

Distribution of a Major Allergen of Rye Grass (*Lolium perenne*) Pollen between Other Grass Species

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Abstract. Polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS) followed by protein staining has shown that extracts from 11 different grass pollens contained proteins with similar molecular weights to that of the allergen R7 from rye grass (*Lolium perenne*) pollen extract (i.e. 29,000-31,000 daltons). Western blotting and detection with polyclonal (rabbit) antibodies raised against the purified R7 (Rye I) allergen indicated that these proteins were antigenically related and their allergenic properties were demonstrated by the binding of human IgE to immunoblots. The distribution of cross-reacting antigenic determinants was further investigated by immunoblotting with 2 mouse monoclonal antibodies, R7M1 and R7M2, produced with purified R7 as the initial immunogen. The 2 monoclonal antibodies were shown to react with 'R7-like' components of grass pollen extracts other than the component from rye grass. Differences in the distribution of R7M1 and R7M2 binding were found indicating that they are directed at separate R7 epitopes.

Introduction

Allergenic cross-reactivity has been shown to exist between allergens derived from different species of grass pollens. Evidence for this has come from a variety of in vitro and in vivo systems, most notably radioallergosorbent test inhibition assays, crossed radioimmuno-electrophoresis and skin prick testing [1-4]. It is now possible to detect cross-reacting allergens with distinct electrophoretic mobilities by a combination of polyacrylamide gel electrophoresis (PAGE), electrophoretic transfer to nitrocellulose and detection with reaginic human sera [5]. More recently hybridoma technology has been employed to produce monoclonal antibodies directed against individual antigenic epitopes on a variety of aeroallergens [6-8]. Such antibodies raised against *Lolium perenne* (rye) grass pollen extract have demonstrated the presence of cross-reacting epitopes on other pollen species [9-11].

Three components, R7 (31,000 daltons), R14a (acidic, 11,000 daltons) R14b (basic, 11,000 daltons) have been isolated from rye grass pollen in purified forms by preparative isoelectric focusing (IEF) [12]. Sodium dodecyl sulphate (SDS)-PAGE of ¹²⁵I rye-hu-

man IgG complexes has shown that a larger proportion of atopic individuals respond to R7 than to R14a or R14b [13]. It has also been established that these 3 components are biochemically and immunochemically similar to those of rye groups I, II and III, respectively, the major allergens of rye grass pollen as defined by Marsh [14].

In the present study a combination of SDS-PAGE, Western blotting and immunodetection with rabbit antibodies raised against rye grass pollen R7 has been used to investigate the distribution of this allergen in 14 other grass species. Mouse monoclonal antibodies have been employed to confirm the existence of single cross-reacting epitopes on 'R7-like' allergens.

Materials and Methods

Buffers and General Reagents

Phosphate-buffered saline (PBS), pH 7.3 (Difco haemagglutination buffer) and PBS containing 0.5% w/v Tween 20 (PBST) were used. Tween 20 and bovine serum albumin (BSA) were obtained from Sigma and other general reagents were from BDH, Sigma, Bio-Rad, Gibco and Flow laboratories.

Pollen Extracts

All pollens were obtained from Bencard (Worthing, Sussex).

Extracts were prepared by a 4-hour aqueous extraction into 10 mM NH_4HCO_3 (pH 7.0), followed by centrifugation (10,000 g for 30 min) and sequential filtration through 1.2 μm down to 0.22 μm membrane filters, and finally freeze-dried without prior dialysis. All the pollen extracts were analysed for protein by the Bio Rad dye-binding assay using BSA as a standard. The pollen proteins R7 (31,000 daltons) and R14a (11,000 daltons) were purified from rye grass pollen extract (*L. perenne*) by preparative IEF as described by Cottam et al. [12].

