

Role of carbohydrate moieties in cross-reactivity between different components of *Parietaria judaica* pollen extract

Mucci N, Liberatore P, Federico R, Forlani F, Di Felice G, Afferni C, Tighino R, De Cesare F, Pini C. Role of carbohydrate moieties in cross-reactivity between different components of *Parietaria judaica* pollen extract. Allergy 1992; 47: 424–430.

Cross-reactivity between the different components in *Parietaria judaica* pollen extract has been investigated by polyclonal as well as monoclonal antibodies before and after chemical deglycosylation obtained by trifluoromethanesulphonic acid (TFMS) treatment of the extract. In western blotting a polyclonal rabbit antiserum, obtained by injecting purified Par j I, was able to recognise many components of the native extract. However, its reactivity was restricted, after chemical deglycosylation of the extract, to the major allergen alone, indicating that its cross-reactivity was due to sugar moieties. Moreover, out of several monoclonal antibodies raised by injecting the whole *Parietaria judaica* extract, one (1A4/2F8) was also able in western blotting to recognise an epitope shared by many components of the extract except the major allergen Par j I. However, in this case the broad reactivity of the antibody was not affected by the deglycosylating procedure. When the reactivity of *Parietaria judaica* extract was tested before and after sugar removal, against specific IgE from a pool of patient sera, no differences could be demonstrated, thus indicating that carbohydrates are not strongly involved in the binding of *Parietaria judaica*-specific IgE. The results indicate that both proteic and carbohydrate cross-reactive epitopes are shared by many components of *Parietaria judaica* pollen extract.

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Key words: *Parietaria judaica*; carbohydrates; monoclonal antibodies; SDS-PAGE; immunoblotting; ELISA.

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Accepted for publication 7 November 1991

The lack of carefully standardised allergenic extracts represents one of the most critical points in the diagnosis and therapy of allergic diseases; for this purpose, many methods have been proposed for lowering the variability between various pollen extract preparations. They generally require the use of polyclonal or monoclonal antisera, raised by injecting the whole extract in animals, or obtained from highly sensitive allergic patients.

However, many of the immunological procedures proposed for the standardization of extracts can be influenced by the presence of several epitopes shared by many different allergens and cross-reacting at the IgE level. In many instances, such cross-reactivity has been detected between quite unrelated allergens, such as foods and plants (1, 5, 33) and grass and trees (21). However, cross-reactions have been more frequently reported between closely related pollens, namely between extracts obtained either from tree pollens, grass pollens or weed pollens (3, 9, 10, 18, 24, 28, 35, 36). Where a detailed investigation has

been carried out, 2 different types of epitopes have been identified as responsible for the cross-reactions, and they were either glycosidic or proteic in nature (1, 5). Besides cross-reactivities detectable between components in extracts obtained from different species, another type of cross-reaction has been described, which could influence many standardization approaches and is related to the presence of cross-reacting structures shared by several components within the extract (2, 7, 13, 16, 29, 36). However, no data are generally available on the chemical nature of these epitopes and their practical relevance. Therefore, despite the fact that individual allergens from many pollens have been purified and characterised, it is often unclear whether the relationships existing between the various components in the pollen extract from a single allergenic species are due to common determinants associated with the same proteic structure or to glycosidic moieties or to both.

The aim of the present study was to investigate the

presence of cross-reacting epitopes shared by various components present in pollen extract from *Parietaria judaica*, including the major allergen Par j I, and to evaluate their relevance in the interaction with either polyclonal or monoclonal animal antibodies as well as with human IgE from *Parietaria*-allergic subjects.

Material and methods

General protocol design

As indicated in the introduction, to evaluate the effect of sugar removal on the antigenicity of the various components of *Parietaria judaica* pollen extract, both untreated and chemically deglycosylated extracts were tested with either monoclonal or polyclonal reagents by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting and enzyme-linked immunosorbent assay (ELISA) inhibition.

