

Crossreactivity of IgE antibodies from sera of subjects allergic to both ryegrass pollen and wheat endosperm proteins: evidence for common allergenic determinants

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Summary

Positive RAST (> 5% radioactive uptakes) to wheat endosperm proteins were found in approximately one-quarter of subjects who had both a positive skin prick and RAST (> 10% radioactive uptake) to ryegrass pollen proteins. Immunoblotting of proteins electrophoretically transferred to nitrocellulose membrane after SDS-PAGE of ryegrass pollen and wheat endosperm proteins confirmed the crossreactive properties of the sera identified by RAST testing. Immunoabsorption of serum IgE onto nitrocellulose membrane, to which ryegrass pollen or wheat endosperm proteins had been adsorbed, removed IgE from crossreactive sera reactive to both ryegrass pollen and wheat endosperm proteins. Elution of the adsorbed IgE from the nitrocellulose membrane after immunoabsorption and probing blotted strips of both ryegrass pollen and wheat endosperm proteins supported the results obtained from the immunoabsorption experiments. This data provides evidence that the crossreactivity of IgE antibodies in sera reacting with both ryegrass pollen and wheat endosperm proteins involves common or related determinants and has implications for the clinical management of these allergic subjects.

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Introduction

There have been several reports of subjects who are hypersensitive to pollen allergens exhibiting reactivity to a number of non-related food substances. In a study of 73 atopic individuals prick tested with inhalant and food allergen extracts, 43 individuals were skin-test positive to grass pollen and, of these, 42 were reactive to cereal grains [1]. In birch pollinosis, a number of non-related fruits, vegetables and nuts cause allergic symptoms, mainly oral itching [2,3,4] and there has been a reported association between mugwort pollinosis and sensitivity to celery [5]. Ragweed pollen-sensitive subjects [6] reported oral itching after eating banana, water-melon and other members of the gourd family such as cantaloupe and honeydew melon. In a study of 262 patients [7], 208 (79.4%) with allergy to vegetables and fruits also had hay fever. Of

these 208, 43.9% were allergic to grass pollens, 39.5% to birch pollen, 8.5% to *Parietaria* pollen and 8.2% to mugwort pollen.

IgE antibodies to wheat flour components have been studied previously [8] and recently reviewed [9]. This paper examines the IgE antibody crossreactivity of sera from ryegrass pollen-allergic subjects with wheat endosperm proteins using radioimmunoassay and protein blotting techniques.

Materials and methods

Preparation of ryegrass pollen and wheat endosperm protein extracts

Pure, dry ryegrass pollen (10 g) (*Lolium perenne*, Greer Laboratories, Inc., Lenoir, U.S.A.) was extracted with 10 volumes of water overnight at 4°C with constant stirring. The mixture was centrifuged at 15 000 r.p.m. (27 500 × g) in the SS34 rotor of the Sorvall RC2B centrifuge for 15 min. The supernatant was removed and dialysed for 2

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days at 4°C against 2 l of water with a total of six changes. The dialysed extract was then freeze dried and stored at room temperature under vacuum.

Total wheat endosperm protein was prepared from wheat flour (10 g) (*Triticum aestivum* cv. Timgalen) by extraction with 50 ml of SDS extracting solution (82.5 mM Tris-HCl pH 6.8, 4% (w/v) sodium dodecyl sulphate, 1.5% (v/v) 2-mercaptoethanol) using end over end mixing, overnight, at room temperature. The mixture was centrifuged, dialysed and freeze dried in the same manner as the ryegrass pollen extract. The albumin and globulin fraction was extracted from 10 g of flour with 50 ml of 10% sodium chloride solution by stirring at room temperature for 4 hr. The suspension was then treated in the same manner for the preparation of the SDS fraction above.

Protein determinations of freeze-dried extracts were carried out using the BCA protein determination method (Pierce Chemical Co., Rockford, Illinois, U.S.A.). Protein percentages of the freeze-dried extracts were 50% for the ryegrass pollen extract, 49% for the wheat endosperm protein extract, and 32.5% for the wheat endosperm albumin and globulin fraction.

Sera

Sera were obtained from the Allergy Clinic, Royal North Shore Hospital and from private patients with their consent (courtesy Dr D. Bass, Sydney, Australia). All subjects had a history of inhalant allergy to ryegrass pollen or inhalant [8,9] and/or ingestion allergy to wheaten products and all exhibited a positive skin-prick test [11] to extracts of ryegrass pollen and/or wheat flour protein. Freeze-dried sera from subjects hypersensitive to either ryegrass pollen or wheat flour proteins were obtained from Dr R. C. Panzani, Laboratoire de Recherches, Marseille, France.