Rabbit Antisera

New Zealand White rabbits were immunized with 2 mg of R7 or R14a emulsified in Freund's complete adjuvant (Difco) on day 0 followed by 2 mg in Freund's incomplete adjuvant on day 10 and a 50 μg aqueous boost on day 21. The immunizing doses were divided between subcutaneous, intramuscular and intradermal sites. A serum pool was prepared from blood samples collected 36, 53 and 60 days after the initial immunization.

The rabbit anti-R14a antiserum was absorbed with R7 coupled to CNBr-activated Sepharose 4B (Pharmacia). Unrelated antigenic specificities were removed from the rabbit anti-R7 serum by absorption with rye grass pollen extract from which R7 and R14a had been removed. This was accomplished by fractionating rye grass pollen extract on a TSK 3,000 (LKB Produkter) high-performance liquid chromatography (HPLC) column. The void volume material, coupled to CNBr-activated Sepharose 4B, was used for the absorption.

Monoclonal Antibodies

Immunization. Male Balb/c mice were immunized subcutaneously with 50 μg of R7 emulsified in Freund's complete adjuvant on day 0 and in Freund's incomplete adjuvant on day 14. Further injections were given in 0.9% NaCl on day 56 (25 μg R7 i.p.) and day 57 (10 μg R7 i.v.). Spleens were removed for fusion 3 days after the final injection.

Cell Fusion. Hybridisation was carried out as described by Kohler [15] using the non-secreting myeloma cell line NSO/1 of Clark, Wright and Milstein [for details see 16], which was kindly provided by the MRC laboratory of Molecular Biology, Cambridge, UK. The cells were cultured with a peritoneal macrophage feeder layer in RPMI 1640 containing 15% myclone fetal calf serum (Gibco) and hypoxanthine, thymidine and aminopterin (HAT medium). Supernatants were screened for antibody production to R7 by an enzyme-linked immunosorbent assay (ELISA) and cells from positive wells were cloned by limiting dilution. Single clones were tested for anti-R7 activity by ELISA and for antigen specificity by immunoblotting.

Ascitic Fluid. Two monoclonal cell lines R7M1 and R7M2, (5×10^6 cells in PBS) were injected into the peritoneum of male Balb c mice, 7 days after an injection of pristane (0.5 ml of 2, 6, 10, 14-tetramethylpentadecane, Aldrich Chemicals), and ascitic fluid was harvested 14–28 days later. DEAE Affi-gel blue chromatography of the R7M1 ascitic fluid was performed as described by Bruck et al. [17] except that a 0- to 500-mM NaCl gradient was employed for elution.

Preparation of Goat Anti-Mouse IgG. An IgG fraction of goat anti-mouse IgG (Cappel Laboratories, Downingtown, USA) was affinity-purified on mouse IgG (DEAE fraction) conjugated to Sepharose.

Iodinations. Protein A (Sigma) and goat anti-mouse IgG were

iodinated using ^{125}I sodium iodide (Amersham International) by the chloramine T method [18].

SDS-PAGE and Immunoblotting. PAGE in SDS was performed on a vertical slab gel apparatus (Bio-Rad Laboratories) using 12.5% w/v polyacrylamide gels according to the method of Laemmli [19]. Molecular weights were estimated using prestained protein standards 3,000–43,000 daltons from Bethesda Research Laboratories, and samples were electrophoresed in the presence of dithiothreitol. Electrophoretic transfer to nitrocellulose was performed as described by Moran et al. [20] and immunodetection with the relevant antisera as described by Sutton et al. [5].

Autoradiography was performed using pre-flashed Fuji RX medical X-ray film (Fuji Film Company, Japan) and match-2 intensification screens.

Immunoassays of Monoclonal Mouse IgG. Monoclonal culture supernatants were screened by an ELISA performed essentially as described by Kohler [15]. Purified allergen R7 at 50 $\mu\text{g} \cdot \text{ml}^{-1}$ was used for coating the micro-ELISA plates (Dynatech). Detection of bound mouse IgG was performed with alkaline-phosphatase-conjugated rabbit anti-mouse IgG (Sigma 50 μl diluted 1:500 into PBST containing 0.5% BSA) and phosphatase substrate (100 $\mu\text{l} \cdot 1 \text{ mg} \cdot \text{ml}^{-1}$ *p*-nitrophenyl phosphate, Sigma). The colour intensity was estimated visually or spectrophotometrically at 405 nm on a Pye Unicam SP1800.