Antisera

Anti-Par j I rabbit antiserum. To obtain enough highly purified Par j I to inject into rabbits, the procedure of Knudsen (22) was followed. Briefly, after preparative SDS-PAGE and blotting, the left and right margins of the nitrocellulose paper were cut out and either stained with Amido black or developed with a human specific IgE pool: the nitrocellulose portion, corresponding to the major allergen Par j I band, was cut out and dissolved in a small amount of DMSO and then emulsified with complete Freund's adjuvant (CFA). The final mixture was injected into the foot-pads of rabbit #482: this regimen was repeated 4 times. To ensure the highest purity of the recovered sample, the Par j I-containing band was cut leaving out a small portion of the band itself. Intermediate and final bleedings were tested by immunoblotting and enzyme-linked immunosorbent assay (ELISA).

Human allergic sera. Human sera were obtained from patients sensitive to *Parietaria judaica* only, as confirmed by skin test and radio-allergo-sorbent-test (RAST). Highly positive sera (RAST class 4) were pooled, aliquoted and stored at -30°C until used. The sera considered for the pool were all reactive with Par j I and with many other allergenic components, as evaluated by immunoblotting.

Monoclonal antibodies were prepared essentially according to the method of Geffer et al. (14). Mice were injected 3 times intraperitoneally with *Parietaria judaica* pollen extract (100 μg of protein for each injection) emulsified in CFA. Three days before the

fusion one mouse was boosted intravenously with the same amount of antigen in saline. After the fusion, cells were seeded in selecting medium and growing hybrids were tested by ELISA and immunoblotting. Hybrids of interest were cloned twice by limiting dilution and expanded *in vitro*. Supernatants were concentrated 10 times by precipitation at 50% ammonium sulphate saturation. Supernatant from clone 1A4/2F8 only was used throughout the experiments, while supernatant from clone 1A6/1D1, specific for the Par j I, together with an irrelevant monoclonal antibody, was occasionally used in immunoblotting as controls.

Parietaria judaica pollen extract was kindly supplied by Lofarma (Milano, Italy). The extract was prepared according to Giuliani et al. (17).

SDS-PAGE was performed according to Laemmli (23) in a vertical slab gel in a 13% acrylamide gel, by applying 100 μg protein/well. The run was carried out in a refrigerated system and the gel was stained with 0.05% Coomassie Brilliant Blue in water:methanol:acetic acid (50:40:10).

Immunoblotting was performed according to Towbin et al. (32) in a BIO-RAD apparatus (BIO-RAD, Richmond, CA). Reactive components were detected by incubation with the rabbit #482 antiserum (dilution 1:100, 2 h at room temperature), with monoclonal antibodies (10 times concentrated 1A4/2F8 or 1A6/1D1 supernatants, 2 h r.t.) and with the human IgE pool (diluted 1:2, overnight at r.t.). After the first incubation period, the bound antibody was detected by adding a peroxidase-labelled goat anti-rabbit antibody (KPL, Gaithersburg, MD) (1:500, 2 h r.t.), a peroxidase-labelled rabbit anti-mouse IgG + IgM (KPL) (1:500, 2 h r.t.) and a ^{125}I -goat anti-human IgE (Pharmacia, Uppsala, Sweden), respectively. The colorimetric reaction was carried out according to the BIO-RAD instructions by using 4-chloro-1-naphthol, while the IgE system was developed by exposing the blots to X-ray film for 3 days.

ELISA inhibitions were performed essentially according to the method of Yman et al. (38). PVC plates (Falcon 3912, Becton Dickinson, Oxnard, CA) were coated with the antigen for 3 h at 37°C and then left overnight at 4°C . For each well, 0.2 ml of 20 $\mu\text{g}/\text{ml}$ of *Parietaria judaica* pollen extract in 0.05 M carbonate buffer, pH 9.6, 0.02% NaN_3 , were added. Before using, the plates were blocked for 2 h at r.t. with 3% gelatin solution in PBS. Three different ELISA inhibitions, and therefore 3 slightly different sets of conditions were used, in relation to the antibody under investigation.