Allergen discs and the radio-allergosorbant test (RAST)

All sera were tested for ryegrass pollen, wheat gluten and wheat albumin and globulin protein-specific IgE antibodies using the RAST. This was carried out using allergen-coated nitrocellulose or paper discs (5 mm diameter) which were incubated for 3 hr with 50 µl of serum, washed with 0.05% Tween 20 in PBS and incubated overnight with ¹²⁵I-labelled anti-human IgE (Pharmacia, Uppsala, Sweden; 20 000 c.p.m./assay). The discs were washed and counted in an LKB 1275 Mini-Gamma counter. Results are expressed as percentage uptake by the discs of radioactive label used in the assay. Ryegrass pollen (*L. perenne*) allergen discs were obtained from Pharmacia and Dr G. Stewart, Princess Margaret

Hospital, Western Australia. Wheat gluten and wheat albumin and globulin protein-coated nitrocellulose discs were prepared by applying 20 µl (200 µg dry weight) of protein solution dissolved in 1% KOH as described previously [10,12]. Cord serum was obtained from the Department of Obstetrics, Royal North Shore Hospital, and used as an 'IgE-free' control.

SDS-PAGE and electroblotting

SDS-PAGE was carried out using 9–27% gradient gels as previously described [13,14]. Protein solutions (2 mg protein/ml) were prepared in 82.5 mM Tris-HCl, pH 6.8, 4% (w/v) SDS, 10% (v/v) glycerol, 0.1 mg/ml bromophenol blue and heated at 100°C for 5 min prior to loading. Protein (600 µg) was loaded across the full width (115 mm) of the stacking gel.

Low molecular weight markers were purchased from Pharmacia and were included in each electrophoretic separation.

Electrophoretic transfer of proteins to nitrocellulose was as described previously [15].

Immunoabsorption of IgE antibodies

Nitrocellulose discs (50 mm diameter) were incubated overnight in 55 mm Petri dishes on a slowly rocking platform with 2 ml of either ryegrass pollen or wheat endosperm total protein extracts (2 mg/ml protein in 25 mM Tris-HCl, pH 8.3, 192 mM glycine, 0.1% SDS, 20% (v/v) methanol). The protein solution was aspirated off and the discs vigorously washed with four lots of 5 ml of 0.5% Tween 20 in PBS for a total time of 1 hr.

Serum from allergic subjects was diluted (1:10) with PBS containing 0.005% Tween 20, 0.5% bovine serum albumin (BSA; Sigma Chemical Co., St Louis, U.S.A.) and 0.1% sodium azide. Two millilitre aliquots of the diluted serum were separately incubated overnight with one of the protein-saturated discs prepared above. After incubation the diluted serum was removed and retained for immunoblotting. The discs were then vigorously washed with four aliquots of 5 ml of 0.05% Tween 20 in PBS for a total time of 1 hr. After washing, the adsorbed IgE antibodies were eluted by four incubations of 1 ml each with 0.1 M glycine-HCl, pH 2.5, 0.5% BSA [16, 17] for 1, 2, 4 and 8 min each. After each elution the solution was removed and immediately neutralized with 3 M Tris-HCl, pH 8.8 to give a final pH of 7.5. The four 1 ml eluates were then combined.

Probing of nitrocellulose membrane strips and autoradiography

Nitrocellulose strips (4-mm wide) obtained from the SDS-PAGE electroblots were incubated overnight with

sera, immunoadsorbed sera (both diluted 1:10 with 0.005% Tween 20, 0.1% sodium azide, 0.5% BSA) or with IgE eluates obtained after immunoadsorption onto the nitrocellulose discs and elution using the method described above. The strips were washed extensively with 0.05% Tween 20 in PBS and incubated overnight with ^{125}I -labelled anti-human IgE (80 000 c.p.m./strip in 0.005% Tween 20 in PBS, 0.1% sodium azide, 0.5% BSA). A control strip which had only been incubated with ^{125}I -labelled anti-human IgE was routinely included. After extensive washing with 0.05% Tween 20 in PBS, the strips were dried, mounted on a cardboard support and loaded into a Kodak X-Omatic cassette with photographic film (Fuji Rx-safety) and exposed at -70°C for 1–14 days.