Raidoimmunoassays were performed in PVC flexible plates (Dynatech) coated with R7 as described for the ELISA. Culture supernatants and ascites were pre-incubated for 2 h at ambient temperature in PBS containing 0.5% BSA either with or without R7 (or R14a) at 1 $\text{mg} \cdot \text{ml}^{-1}$, transferred to the R7-coated plates for a further 2 h and bound monoclonal IgG was detected with ^{125}I goat anti-mouse IgG (100,000 cpm, 4–8 ng).

The IgG subclass was determined by ELISA using affinity-purified rabbit anti-mouse subclass antisera (Miles) at a 1:20 dilution.

Results

Comparison of Grass Pollens by Protein Staining and Immunodetection with Polyclonal Antisera

After SDS-PAGE and protein staining of rye grass pollen extract (fig. 1, lane 1), intensely staining bands were seen at molecular weights of 31,000 and 11,000 daltons corresponding to the allergens R7 (Rye I) and R14a/b (Rye II and III), respectively. Comparing this profile with those of 11 other grass pollen extracts (fig. 1, lanes 2–12) a component of 31,000 daltons was clearly visible in 8 of these extracts (fig. 1, lanes 2–6, 8–10). In all 11 grass pollen extracts an intensely stained band of 11,000–12,000 daltons was observed in the same position as the R14a/b component of rye grass pollen extract. The 29,000- to 31,000-dalton component found in the 11 grass pollen extracts (fig. 1, tracks 2–12) was shown to have the same allergenic property as the R7 allergen of rye grass by immunoblotting with a serum pool from grass-pollen-sensitive human subjects.

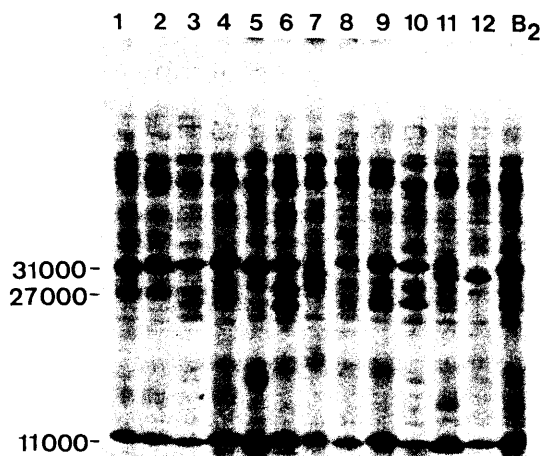


Fig. 1. Protein-stained profiles of grass pollen extract after SDS-PAGE under reducing conditions. Pollen extracts were loaded onto a 12.5% (w/v) polyacrylamide gel in the following order: (1) Rye grass (*L. perenne*); (2) fescue (*Festuca pratensis*); (3) cocksfoot (*Dactylis glomerata*); (4) bent (*Agrostis tenuis*); (5) sweet vernal (*Anthoxanthum odoratum*); (6) false oat (*A. elatius*); (7) brome (*Bromus* spp.); (8) meadow grass (*Poa pratensis*); (9) Yorkshire fog (*H. lanatus*); (10) dogtail (*C. cristatus*); (11) timothy (*P. pratense*); (12) foxtail (*A. pratensis*); (B₂) mixture of all 12. In each case 80 μ g of protein, as determined by the Bio-Rad dye-binding assay, was electrophoresed.