Rabbit #482 serum, prediluted 1:2000 on the basis of preliminary experiments, was pre-incubated 2 h at r.t. with 2-fold serial dilutions of the inhibitors, ranging from 5 µg protein/ml to 0.02 µg/ml; after this period, the mixture was transferred in the PVC plates and further incubated for a 2-h period: after washings, a peroxidase-labelled anti-rabbit IgG (KPL) was used at a dilution of 1:5000, and incubated 2 h at r.t. The plates were washed again and developed for 30 min by adding 0.2 ml of ortho-phenylenediamine solution (0.5 mg/ml in 0.1 M citrate/0.2 M phosphate buffer, pH 7.2, containing 0.01% hydrogen peroxide). The reaction was stopped by adding 50 µl 0.5 N sulphuric acid and the plates read at 495 nm in a Titertek Multiskan ELISA plate reader (Flow Laboratories, Milano, Italy).

The 10-times concentrated supernatant from clone 1A4/2F8 was pre-diluted 1:50 and incubated for 1 h at r.t. with 2-fold serial dilutions of inhibitors at a concentration ranging from 50 µg protein/ml down to 1.6 µg/ml. The mixtures were then transferred to the plates and further incubated for 1 h at r.t. After washings, the plates were incubated for 1 h at 37°C with a peroxidase-labelled rabbit anti-mouse IgM + IgG (KPL) (1:5000) and finally developed as reported above for the rabbit system.

Inhibition of specific IgE was carried out by incubating the human pool, diluted 1:5, for 3 h with 2-fold serial dilutions of inhibitors ranging from 5 µg protein/ml to 0.02 µg/ml. After this period, the mixture was transferred to the plates and further incubated for 3 h at r.t., followed, after washings, by an overnight incubation with a peroxidase-labelled rabbit IgG anti-human IgE (KPL) (1:2000). The plates were developed as above.

Data were expressed in terms of the protein concentration of each inhibitor producing 50% inhibition.

Sugar analysis was carried out essentially according to the method developed by Mopper (27). The samples were hydrolysed by heating 2 h at 120°C in 2N trifluoroacetic acid, reacted with dansyl-hydrazine and analysed by HPLC (Waters, Model 501 pump, Millipore) equipped with a reverse-phase column (Supelco LC-18). The analysis was carried out at room temperature and at a flow rate of 1.0 ml/min. Ten µl of sugar standards containing 10 µg of total sugar, and 10 µl of samples, containing about 30 µg of protein, were injected. The elution phase (acetonitrile plus 0.08 M acetic acid, 17:83) and the washing phase (acetonitrile plus 0.08 M acetic acid, 75:25) were used for 60 min and 30 min, respectively. The eluted derivatized sugars were detected at 253 nm.

Protein assay was performed according to Bradford (4).

Chemical deglycosylation of pollen extract was carried out by the method of Edge et al. (11). This method was preferred to the periodate treatment since many components contained in *Parietaria judaica* pollen extract are not soluble at pH 5.0 in acetate aqueous buffer, which is the optimum pH for the periodate treatment.

Results

We have demonstrated that several components of a *Parietaria judaica* whole extract, separated by analytical polyacrylamide gel electrophoresis, share common epitopes. When a polyclonal antiserum raised by injecting a rabbit with the purified major allergen (10 000 M_r) was analysed in western blotting, the antibodies produced were able to recognise a very broad range of components, from 10 000 M_r up to 100 000 M_r (Fig. 1, lane c). A similar result was obtained using the monoclonal antibody 1A4/2F8, which displayed a clear broad reactivity with several components except the major allergen (lane d). All the components recognised by the monoclonal antibody were proved to be allergens since they were reactive also with the human IgE, as demonstrated by comparing the immunoblotting developed with the clone 1A4/2F8 with that developed with human pool (lane b).

