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Allergenic relationship between taxonomically diverse pollens

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Summary

Background Skin tests and tests for IgE antibodies show that subjects are usually sensitive to a number of different pollens, frequently from taxonomically diverse species which are assumed to be allergenically non-crossreactive. This suggests that the presence of IgE antibody-reactivity to an individual pollen may not necessarily have resulted from contact with that pollen or even with a taxonomically closely related species.

Objective Since this has important consequences for allergen avoidance and desensitization of patients, we attempted to define allergenic relationships between diverse pollen species.

Methods Sera from subjects were examined in direct IgE antibody binding experiments and by quantitative inhibition, protein blotting and adsorption and elution studies.

Results Sera from subjects diagnosed as allergic to white cypress pine, Italian cypress, ryegrass or birch pollen were shown to have IgE antibodies that reacted with pollens from these four species and from cocksfoot, couch grass, lamb's quarter, wall pellitory, olive, plantain and ragweed. These reactions were confirmed in protein blotting and adsorption and elution studies where numerous IgE-binding bands were detected in all 11 different pollen extracts with sera from each of the different allergic categories. Further evidence of allergenic (i.e. IgE-binding crossreactivity between the different pollens was provided by inhibition studies in which clear-cut inhibitions of IgE binding to the different pollen allergen discs were obtained with comparable amounts of the different pollen extracts.

Conclusion We conclude that the presence of pollen reactive IgE antibodies may not necessarily be a true reflection of sensitizing pollen species.

Keywords: pollens, allergens, cross reactivity, pollen allergy, protein blotting

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Introduction

The allergenic role of pollens in allergic rhinitis is well known and pollens also appear to be a major cause of chronic bronchial hyperreactivity. Clinically, it is often observed that patients with pollen asthma develop what appears to be 'non-specific' hyperreactivity during the pollen season [1]. It should be remembered, however, that allergens responsible for provoking hypersensitivity may not always be recongized by patients and their clinicians as a provoking factor because doses inhaled were too low to produce an acute reaction [2,3].

Although the primary treatment for asthma and allergic rhinitis today is pharmacological [4], allergen avoidance should always be attempted and desensitization with the

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appropriate allegen(s) may be considered in severe cases. Both of these measures, of course, require a knowledge of the sensitizing agents and with pollens this may be more difficult than it first seems. Although allergenic crossreactivity between pollens is well recognized [5], such relationships are generally thought to occur between taxonomically closely-related species [6,7]. Skin and serum tests, however, reveal that pollen-allergic subjects are rarely sensitive only to a single species—sensitivity to a number of different pollens from grasses, weeds and trees is frequently seen even when some of the species have not been encountered [8,10]. Since this has important implications for allergen avoidance and desensitization of pollen-allergic patients, we studied the allergenic relationship between diverse pollen species in an attempt to define the extent and, ultimately, the basis of cross-reactions and to aid clinical management of subjects.

Materials and methods

Pollen extracts

Callitris glaucophylla, *Cupressus sempervirens*, *Lolium perenne*, *Dactylis glomerata*, *Cynodon dactylon*, *Chenopodium album*, *Parietaria judaica*, *Betula pendula*, *Olea europea*, *Plantago lanceolata* and *Ambrosia elatior* pollens were obtained and extracted as previously described [8–14]. The protein content of the dialysed extracts was determined with the Pierce BCA (Bicinchoninic acid) Protein Assay Reagent [15]. Lyophilized extracts were stored at −20°C.

Sera

Sera from subjects initially clinically diagnosed on the basis of patient histories and skin-prick tests (positive test $\geqslant$ 3 mm weal) as allergic to *C. glaucophylla*, *C. sempervirens*, *L. perenne* or *B. pendula* pollens were collected in Dubbo and Dunedoo, New South Wales, Australia (*C. glaucophylla*), Provence, France (*C. sempervirens*) and Canberra and Sydney, Australia (*L. perenne* and *B. pendula*). *C. glaucophylla* skin-prick test solution was as described [16]. Other skin-prick test solutions (1 : 20 w/v) were from Miles Inc, Ekhart IN, USA. Control sera were obtained at Royal North Shore Hospital (RNSH), St Leonards, Australia from subjects allergic to food or house dust mites (*Dermatophagoides pteronyssinus*) but not to pollens, from non-allergic subjects with high total serum IgE levels and normal non-allergic laboratory workers. Cord serum was from the Department of Obstetrics, RNSH and used as an IgE-'free' control. Tests for IgE antibodies to all four pollens were all carried out side-by-side in the laboratory at RNSH using the materials and methods described [9].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransfer to nitrocellulose

SDS-PAGE [17] was carried out as previously described [18]. Separated pollen proteins in polyacrylamide gels were electrotransferred to nictrocellulose as previously described [11,19].