Further to this, a rabbit antiserum raised against the R7 allergen was employed to investigate the distribution of 'R7-like' antigenic determinants. The results of electroblotting rye and 11 other grass pollen extracts and immunoanalysis with rabbit anti-R7 (pre-adsorbed with other rye grass pollen proteins) are shown in figure 2. The 29,000- to 31,000-dalton component in all 11 grass pollen extracts is recognised by the rabbit anti-R7 serum (fig. 2, lanes 2-12). From comparisons of the autoradiographic intensity with that of serially diluted R7 (fig. 2) it was estimated that up to 10% (100 ng in 1,000 ng) of the pollen extract protein is 'R7-like'. In addition to the 11 pollen extracts shown in figure 2, the rabbit anti-R7 antibodies recognised a 29,000- to 31,000-dalton material in cultivated rye (*Secale cereale*), Bermuda grass (*Cynodon dactylon*) and to a lesser extent in maize (*Zea mays*). No binding to ragweed (*Ambrosia elatior*) or *Parietaria* pollen proteins was found under these conditions.

The distribution of 'R14a-like' specificities was more restricted. Strong positive binding of R7-ad-



Fig. 2. Binding of adsorbed rabbit anti-R7 to 'R7-like' components of 12 grass pollens analysed by immunoblotting and autoradiography. Extracted pollen protein (1 μ g) was electrophoresed under reducing conditions and electroblotted. The resulting nitrocellulose sheet was blocked with 5% BSA in PBS, incubated with adsorbed rabbit anti-R7 antiserum (1:1,000 dilution) in PBST containing 0.5% BSA for 2 h at ambient temperature, washed in 6 changes of PBST, developed with ¹²⁵I protein A (10⁵ cpm $\cdot$ ml⁻¹, 2-4 ng $\cdot$ ml⁻¹) in 0.5% BSA/PBST (2 h), washed and autoradiography was performed. Tracks 1-12 contain grass pollen extracts in identical order to that seen in figure 1.

sorbed rabbit anti-R14a to an 11,000- to 12,000-dalton species occurred in only 7 out of the 12 grass pollen extracts seen in figure 1 (i.e. lanes No. 1, 3, 4, 6, 8, 10 and 12) [data not shown].

Production of R7-Specific Mouse Monoclonal Antibodies

When whole rye grass pollen extract was employed as the immunogen no clones were produced secreting antibodies that recognised rye components by immunoblotting even though rye-specific antibodies can be detected by ELISA (using whole-extract-coated plates). Therefore R7 was used as the immunogen to increase the likelihood of inducing the required clones. The culture supernatants were tested by ELISA 15 days post fusion and a number of positive supernatants were detected when R7, but not whole rye grass pollen extract, was used to coat the ELISA plates.

Clonal Expansion

After cloning by limiting dilution, the R7-positive

Table I. Direct binding and inhibition radioimmunoassays for the R7 monoclonal antibodies (R7M1 and R7M2)

Clone	Dilution	Percent uptake of ^{125}I goat anti-mouse IgG onto R7-coated PVC plate			Percent inhibition of specific R7 binding by R7
		no inhibitor	+ R7 (1 mg·ml ⁻¹)	+ R14a (1 mg·ml ⁻¹)	
R7M1 supernatant	1:10	15.8	11.4	16	33
R7M2 supernatant	1:10	2.6	1.8	-	32
R7M1 supernatant	1:100	11.3	4.6	-	60
R7M1 ascites	1:10 ⁵	16.8	3.2	-	81

Flexible microtitre plates were coated with R7. The culture supernatants or ascites, which had been preincubated in the absence or presence of inhibitor, were added and the uptake of the monoclonal antibody was detected with ^{125}I goat anti-mouse IgG. Results are expressed as cpm bound divided by cpm added, giving percent uptake onto the plate.

single clones (as determined by ELISA) were shown to produce antibody that was inhibited by R7 in the inhibition radioimmunoassay and were clearly R7-specific on immunoblotting with whole rye grass pollen extract. Only 2 clones named R7M1 and R7M2 were studied in detail. Both produced monoclonal antibodies of the mouse IgG 2b class. The fluid produced when the R7M1-positive clones were expanded as mouse ascites was purified by DEAE Affi-gel blue chromatography. Application of a linear gradient of sodium chloride (0–500 mM) to the Affi-gel blue column eluted anti-R7 activity between 75 and 100 mM NaCl. Although only a partial purification was achieved, as judged by SDS-PAGE, the monoclonal antibody was free of transferrin and most of the other contaminating proteins apart from mouse albumin.