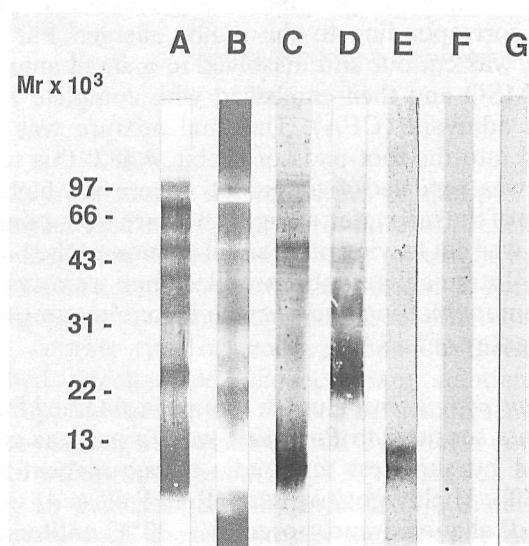


Fig. 1. SDS-PAGE and immunoblotting pattern of *Parietaria judaica* pollen extract. Lane a: Coomassie Blue staining; lane b: immunoblotting performed with pooled human sera from *Parietaria* allergic patients; lane c: immunoblotting performed with rabbit #482 antiserum; lane d: immunoblotting performed with mouse monoclonal antibody 1A4/2F8; lane e: immunoblotting performed with mouse monoclonal antibody 1A6/1D1; lane f: immunoblotting performed with prebleeding from rabbit #482; lane g: immunoblotting performed with an irrelevant monoclonal antibody.

The broad reactivity of the monoclonal and polyclonal reagent could be due either to the presence of common sugars or proteic structure on different components, or to different degrees of aggregation of each component yielding different final molecular weight. To test these hypotheses, the extract was first submitted to a deglycosylating procedure by TFMS, and the product analysed before and after the treatment (Fig. 2). Six sugars (galacturonic acid, galactose, glucose, mannose, arabinose and fucose) could be identified in the whole extract before the treatment, and they were no longer detectable after the deglycosylation procedure had been carried out. After this treatment, the extract was again analysed by SDS-PAGE and immunoblotting. The Coomassie staining of the deglycosylated product revealed only a decrease in the definition of some high molecular weight major bands and the disappearance of a few minor components (Fig. 3, lane a). In a similar way, the reactivity of the monoclonal antibody 1A4/2F8 against the deglycosylated extract did not show any significant pattern change (Fig. 3, lane c). On the contrary, the rabbit antiserum failed to detect the higher molecular weight cross-reactive components in the TFMS-treated extract but was still able to recognise the major allergen Par j I (Fig. 3, lane b).

The differences observed in the reactivity with the polyclonal and monoclonal antibodies between TFMS-treated and -untreated extract by using SDS-

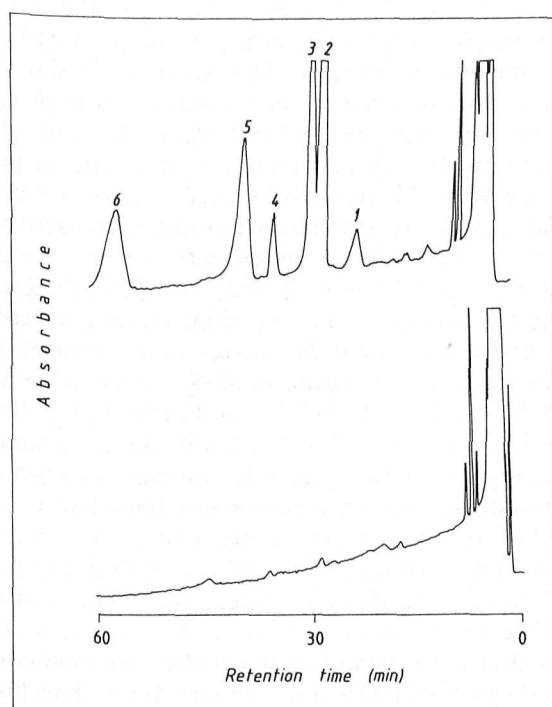


Fig. 2. HPLC analysis of the carbohydrate in the native (top) and deglycosylated (bottom) *Parietaria judaica* pollen extract. (1) galacturonic acid, (2) galactose, (3) glucose, (4) mannose, (5) arabinose, (6) fucose.