Probing of nitrocellulose membrane strips and autoradiography

Materials and methods for identification of IgE-binding proteins by autoradiography were as previously described [20], except that prior to probing with sera, strips in cases of *C. glaucophylla* and *C. sempervirens* were fixed with 0.5% glutaraldehyde (1 : 50 of 25% solution) in phosphate buffered saline (PBS) for 15 min, followed by a 30 s wash with PBS, and then incubation in 1% ethanolamine in PBS for 1 h. Detection of IgE-binding proteins was achieved by the addition of ^{125}I-anti-human-IgE (Bioclone, Australia).

Adsorption and elution experiments

A 5 cm diameter cyanogen bromide (CNBr)-activated paper disc was soaked overnight with agitation at room temperature in a solution (2 mg protein/mL) of *C. glaucophylla*, *C. sempervirens*, *L. perenne* or *B. pendula* pollen extract in 0·1 M $NaHCO_3$ freshly made. The disc was washed with 0·1 M $NaHCO_3$, blocked with 0·1 M ethanolamine in 0·1 M $NaHCO_3$ pH 9·0 for 5 h and followed by washing with 0·1 M acetate buffer pH4 and then with PBS. The disc was incubated overnight with rocking at room temperature with well characterized serum from an allergic individual (diluted 1 : 10 with 0·5% of BSA in 0·005% Tween-20, 0·1% sodium azide in PBS). The eluted acidic solution containing IgE antibodies was neutralized with 3 M Tris base to pH 7·5. Eluted IgE antibodies were used to probe western blot strips of whole pollen extracts, followed by ^{125}I-anti-human IgE and autoradiography.

Results

Detection binding of pollen-reactive IgE antibodies

Direct binding radioimmunoassays (RIAs)

Sera from subjects clinically diagnosed in the first

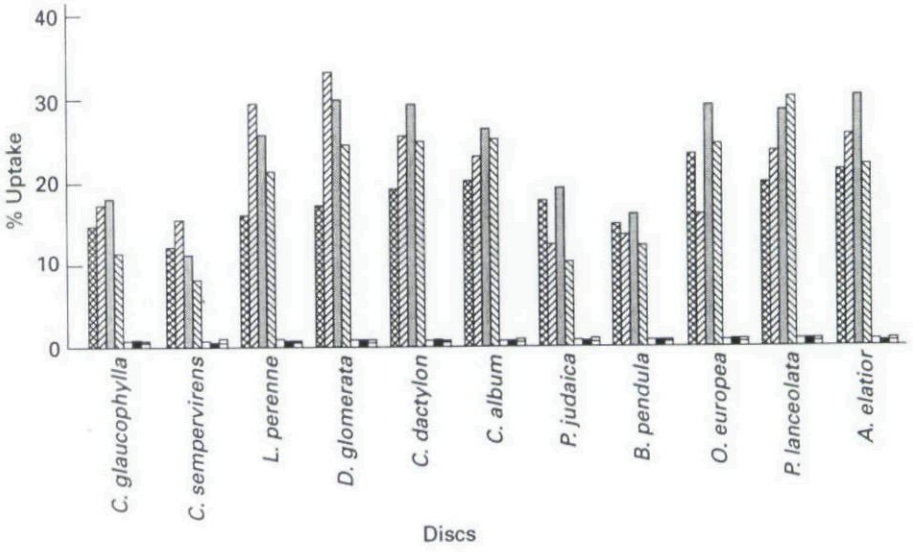

Fig. 1. Direct binding of serum IgE antibodies to pollen proteins from 11 different species including grasses, weeds and trees. Sera from subjects allergic to (■) *C. glaucophylla* (serum 2593), (▨) *C. sempervirens* (serum CY13), (▤) *L. perenne* (serum 2196), (▥) *B. pendula* (serum 2020), and from (□) cord blood (IgE-'free' control), (■) a normal non-allergic subject and (▤) serum with a high total IgE level but no IgE to pollens, were incubated with nitrocellulose discs coupled to pollen proteins from the species shown. Reactions were measured by recording the uptakes of ^{125}I-anti-IgE after the addition of ^{125}I-anti-human IgE as a second antibody.