Antigenic Specificity of R7M1 and R7M2

The specificities of the anti-R7 monoclonal antibodies were determined both by direct binding and by inhibition radioimmunoassays employing ^{125}I goat anti-mouse IgG. Antibodies from the R7M1 culture supernatant were taken up onto an R7-allergen-coated plate, whereas very little binding was observed when the 11,000-dalton rye allergen (R14a) was used to coat the microtitre plate. Binding to R7 was inhibited by R7 itself but not by R14a (table I). Dilution of R7M1 from 1:10 to 1:100 decreased its uptake but increased the level of inhibition by R7 from 33 to 60%. R7M2 was less active in the R7 direct binding radioimmunoassay but was as readily inhibited by R7 (table I). The better explanation of this observation is

that the R7M2 monoclonal antibody is directed against an epitope which has a lower density of distribution on R7 than that of the epitope recognised by R7M1 rather than that it is at a lower concentration or is less well recognised by the ^{125}I goat anti-mouse IgG.

The R7M1 ascitic fluid also showed strong R7 specificity in the inhibition radioimmunoassay (table I). A higher uptake onto R7-coated PVC plates was found with the Affi-gel-blue-purified antibody than the starting ascites when both were compared at the same protein concentration, assessed by absorbance at 280 nm.

Immunoblotting with R7M1 and R7M2 Monoclonal Antibodies

Specificity for R7. Immunoblotting with whole rye grass pollen extract (10 μg i.e. 10-fold increase on the concentration normally employed) indicated that both R7M1 and R7M2 recognised only the 31,000-dalton band (R7). The apparent weaker response of the R7M2 culture supernatant for R7 compared with that of R7M1 was also observed on immunoblotting. The possibility that R7M2 was recognising a more restricted number of R7 isoallergens was explored using R7 fractions separated by HPLC ion exchange chromatography. These fractions had slightly differing pIs on analytical IEF, but the same apparent molecular weights on SDS-PAGE. R7M1 showed clear positive binding to all the isoallergen fractions; R7M2 appeared to give similar results but the binding was weaker.

Some non-specific binding to rye pollen proteins

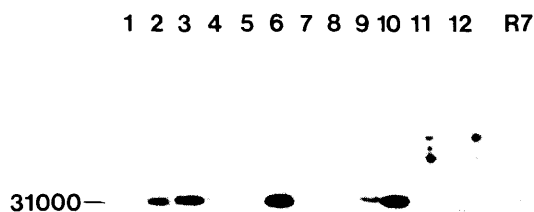


Fig. 3. Autoradiograph showing the distribution of R7M2 monoclonal antibody binding sites between grass pollen extracts. Twelve grass pollen extracts (tracks 1–12) were electrophoresed in the same order as in figure 1, 2 μ g of each extract being loaded. After electrophoretic transfer onto nitrocellulose the sheet was overlaid with the R7M2 culture supernatant (1:5 dilution and incubation at ambient temperature for 6 h) followed by ^{125} I goat anti-mouse IgG (10^5 cpm $\cdot$ ml $^{-1}$, 4–8 ng $\cdot$ ml $^{-1}$) for 18 h at 4°C.

other than R7 was observed with the R7M1 mouse ascites but this was abolished by partial purification on Affi-gel blue.