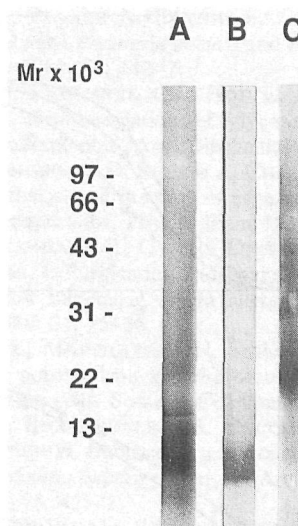


Fig. 3. SDS-PAGE and immunoblotting of *Parietaria judaica* pollen deglycosylated extract. Lane a: Coomassie staining of TFMS-treated extract; lane b: immunoblotting performed with rabbit #482 antiserum; lane c: immunoblotting performed with mouse monoclonal antibody 1A4/2F8.

PAGE and immunoblotting were clearly confirmed by ELISA inhibition experiments using freshly dissolved *Parietaria judaica* whole extract, TFMS-treated extract and mock control as inhibitors. The inhibition curves obtained confirmed that the cross-reactive epitopes recognised by the monoclonal antibody 1A4/2F8 are insensitive to the deglycosylating procedure since the amount of inhibitor producing 50% inhibition was 10 µg/ml for the freshly dissolved extract, 15 µg/ml for the deglycosylated one and 8 µg/ml for the mock control (Fig. 4a, top), while the ability to inhibit the binding of the rabbit antiserum was strongly reduced in the TFMS-treated sample (the inhibition never reaches 50%, compared with the mock control which gives this value at 0.22 µg/ml and with the native extract which gives this value at 0.35 µg/ml) (Fig. 4a, bottom).

The removal of sugar moieties from the allergenic extract does not interfere with the binding of human IgE, indicating that the epitopes recognised by human IgE are essentially TFMS-resistant (Fig. 4b). No relevant differences could be observed in the IgE-inhibition assay between the 3 inhibitors (0.19 µg/ml of native extract, 0.34 µg/ml of TFMS-treated extract and 0.25 µg/ml of mock control).

Discussion

This paper reports on the presence of common epitopes shared by several allergenic components in the *Parietaria judaica* pollen extract, and our data suggest that these epitopes are both proteic and glycidic in nature.

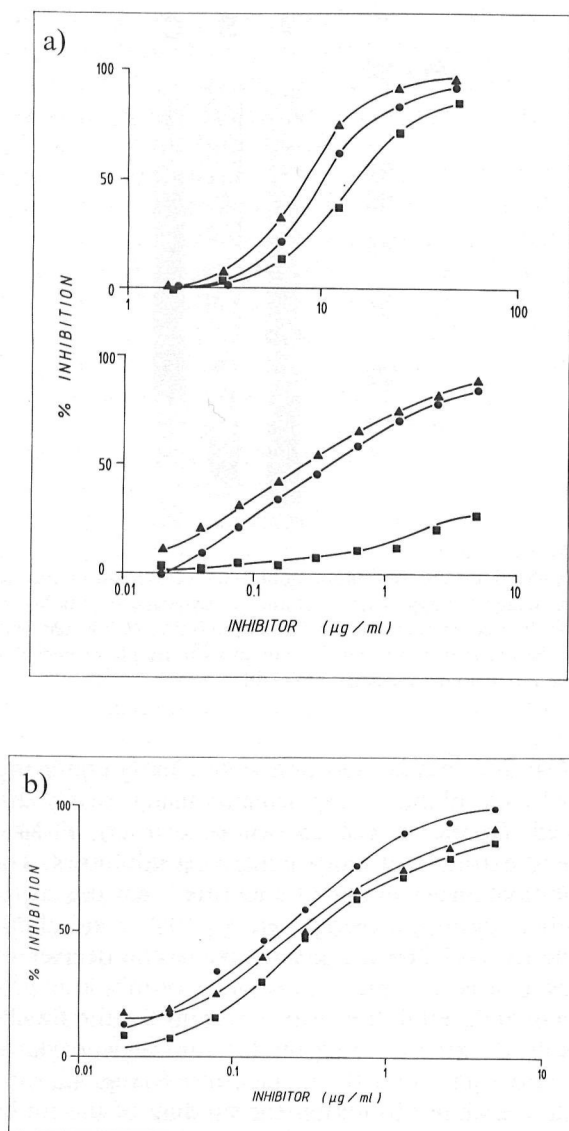


Fig. 4. ELISA Inhibition pattern of a) monoclonal antibody 1A4/2F8 (top) and rabbit #482 antiserum (bottom) and of b) pooled human sera from *Parietaria* allergic patients. The inhibitors used were: (●) *Parietaria judaica* native extract; (▲) mock control; (■) *Parietaria judaica* deglycosylated extract.