Results

Clinical history of allergic subjects

Subjects were defined as 'allergic' on the basis of their reported symptoms and presenting a positive skin-prick test [11] to either or both ryegrass pollen or wheat endosperm protein extracts. All the subjects' sera were RAST tested for IgE antibodies to both ryegrass pollen and wheat endosperm proteins. In some instances subjects allergic to both ryegrass pollen and wheat proteins showed evidence of lessening of their allergic symptoms when wheaten products were removed from their diets.

Selection of serum by RAST

Of 78 sera from subjects who gave a positive skin-prick test to ryegrass pollen proteins and who gave a RAST uptake of greater than 10% with ryegrass pollen extract, 19 gave a RAST uptake with wheat gluten of 5% or greater and 11 greater than 10%. Within the same 78 subjects 20 gave RAST values of greater than 5% uptake to wheat albumin and globulin proteins and 11 greater than 10%. Only sera having RAST uptakes of greater than 10% for either or both ryegrass pollen and wheat endosperm proteins were used for SDS-PAGE/blotting. The selection of sera on this basis was to ensure that there were sufficient IgE antibodies for clear and unambiguous results from the blotting experiments reported below. With the extracts and sera used, RAST values below 5% uptake often failed yield to satisfactory blotting results, and, in our hands, represented a baseline below which results were considered equivocal.

Immunoprobings of nitrocellulose membrane strips with sera from crossreactive and non-crossreactive subjects

Diluted sera were used to probe nitrocellulose strips prepared from SDS-PAGE and blotting of ryegrass

pollen or wheat endosperm total proteins (Fig. 1). Nine of the subjects' (a–h, k) sera had RAST uptakes of greater than 10% for both ryegrass pollen and wheat endosperm proteins, four (l–o) for ryegrass pollen extracts only and four (i, j, p, q) for wheat endosperm proteins only.

The IgE-binding patterns of the ryegrass pollen–wheat endosperm protein crossreactive sera (a–i, k) gave consistent patterns with wheat endosperm total protein blots (Fig. 1B). Most of the IgE-binding was observed in the molecular weight range of 30–80 kD and corresponded in size to molecular weight values of many of the albumin and globulin classes of proteins in addition to all of the gliadin and reduced low molecular weight glutenin proteins [18–20] of wheat endosperm which account for about 90% of the total endosperm proteins. The IgE-binding patterns of sera whose IgE bound only to wheat endosperm proteins (Fig. 1B; i, j, p, q), also lay within the range 30–80 kD.

The IgE-binding patterns observed with crossreacting sera on ryegrass pollen protein blots (Fig. 1A) were more variable than those observed for wheat endosperm total proteins. Sera whose IgE bound only to ryegrass pollen proteins (l–o) produced similar profiles to many of the crossreactive sera (e.g. Fig. 1a; compare a and l, m and o).

The total wheat endosperm protein comprises the albumin and globulin fraction accounting for approximately 10–20% of the protein and the gliadins and glutenins, approximately in equal proportions, and comprising approximately 80–90% of the protein [18,21]. Classical fractionation of the proteins of wheat endosperm [21] often results in the sparingly water soluble gliadins and insoluble glutenins forming a gluten complex which cannot be solubilized, even in the presence of SDS and reducing agents. The SDS-PAGE profile of the total proteins is the only practically simple means of representing the gliadins and glutenins in proportions corresponding to their representation in the endosperm.

Representatives of the panel of sera (a–q) were used to probe SDS-PAGE blots of the wheat endosperm total proteins (Fig. 2A) and the wheat albumin and globulin fraction prepared by 10% NaCl extraction of flour (Fig. 2B). The major IgE-binding protein bands in the albumin and globulin fraction were within the 30–80 kD range in common with the result obtained with the total protein blots. Many of these IgE-binding bands were also clearly visible in the blots of the wheat endosperm total proteins as would be expected, however, there were many bands represented as major IgE-binding albumin and globulin bands which were barely visible in the total endosperm blots. A large number of bands strongly represented in the total protein extract were either very faint or not represented in the blots of the albumins and globulins indicating that they were most likely to be