Binding to Other Grass Pollen Extracts. Clear differences in binding to the various grass pollen extracts were apparent (fig. 3) when the R7M2 culture supernatant was used in immunoblotting experiments, employing the same concentrations of extract as in the rabbit antibody studies. When the autoradiographic intensities relative to the R7 band of rye grass pollen were compared (fig. 3, lane 1), there appeared to be a higher concentration of R7M2 antigenic determinants on 5 pollen extracts other than on rye grass itself (fig. 3, lanes 2, 3, 6, 9 and 10). Hence it seems likely that there is a lower density of distribution of the epitope recognised by R7M2 on the R7 component of rye than on the 'R7-like' component in these other grass pollen extracts.

The R7M1 monoclonal antibody (culture supernatant) recognised the 'R7-like' component in 8 out of the 12 grass pollens seen in figure 1 (tracks No. 1–5, 8–10). The R7M1 partially purified antibody from mouse ascites gave a result identical to that found with the culture supernatant (fig. 4).

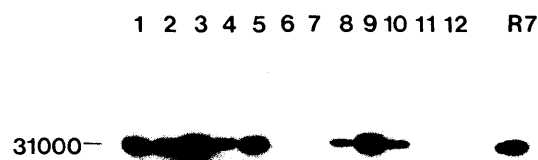


Fig. 4. Analysis of the binding of the R7M1 to antigenically identical components of 12 grass pollens by immunoblotting and autoradiography. Grass pollen proteins (2 μ g) were electrophoresed, electroblotted and the nitrocellulose was overlaid with the R7M1 monoclonal antibody purified from ascites by Affi-gel blue chromatography. The immunoblot was developed with ^{125} I goat anti-mouse IgG and autoradiographed. Tracks 1–12 contain the pollen extracts in the same order as shown in figure 1.

On comparing the distribution of R7M1 antigenic determinants between the 29,000- to 31,000-dalton allergen of 12 grass pollen extracts (fig. 4) and that for the R7M2 monoclonal (fig. 3), some striking differences were noted. No binding of the R7M1 monoclonal antibody was observed to the 'R7-like' species in false oat (*Arrhenatherum elatius*) relative to rye R7 (fig. 4: compare tracks 1 and 6), whereas clear binding was obtained with R7M2 (fig. 3, compare tracks 1 and 6). The autoradiographic intensity with R7M1 was greater for Yorkshire fog (*Holcus lanatus*) than for dogstail (*Cynosurus cristatus*) (fig. 4, tracks 9 and 10), while the situation was reversed for R7M2 (fig. 3, tracks 9 and 10). The R7M2 monoclonal binds to an antigenic site on the 'R7-like' component of timothy (*Phleum pratense*) and foxtail (*Alopercurus pratensis*) to the same extent as to the R7 itself, whereas R7M1 does not.

Neither R7M1 nor R7M2 appeared to bind to the components of cultivated rye, Bermuda, ragweed or *Parietaria* under the immunoblotting conditions employed. Binding was observed to a 29,000- to 31,000-dalton component in maize with R7M1, but not with R7M2.

Discussion

Protein-staining bands of 29,000–31,000 daltons found in 11 grass pollen extracts were recognised by human IgE on immunoblotting, suggesting that they contain allergens similar to the R7 allergen of rye grass pollen. Antigenic cross-reactivity was found between these pollen proteins and R7 when using immunoblotting with rabbit anti-R7 antiserum (pre-adsorbed with other rye pollen proteins). Two monoclonal antibodies R7M1 and R7M2 raised against the R7 component of rye grass pollen extract also reacted with the 29,000- to 31,000-dalton protein found in grass pollens other than rye indicating the presence of single 'cross-reacting' epitopes in these 'R7-like' allergens.

Differences in degrees of binding to the 'R7-like' proteins in different grass pollens suggested that these 2 monoclonal antibodies had different antigenic specificities. Although confirmatory studies have not yet been performed, further work using a competitive binding assay [21] employing nitrocellulose-bound R7 should provide the necessary confirmation.