Removal by treatment with TFMS of the carbohydrate moieties produced a strong reduction in the number of components recognised by the rabbit antiserum obtained by injecting pure Par j I and displaying a high degree of reactivity with many extract components before TFMS treatment. This finding indicates that the major allergen of *Parietaria judaica*, Par j I, used to inject the rabbit, contains sugar moieties shared by many other components of the extract and able to induce specific antibodies. Carbohydrates were therefore investigated in the whole pollen extract, and found to be similar to those commonly found in plants (12); moreover a few of them have also been associated with very broad

cross-reactivities between different components of plant cell wall extracts obtained with polyclonal or monoclonal antibodies (26, 34).

The sugars in the pollen extract and shared by many components could be responsible, at least in part, for the high degree of heterogeneity detectable in *Parietaria judaica*. Although the presence of many glycoproteic components in allergenic extracts obtained from various pollens has been clearly demonstrated (19, 30), in many instances the role of the glycosidic moieties is still poorly understood from a biochemical and immunochemical point of view. However, when investigated, carbohydrates have been considered responsible for producing the high degree of heterogeneity detectable in extracts by immunoblotting with monoclonal antibodies (37), and also in Japanese cedar (31), where a family of isoallergens has been split into several structures which are identical in terms of sequence: the authors' interpretation was that the electrophoretic differences observed could be due to a different degree of glycosylation.

However, in the present study, TFMS-resistant cross-reacting epitopes shared by many components of the extract have been also detected, as demonstrated by the 1A4/2F8 monoclonal antibody reactivity before and after removing the carbohydrates. These results obtained with our monoclonal antibody confirm those of Corbi' et al. (7), who were able to purify by immunoaffinity procedures by using their monoclonal antibody specific for Par j I, 4 cross-reactive peptides sharing a common epitope with the major allergen. The authors (7) did not demonstrate whether the cross-reactive epitope recognised by their monoclonal antibody, and also present on the major allergen, was glycosidic or proteic in nature. Neither Corbi' et al. (7) nor our data could rule out the possibility that the heterogeneous pattern of reactivity observed with the monoclonal antibodies could merely be due to different degrees of aggregation of each component yielding different final molecular weight. However, on the basis of our results this would seem unlikely, since after the TFMS treatment (acidic conditions), the pattern with 1A4/2F8 monoclonal antibody did not change.

As regards the human IgE binding to *Parietaria judaica* pollen extract, we could not demonstrate any effect of the deglycosylation procedure, since no differences were observed in terms of inhibiting capacity between the deglycosylated, the mock control and the freshly dissolved whole extract. This suggests that the human IgE antibodies are mainly directed against TFMS-resistant structures. This finding is in agreement with the conclusions of similar studies carried out with allergenic extract from other sources and focussed on the interaction of human IgE antibodies with the carbohydrate structure

present in the extracts. Such conclusions indicate that IgE antibodies are generally directed against non-carbohydrate epitopes, since the procedures for removing sugars were ineffective in reducing the binding properties of various allergens to human IgE (5, 20, 30). However, in some cases, IgE antibodies reacting with periodate-sensitive and heat-stable structures have been identified (1, 5, 38) and these structures have been related to the high degree of cross-reactivity between a large number of unrelated allergens (foods and pollens).

Our results have interesting practical implications, particularly when immunochemical analysis and extract standardization are carried out with monoclonal and polyclonal reagents other than human IgE (6, 8, 15, 25). In these cases, unless the fine specificity of the immunological reagents employed has been clearly demonstrated, great care should be taken in the interpretation of data, taking into consideration that the monoclonal or polyclonal reagents could be able to interact with common glycosidic epitopes shared by several components in the extract.