instance as allergic to white cypress pine, Italian cypress, ryegrass or birch pollen, were shown to have serum IgE antibodies that reacted with allergen discs prepared from extracts of pollens from white cypress pine (*C. glaucophylla*), Italian cypress (*C. sempervirens*), ryegrass (*L. perenne*), cocksfoot (*D. glomerata*), couch grass (*C. dactylon*), lamb's quarter (*C. album*), wall pellitory (*P. judaica*), birch (*B. pendula*), olive (*O. europea*), plantain (*P. lanceolata*) and ragweed (*A. elatior*). Figure 1 shows typical results obtained when a serum from each of the four different diagnosed groups was used with the above pollen protein solid phases. Specific uptakes of ^{125}I-anti-IgE in the range ~8–33% were recorded for the test sera with each of the different pollen discs while control sera consisting of cord serum, sera from 'normal', non-allergic subjects and sera from subjects with high total IgE levels but who were not allergic to pollens, produced uptakes of only 0·9–1·5%.

RIA inhibition studies

Further evidence of allergenic (i.e. IgE-binding) crossreactivity between the different pollens was provided by inhibition studies with diluted sera in which clear-cut inhibitions of IgE-binding to the different pollen allergen discs were obtained with comparable amounts of the different pollen extracts. Table 1, for example, shows the per cent inhibitions obtained when 50 μg of the 11 different pollen protein extracts were used with sera from subjects allergic to *C. glaucophylla*, *C. sempervirens*, *L. perenne* or *B. pendula* and discs prepared

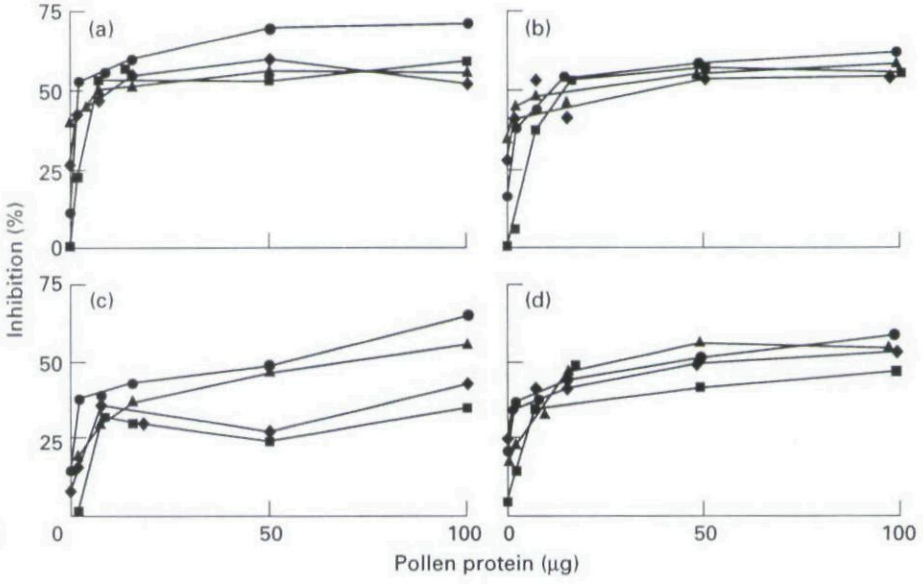

Fig. 2. Quantitative inhibition curves obtained with serum (from subject 2593) allergic to *C. glaucophylla* pollen using nitrocellulose discs coupled to (a) *C. glaucophylla*, (b) *C. sempervirens*, (c) *L. perenne* and (d) *B. pendula* pollen proteins. Uptakes of IgE antibodies by the discs (measured as uptakes of the second antibody ^{125}I-anti-human IgE) were inhibited by preincubation of the diluted serum with (●) *C. glaucophylla*, (■) *C. sempervirens*, (▲) *L. perenne* and (◆) *B. pendula* pollen proteins.

Table 1. Pollen crossreactivity spectra of sera from subjects allergic to *C. glaucophyllia, C. sempervirens. L. perenne* or *B. pendula* pollen. RAST inhibition studies with pollens from grasses, weeds and trees