Evidence that the R7M2 monoclonal antibody may be directed at an antigenic site of lower density distribution on rye R7 than that recognised by R7M1 was obtained from inhibition radioimmunoassays and immunoblotting experiments. However R7M2 showed some binding to all the isoallergen fractions of R7 isolated by HPLC ion exchange chromatography. Since R7 is known to be heterogeneous on analytical IEF [12], a combination of this technique with immunoblotting [22] may produce clearer data on the relative binding of R7M1 and R7M2 to R7 (Rye I) isoallergens.

Partial purification of mouse IgG with anti-R7 activity was achieved by passage of the R7M1 ascitic fluid down a DEAE Affi-gel blue column and elution with 75–100 mM NaCl. This sodium chloride concentration is somewhat higher than that reported by other workers [17, 23] for obtaining mouse IgG free from other proteins in the ascitic fluid. Since R7M1 belongs to the IgG2b subclass, purification on a protein A affinity column [23] should be possible and may result in a preparation free of mouse albumin, although increasing the risk of denaturation.

The monoclonal antibody FMCA1 produced by Smart et al. [11] has been shown to bind to an allergen of rye grass pollen of about 30,000 daltons which is most probably Rye group I. Some of the pollen ex-

tracts recognised by FMCA1 in the dot blotting assay [9] also bind R7M1: e.g. maize and cocksfoot; however, there are notable differences: e.g. R7M1 does not bind to allergens of cultivated rye. The anti-Rye I monoclonal antibody of Kahn and Marsh [10] also showed some similarities both to R7M1 and to R7M2. The purified allergen AgD1 of cocksfoot displays physico-chemical properties similar to those of rye R7, and is recognised by an anti-Rye I monoclonal antibody [24]. It seems likely therefore that the 30,000-dalton 'R7-like' allergen found in cocksfoot (fig. 2, 3, and 4, track 3) is AgD1.

Cross-reacting antigenic determinants have been defined on other aeroallergens using monoclonal antibodies. *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* P1 and Fr1 allergens, respectively, contain common epitopes [25] and a monoclonal antibody which recognises a 42,000-dalton allergen from *D. pteronyssinus* has been used to demonstrate cross-reactivity with a 30,000-dalton allergen from *D. farinae* [7] hence cross-reacting epitopes are thus not necessarily found on allergens of identical molecular weights. Common antigenic determinants have also been found in allergens derived from different species of *Parietaria* pollen [8].

It has been suggested that monoclonal-antibody-based immunoassays could be used in allergen standardisation [6, 8] as a replacement for the more conventional immunological techniques. The most widely employed in vitro methods for standardizing pollen extracts are either radioallergosorbent test inhibition assays, which measure allergenic potency; or crossed radioimmuno-electrophoresis (CRIE), which gives a semiquantitative assessment of the relative amounts of individual allergen components [26–28]. Identification of allergenic components by SDS-PAGE and immunoblotting with reaginic sera, followed by densitometry for quantitation of each component has been suggested as a viable alternative to CRIE [29]. Another approach has been to identify a 'major allergen' and standardize allergen extracts in terms of the content of this marker allergen by a radioimmuno-inhibition assay employing polyclonal rabbit antisera [30]. This type of inhibition assay, employing monoclonal antibodies, can be used to standardize pollen extracts in terms of species-specific epitopes [31] and cross-reacting determinants [10]. Although the monoclonal antibodies R7M1 and R7M2 recognise a range of cross-reacting epitopes on 'R7-like' allergens in grass pollens other than rye, the

polyclonal rabbit anti-R7 reacts with a much wider range of grass pollens. Hence for standardisation of pollen extracts in terms of R7 (Rye I) content the adsorbed rabbit antiserum is of much greater value. Preliminary data suggest that an assay involving inhibition of the uptake of ^{125}I -labelled R7 by rabbit anti-R7 will prove a highly useful tool in standardisation of extracts in terms of R7 marker allergen content. The monoclonal antibodies have provided valuable information on the cross-reactivity of grass pollen extracts, and could be of further value to purification and characterisation of the 'R7-like' allergens from extracts of pollens other than rye.

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