Acknowledgements

We would like to thank Mr. Luigi Bernardini, Mr. Giorgio Rizzotto, Mr. Quinto Di Luzio and Mr. Nazareno Di Carlo for their technical assistance.

References

1. AALBERSE RC, KOSHTÉ V, CLEMENS JGJ. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and *Hymenoptera* venom. *J Allergy Clin Immunol* 1981; 68: 356–64.
2. ANSARI AA, KIHARA TK, MARSH DG. Immunochemical studies of *Lolium perenne* (rye grass) pollen allergens, Lol p I, II, and III. *J Immunol* 1987; 139: 4034–41.
3. BOUSQUET J, GUERIN B, HEWITT B, LIM S, MICHEL FB. Allergy in the Mediterranean area III: cross reactivity among *Oleaceae* pollens. *Clin Allergy* 1985; 15: 439–48.
4. BRADFORD MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248–54.
5. CALKHOVEN PG, AALBERS M, KOSHTÉ VL, POS O, OEI HD, AALBERSE RC. Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 1987; 42: 382–90.
6. CHAPMAN MD. Allergen specific monoclonal antibodies: new tools for the management of allergic disease. *Allergy* 1988; 43, Suppl. 5: 7–14.
7. CORBI' AL, AYUSO R, CARREIRA J. Identification of IgE binding polypeptides cross-reactive with the *Parietaria judaica* main allergenic polypeptide. *Molec Immunol* 1986; 23: 1357–63.
8. CORBI' AL, AYUSO R, LOMBARDERO M, DUFFORT O, CARREIRA J. A competitive solid-phase radioimmunoassay for quantitation of the major allergen of *Parietaria* pollen. *J Immunol Methods* 1985; 83: 83–8.
9. CORBI' AL, CORTES C, BOUSQUET J, et al. Allergenic cross-reactivity among pollens of *Urticaceae*. *Int Arch Allergy Appl Immunol* 1985; 77: 377–83.
10. CORBI' AL, PELAEZ A, ERRIGO E, CARREIRA J. Cross reactivity between *Parietaria judaica* and *Parietaria officinalis*. *Ann Allergy* 1985; 54: 142–7.
11. EDGE ASB, FALTYNEK CR, HOF L, REICHERT LE JR, WEBER P. Deglycosylation of glycoproteins by trifluoromethanesulfonic acid. *Anal Biochem* 1981; 118: 131–7.
12. FAYE L, JOHNSON KD, STURM A, CHRISPEELS MJ. Structure, biosynthesis, and function of asparagine-linked glycans on plant glycoproteins. *Physiol Plant* 1989; 75: 309–14.
13. FEO S, COCCHIARA R, GERACI D. Allergens of *Parietaria judaica* pollen. I. Purification and characterization of a hapten and a low molecular weight allergenic peptide. *Molec Immunol* 1984; 21: 25–36.
14. GEFTER ML, MARGULIES DH, SCHARFF MD. A simple method for polyethylene glycol-promoted hybridization of mouse myeloma cells. *Somatic Cell Genet* 1977; 3: 231–6.
15. GERACI D, BILLESBOLLE KB, COCCHIARA R, LOWENSTEIN H, IPSEN H. Immunochemical characterization of antigens of *Parietaria judaica* pollen. *Int Arch Allergy Appl Immunol* 1985; 78: 421–8.
16. GERACI D, ORESTE U, RUFFILLI A. Purification and characterization of allergens from *Parietaria officinalis* pollen. *Immunochimistry* 1978; 15: 491–8.
17. GIULIANI A, PINI C, BONINI S, MUCCI N, FERRONI L, VICARI G. Isolation and purification of a major allergen from *Parietaria officinalis* pollen. *Allergy* 1987; 42: 434–40.
18. GONZALEZ RM, CORTES C, CONDE J, et al. Cross-reactivity among five major pollen allergens. *Ann Allergy* 1987; 59: 149–54.
19. HAAVIK S, SMESTAD PAULSEN B, WOLD JK. Glycoprotein allergens in pollen of timothy. I. Investigation of carbohydrates extracted from pollen of timothy (*Phleum pratense*) and purification of a carbohydrate-containing allergen by affinity chromatography on Concanavalin A-Sepharose. *Int Arch Allergy Appl Immunol* 1985; 78: 197–205.
20. HAAVIK S, SMESTAD PAULSEN B, WOLD JK, GRIMMER O. Arabinogalactans in a purified allergen preparation from pollen of timothy (*Phleum pratense*). *Phytochemistry* 1982; 21: 1913–9.
21. HOWLETT BJ, HILL DJ, KNOX RB. Cross-reactivity between *Acacia* (wattle) and rye grass pollen allergens. *Clin Allergy* 1982; 12: 259–68.
22. KNUDSEN KA. Proteins transferred to nitrocellulose for use as immunogens. *Anal Biochem* 1985; 147: 285–8.
23. LAEMMLI UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680–5.
24. LOWENSTEIN H. Cross reactions among pollen antigens. *Allergy* 1980; 35: 198–200.
25. MAASCH HJ, HAUCK PR, OLIVER JD, GEISSLER W, WAHL R, WINTER H-G. Allergenic activity of short ragweed pollen (*Ambrosia elatior*) from different years and/or suppliers: criteria for the selection of an in-house allergen reference preparation. *Ann Allergy* 1987; 58: 429–34.
26. McMANUS MT, McKEATING J, SECHER DS, et al. Identification of a monoclonal antibody to abscission tissue that recognises xylose/fucose-containing N-linked oligosaccharides from higher plants. *Planta* 1988; 175: 506–12.
27. MOPPER K, JOHNSON L. Reversed-phase liquid chromatographic procedures and applications to natural samples. *J Chromatogr* 1983; 256: 27–38.
28. ØSTERBALLE O. Immunological partial identity between pollen allergens. *Allergy* 1985; 40, Suppl. 3: 67–9.
29. RUFFILLI A, ORESTE U, SANTONASTASO V, SCOTTO D'ABUSCO A, SACERDOTI G. Low molecular weight allergens of the pollen of *Parietaria officinalis*. *Molec Immunol* 1987; 24: 305–12.
30. SWARD-NORDMO M, SMESTAD PAULSEN B, WOLD JK. Immunological studies of the glycoprotein allergen Ag-54 (Cla