	Inhibition (%) of IgE binding to *C. glaucophylla, C. sempervirens, L. Perenne* and *B. pendula* pollen protein-solid phases** with 50 μg of pollen proteins and sera from subjects:															
	(1) 2593				(2) CY13				(3) 2196				(4) 2020			
Pollen protein extract from	*C. glaucophylla* discs	*C. sempervirens* discs	*L. perenne* discs	*B. pendula* discs	*C. glaucophylla* discs	*C. sempervirens* discs	*L. perenne* discs	*B. pendula* discs	*C. glaucophylla* discs	*C. sempervirens* discs	*L. perenne* discs	*B. pendula* discs	*C. glaucophylla* discs	*C. sempervirens* discs	*L. perenne* discs	*B. pendula* discs
C. glaucophylla	70	61	48	52	24	59	44	61	48	60	41	38	47	33	21	41
C. sempervirens	54	59	24	43	32	29	36	46	51	60	37	47	57	38	23	36
L. perenne	56	57	47	57	34	28	64	49	62	60	45	44	35	43	64	46
D. glomerata	50	67	69	71	38	19	60	44	66	70	71	46	0	9	54	0
C. dactylon	59	63	46	54	29	54	42	59	61	65	54	53	58	50	45	51
C. album	63	59	36	54	14	23	40	52	49	56	53	33	53	39	27	35
P. judaica	55	48	36	50	19	25	40	50	53	59	50	28	52	47	33	44
B. pendula	61	56	26	51	26	47	37	50	63	74	48	59	57	43	29	50
O. europea	60	56	36	42	46	29	38	45	63	71	47	48	55	34	34	41
P. lanceolata	48	44	53	48	33	30	56	52	48	73	45	39	34	30	43	31
A. elatior	26	56	51	53	10	27	23	10	30	45	49	37	45	57	49	45

* Numbers in the columns are the percentage inhibition values obtained in the uptakes by the allergen discs of a ^{125}I-anti-human IgE and hence in the uptakes of IgE antibodies in the subjects' sera.

** Nitrocellulose 6 mm discs activated with mercapto ethanol (5%)-treated pollen proteins, 20 μg protein/disc.

(1) Serum used at a dilution of 1–6 with all discs.

(2) Serum used at a dilution of 1–4 with *C. glaucophylla* and *C. sempervirens*, 1–5 with *B. pendula* and 1–20 with *L. perenne* discs.

(3) Serum used at a dilution of 1–2 with *C. glaucophylla* and *C. sempervirens*, 1–4 with *B. pendula* and 1–40 with *L. perenne* discs.

(4) Serum used at a dilution of 1–3 with *C. sempervirens*, 1–4 with *B. pendula*, 1–8 with *C. glaucophylla* and 1–25 with *L. perenne* discs.

from extracts of these four pollens. Figure 2 shows the detailed results obtained with one of the sera, serum 2593 from a subject allergic to *C. glaucophylla* pollen. Clear, dose-dependent inhibitions were obtained with the four different pollen proteins in the range (1–100 μg) tested.

Protein blotting

Direct binding IgE antibody reactions detected in RIA studies were confirmed in direct blotting experiments where numerous IgE-binding bands were detected in all 11 different pollen extracts with sera from each of the four different pollen-allergic categories (Fig. 3). The band pattern seen with a particular pollen was almost identical regardless of the category of serum used, for example, the reactive band patterns seen on *O. europea* blots (Fig. 3I) were the same with sera from subjects allergic to *C. glaucophylla, C. sempervirens, L. perenne* or *B. pendula*. No bands were detected on blots when sera from allergic subjects, but not to pollens, were used to probe strips or when the sera from the pollen-allergic subjects were used to probe nitrocellulose transfers of house dust mite (*D. pteronyssinus*) or *Candida albicans* proteins (Fig. 3).

Adsorption and elution experiments Using the experimental strategy summarized diagrammatically in Fig. 4, findings were consistent with results of direct binding to protein blots (see Fig. 3). Banding patterns, even though fainter and with some bands only just visible or perhaps even absent, were consistent with the characteristic protein 'fingerprints' of the different pollen species were seen (Fig. 5). Again, no bands were seen with the non-pollen proteins examined.

