27:1047-56.
36. De La Hoz, Polo FF, Moscoso del Prado J, Selles J, Carreira J. Purification of Art v 1, a relevant allergen of *Artemisia vulgaris*. *Mol Immunol* 1990;27:651-7.
37. Nilsen BM, Sletten K, Smestad B, O'Neill M, van Halbeek H. Structural analysis of the glycoprotein allergen Art v II from the pollen of mugwort. *J Biol Chem* 1991;266:2660-8.
38. Breiteneder H, Hoffmann-Sommergruber K, O'Riordain G, Susani M, Ahorn H, et al. Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens. *Eur J Biochem* 1995;233:484-9.
39. King TP, Norman PS, Connell JT. Isolation and characterization of allergens from ragweed pollen. *Biochemistry* 1964;3:458-68.
40. Adolphson C, Goodfriend L, Gleich GJ. Reactivity of ragweed allergens with IgE antibodies. *J Allergy Clin Immunol* 1978;62:197-210.
41. King TP. Immunochemical studies of ragweed pollen allergens. *Allergy* 1980;35:187-8.
42. Klapper DG, Goodfriend L, Capra JD. Amino acid sequence of ragweed allergen Ra3. *Biochemistry* 1980;19:5729-34.
43. Ghosh B, Perry MP, Marsh DG. Cloning the cDNA encoding the Amb t V allergen from giant ragweed pollen. *Gene* 1991;101:231-8.
44. Griffith IG, Pollock J, Klapper DG, Rogers BL, Nault A. Sequence polymorphism of Amb a I and Amb a II, the major allergens in short ragweed. *Int Arch Allergy Appl Immunol* 1991;96:296-304.
45. Bond JF, Garman RD, Keating KM, Briner TJ, Rafnar TR, Klapper DG. Multiple Amb a I allergens demonstrate specific reactivity with IgE and T cells from ragweed-allergic patients. *J Immunol* 1991;146:3380-5.
46. Ghosh B, Perry MP, Marsh DG. Cloning the cDNA encoding the Amb t V allergen from giant ragweed (*Ambrosia trifida*) pollen. *Gene* 1991;101:2318.
47. Rafnar T, Ghosh B, Metzler WJ, Huang SK, Perry MP, Mueller L, et al. Expression and analysis of recombinant Amb a V and Amb t V

- allergens. Comparison with native proteins by immunological assays and NMR spectroscopy. *J Biol Chem* 1992;267:21119-23.
48. Ghosh B, Perry MP, Rafnar T, Marsh DG. Cloning and expression of immunologically active recombinant Amb a V allergen of short ragweed (*Ambrosia artemisiifolia*) pollen. *J Immunol* 1993;150:5391-9.
 49. Kuo MC, Zhu XJ, Koury R, Griffith IJ, Klapper DG, Bond JF, et al. Purification and immunochemical characterization of recombinant and native ragweed allergen Amb a II. *Mol Immunol* 1993;30:1077-87.
 50. Zhu X, Greenstein JL, Rogers BL, Kuo MC. T cell epitope mapping of ragweed pollen allergen *Ambrosia artemisiifolia* (Amb a 5) and *Ambrosia trifida* (Amb t 5) and the role of free sulfhydryl groups in T cell recognition. *J Immunol* 1995;155:5064-73.
 51. Wüthrich B, Dietschi R. Das Sellerie-Karotten-Beifuss-Gewürz-Syndrom: Hauttest- und RAST-Ergebnisse. *Schweiz Med Wochenschr* 1985;115:258-64.
 52. Sone T, Komiyama N, Shimizu K, Kusakabe T, Morikubo K, Kino K. Cloning and sequencing of cDNA coding for Cry j 1, a major allergen of Japanese cedar pollen. *Biochem Biophys Res Commun* 1994;199:619-28.
 53. Suzuki M, Komiyama N, Itoh M, Sone T, Kino K, Takagi I, et al. Purification, characterization and molecular cloning of Cha o 1, a major allergen of *Chamaecyparis obtusa* (Japanese cypress) pollen. *Mol Immunol* 1996;33:451-60.
 54. Turcich MP, Hamilton DA, Mascarenhas JP. Isolation and characterization of pollen-specific maize genes with sequence homology to ragweed allergens and pectate lyases. *Plant Mol Biol* 1993;23:1061-5.