- h II) in *Cladosporium herbarum* with special attention to the carbohydrate and protein moieties. *Int Arch Allergy Appl Immunol* 1989; 90: 155-61.
31. TANIAI M, ANDO S, USUI M, et al. N-terminal amino acid sequence of a major allergen of Japanese cedar pollen (Cry j I). *FEBS Letters* 1988; 239: 329-32.
 32. TOWBIN H, STAELIN T, GORDON J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci* 1979; 76: 4350-4.
 33. VALLIER P, DECHAMP C, VIAL O, DEVILLER P. A study of allergens in celery with cross-sensitivity to mugwort and birch pollens. *Clin Allergy* 1988; 18: 491-500.
 34. VOIGT J. The lithium-chloride-soluble cell wall layers of *Chlamydomonas reinhardtii* contain several immunologically related glycoproteins. *Planta* 1988; 173: 373-84.
 35. WEBER RW. Cross-reactivity among pollens. *Ann Allergy* 1981; 46: 208-15.
 36. WEBER RW, NELSON HS. Pollen allergens and their inter-relationships. *Clin Rev Allergy* 1985; 3: 291-318.
 37. WESTPHAL W, BECKER W-M, SCHLAACK M. Analysis of rye pollen (*Secale cereale*) allergens using patients' IgE, immunoprint, western blot and monoclonal antibodies. *Int Arch Allergy Appl Immunol* 1988; 86: 69-75.
 38. WOLDEN B, SMESTAD PAULSEN B, WOLD JK, GRIMMER O. Characterization of the carbohydrate moiety in a purified allergen preparation from the mite *Dermatophagoides farinae* and its importance for allergenic activity as tested by the RAST-inhibition method. *Int Arch Allergy Appl Immunol* 1982; 68: 144-51.
 39. YMAN L, PONTERIUS G, BRANDT R. RAST-based allergen assay methods. *Dev Biol Stand* 1975; 29: 151-65.

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