Discussion

Inconsistent with the belief that pollen crossreactions are

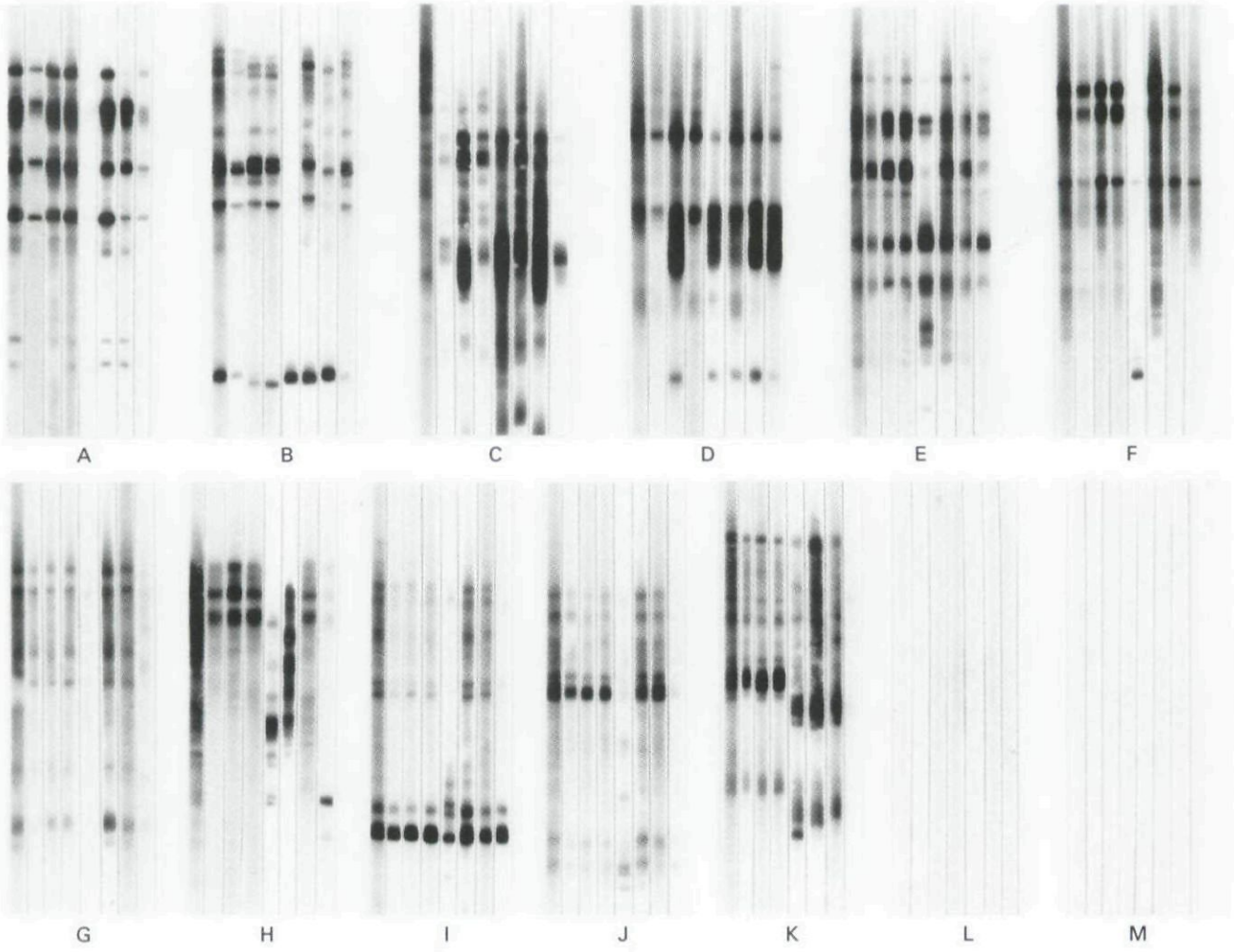

Fig. 3. Direct protein blots showing similar IgE-binding patterns for each pollen protein extract examined. For each set of eight strips: strips 1 and 2: sera from subjects 2593 and 2600 allergic to *C. glaucophylla* pollen; strips 3 and 4: from subjects CY13 and CY45 allergic to *C. sempervirens* pollen; strips 5 and 6: from subjects 2036 and 2196 allergic to *L. perenne* pollen; strips 7 and 8: from subjects 2020 and 2464 allergic to *B. pendula* pollen. Sera were used to probe nitrocellulose strips with transferred proteins from: A. *C. glaucophylla*, B. *C. sempervirens*, C. *L. perenne*, D. *D. glomerata*, E. *C. dactylon*, F. *C. album*, G. *P. judaica*, H. *B. pendula*, I. *O. europea*, J. *P. lanceolata*, K. *A. elatior* pollens. Strips L amd M: house dust mite (*D. pteronyssinus*) and *C. albicans* proteins respectively.

restricted to taxonomically closely related species [6,7] are our findings with serum IgE antibodies and skin-prick tests suggesting that pollen allergenic crossreactivity is much more wide-ranging, encompassing species as diverse as monocots such as ryegrass, 'weeds' like plantain and ragweed, and trees such as birch, olive and cypress. We find that subjects showing both clinical sensitivity and serum IgE antibodies to only one species of pollen are rare. More often, positive skin-prick tests and IgE antibodies are found to a variety of apparently unrelated pollen species even in cases where a single sensitizing pollen species could be implicated and where other skin-test- and RAST-positive species had apparently never been encountered by the patient [8–10].

Results of direct binding RIAs, RIA inhibition, protein blotting, and adsorption and elution studies described here all suggested that the IgE antibodies to one pollen species (white cypress pine, Italian cypress, ryegrass or birch) also reacted with pollen proteins from the other 10 species tested. Many of the crossreactions cannot be explained on the basis of taxonomic relatedness and separate sensitization was either not involved or extremely unlikely. Results from another recent study [10] on subjects allergic to indigenous white cypress pine pollen in central New South Wales, Australia further support this conclusion.

From a detailed analysis of IgE reactivity patterns of birch pollen-allergic subjects from Norway and

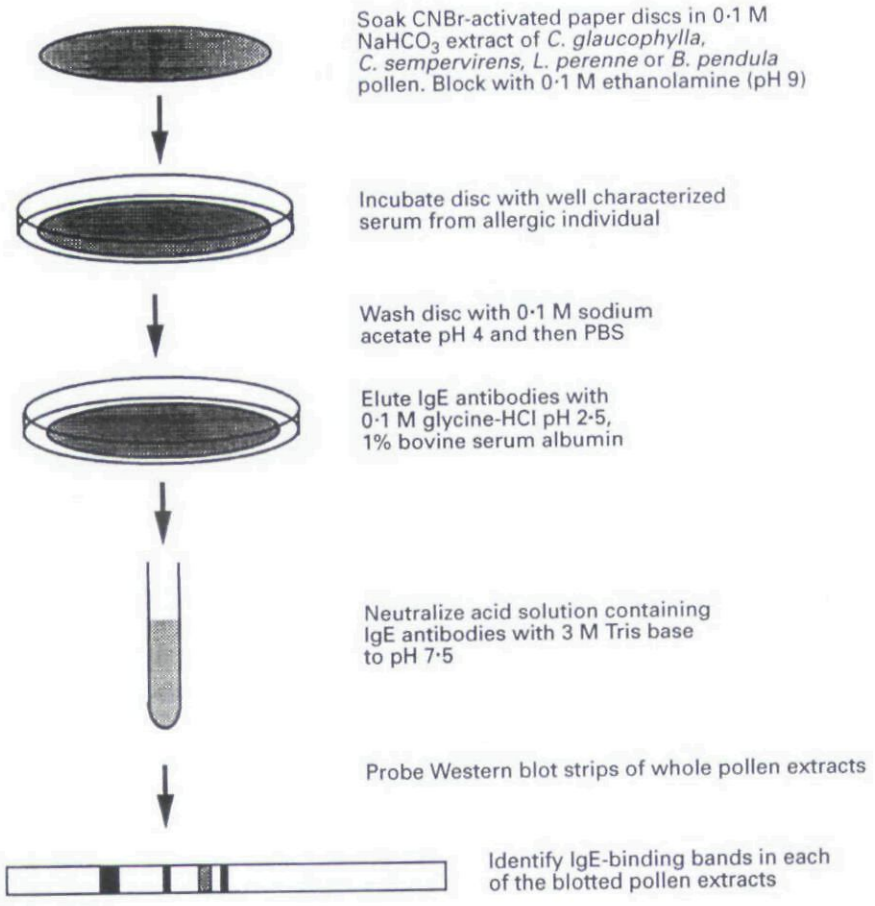

Fig. 4. Diagrammatic representation of the procedure followed for the adsorption and elution of IgE antibodies from pollen proteins bound to a solid phase and the subsequent probing of separated pollen proteins with the eluted antibodies.

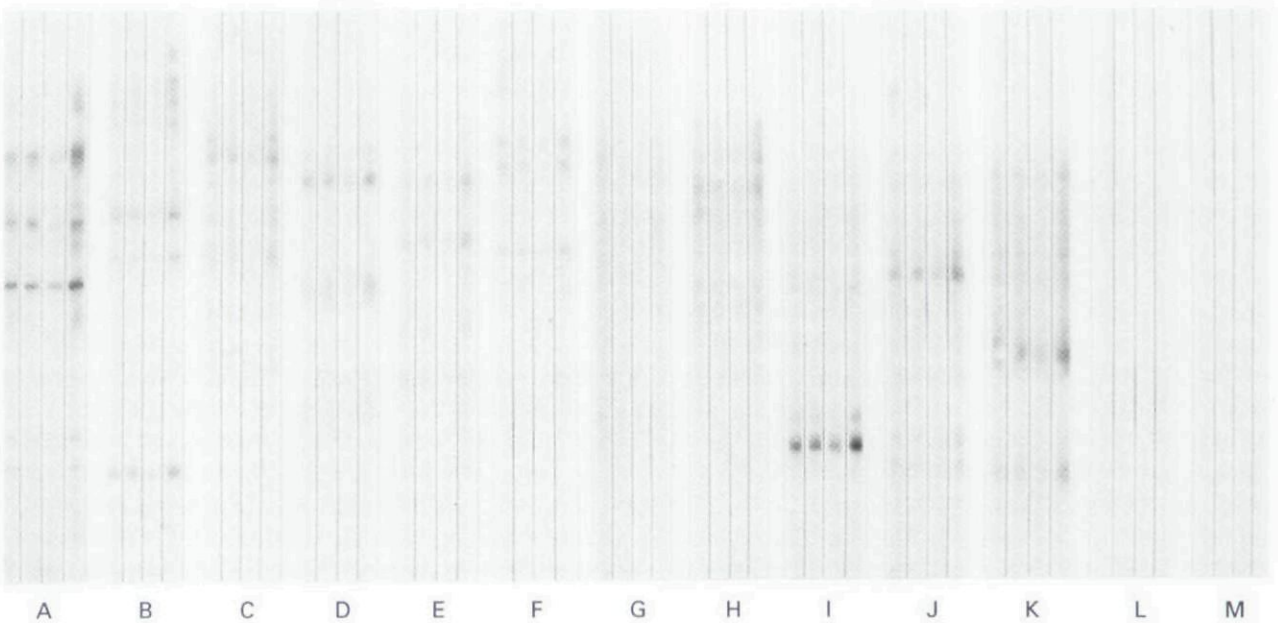

Fig. 5. Autoradiographs of blots of separated pollen proteins probed with eluted IgE antibodies followed by ^{125}I-anti-human IgE. In each set of four strips: strip 1, probed with IgE antibodies eluted from *C. glaucophylla* pollen protein-solid phase. Strip 2, probed with IgE from *C. sempervirens* pollen protein-solid phase. Strip 3, probed with IgE from *L. perenne* pollen protein-solid phase. Strip 4, probed with IgE from *B. pendula* pollen protein-solid phase. Nicrocellulose strips with transferred protein from: A. *C. glaucophylla*, B. *C. sempervirens*, C. *L. perenne*, D. *D. glomerata*, E. *C. dactylon*, F. *C. album*, G. *P. judaica*, H. *B. pendula*, I. *O. europea*, J. *P. lanceolata* and K. *A. elatior*. L. and M. proteins from house dust mite *D. pteronyssinus* and *C. albicans* respectively.

Australia, we concluded that birch pollen-sensitivity in the Australian subjects resulted from exposure to allergens, probably from grass pollens, that crossreact with birch pollen proteins [8]. In a study of Italian, French, and Australian subjects allergic to olive pollen, adsorption and elution and inhibition experiments clearly demonstrated allergenic crossreactivity between olive, privet, ryegrass and couch grass pollen components [9]. As with our findings in the birch study, we concluded that the presence of pollen-reactive IgE antibodies may not necessarily be a true reflection of a sensitizing pollen species.

Allergenic and antigenic crossreactivity has recently been reported for recombinant *Poa pratensis* (*Poa p*) group IX allergens and the group IX grass pollen allergens of 12 different grasses [21]. Recent studies on a family of cytoskeleton proteins, the profilins, have shown them to be an allergenically crossreactive family of proteins having molecular masses of approximately 14 kDa that are present in several different pollen species over a wide taxonomic range [22]. Studies with recombinant birch pollen profilin indicated that it shared common IgE epitope with timothy grass and mugwort profilins.

We conclude that the presence of IgE antibody reactivity to an individual pollen may not necessarily have arisen by contact with that pollen or even with a taxonomically closely related species. This had important consequences for allergen avoidance by pollen-allergic subjects and some asthmatics and for desensitization of patients. Although species-specific pollen allergens almost certainly exist our results suggest the existence of some chemically similar proteins that are distinct from the profilins [22] and the group IX grass pollen allergens [21] in the different species. These proteins may have similar functions, for example, structural or enzymic, that are determined by conserved structures and it may be these structures that are responsible for the observed crossreactions. Strategies designed to identify the molecular basis of the crossreactions and the allergenic structures should now be pursued.

Acknowledgements

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References

1 Altounyan REC. Changes in histamine and atropine responsiveness as a guide to diagnosis and evaluation of therapy in obstructive airways disease. In: Pepys J, Frankland AW, eds. Disodium Cromoglycate in Allergic Airways Disease. London: Butterworth, 1970: 47.
2 Fish JE, Ankin MG, Kelly JF, Peterman VI. Comparison of responses to pollen extract in subjects with allergic asthma and non asthmatic subjects with allergic rhinitis. J Allergy Clin Immunol 1980; 65:154–61.
3 Platts-Mills TAE. Type 1 or immediate hypersensitivity: Hay fever and asthma. In: Lachmann PJ, Peters DK, eds. Clinical Aspects of Immunology. Oxford: Blackwell Scientific Publications, 1982:579–686.
4 Woolcock AJ. Inhaled drugs in the prevention of asthma. Am J Resp Dis 1977; 115:191–4.
5 Baldo BA, Wrigley CW. Allergies to cereals. In: Pomeranz Y, ed. Advances in Cereal Sciences and Technology, Vol 6, Amer Assoc Cereal Chemists, St Paul, Mn, 1984: 289–356.
6 Wodehouse RP. Hayfever plants—their appearance, distribution, time of flowering and their role in hayfever. New York: Hafner, 1971.
7 Baldo BA, Sutton R, Wrigley CW. Grass allergens, with particular reference to cereals. Progr Allergy 1982; 30: 1–66.
8 Hemmens VJ, Baldo BA, Elsayed S, Bass D. Allergic response to birch and alder pollen allergens influenced by geographical location of allergic subjects. Int Arch Allergy Appl Immunol 1988; 87:321–8.
9 Baldo BA, Panzani RC, Bass D, Zerboni R. Olive (Olea europea) and privet (Ligustrum vulgare) pollen allergens. Identification and cross-reactivity with grass pollen proteins. Molec Immunol 1992; 29:1209–18.
10 Pham NH, Baldo BA, Bass DJ. Cypress pollen allergy. Identification of allergens and cross-reactivity between divergent species. Clin Exp Allergy 1994; 24:558–65.
11 Ford SA, Tovey ER, Baldo BA. Identification of orchard grass (Dactylis glomerata) pollen allergens following electrophoretic transfer to nitrocellulose. Int Arch Allergy Appl Immunol 1985; 78:15–21.
12 Ford SA, Baldo BA, Geraci D, Bass D. Identification of Parietaria judaica pollen allergens. Int Arch Allergy Appl Immunol 1986; 79:120–6.
13 Ford SA, Baldo BA. Identification of bermuda grass (Cynodon dactylon) pollen allergens by electroblotting. J Allergy Clin Immunol 1987; 79:711–20.
14 Ford SA, Baldo BA, Panzani R, Bass D. Cypress (Cupressus sempervirens) pollen allergens. Identification by protein blotting and improved detection of specific IgE antibodies. Int Arch Allergy Appl Immunol 1991; 95:178–83.
15 Smith PK, Krohn RI, Hermanson GT et al. Measurement of protein using bicinchoninic acid. Analyt Biochem 1985; 150:76–85.
16 Bass D, Baldo BA, Pham NH. White cypress pine pollen: an important seasonal allergen source in rural Australia. Med J Aust 1991; 155:572.
17 Laemmli UK. Cleavage by structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227:680–5.
18 Tovey ER, Baldo BA. Comparison of electroblotting of IgE-binding components in extracts of house dust mite

bodies and spent mite culture. J Allergy Clin Immunol 1987; 79:93–102.

19 Tovey ER, Baldo BA. Standardization of allergens: qualitative definition of house dust mite extracts following electroblotting and detection of components with antibody and lectin probes. Int Arch Allergy Appl Imunol 1984; 75:322–9.

20 Tovey ER, Ford SA, Baldo BA. Enhanced immunodetection of blotted house dust mite protein allergens on nitrocellulose following blocking with Tween-20. Electrophoresis 1989; 10:243–9.

21 Zhang L, Kisil FT, Sehon AH. Mohapatra SS. Allergenic and antigenic crossreactivities of group IX grass pollen allergens. Int Arch Allergy Appl Immunol 1991; 96: 28–34.

22 Valenta R, Duchene M, Ebner C et al. Profilins constitute a novel family of functional plant pan-allergens. J Exp Med 1992; 175:377–85.

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