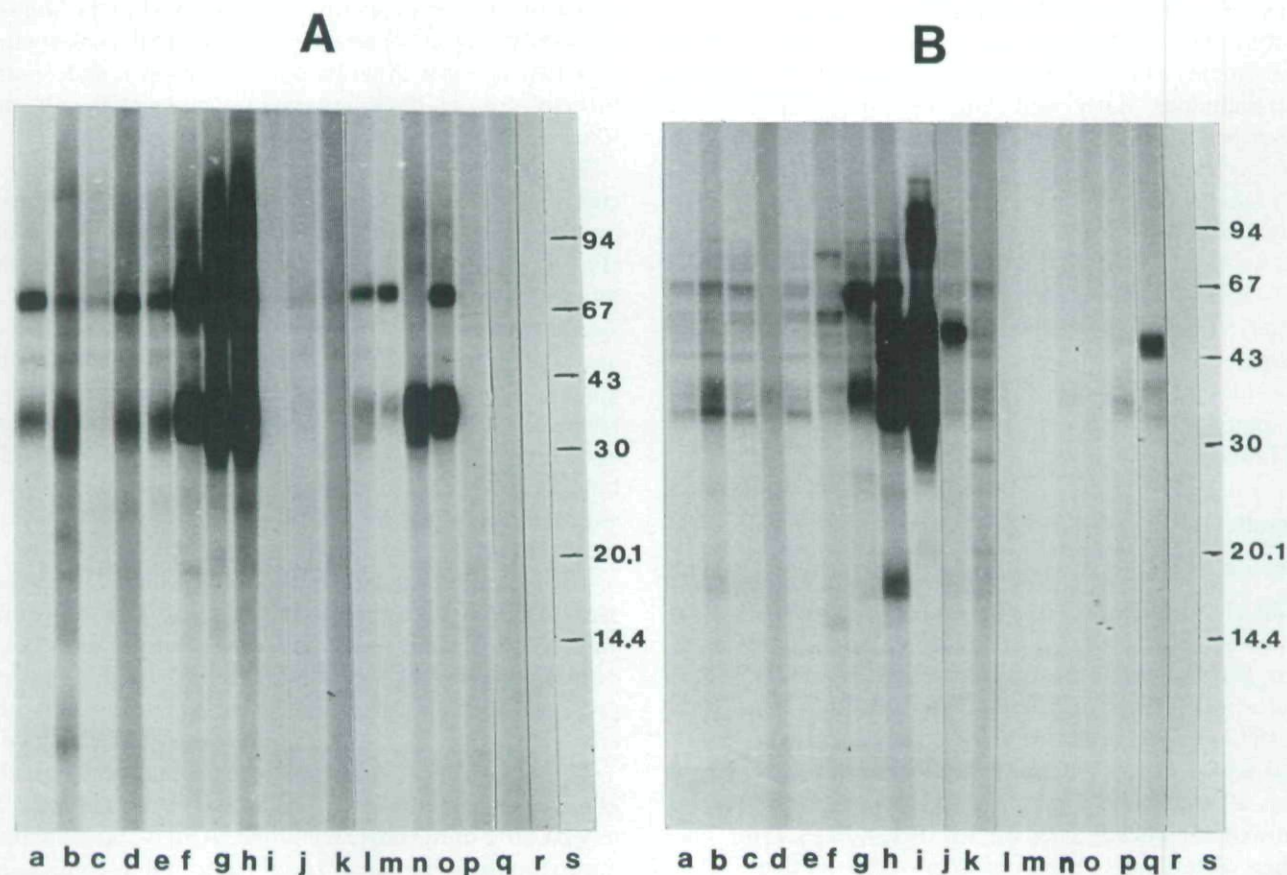


Fig. 1. IgE-antibody binding to Western blots of ryegrass pollen and wheat endosperm total proteins. (A) Ryegrass pollen proteins; (B) wheat endosperm total proteins. (a–q) Serum samples from ryegrass pollen-allergic subjects; (r) control strip incubated with [¹²⁵I]anti-human IgE only; (s) molecular weight markers (kD).

gliadins or glutenins. Because the gliadins and glutenins are represented in about a fourfold excess over the albumins and globulins they represent an important fraction.

Adsorption of IgE from sera of allergic subjects using ryegrass pollen extract and wheat endosperm protein extract adsorbed to nitrocellulose membranes

Four subjects' sera (I–IV) were selected for further study based upon their clinical histories of allergic symptoms, skin prick and RAST testing (Table 1) and the IgE-binding profiles obtained from blotting studies. Two subjects (I and II) were selected because their sera crossreacted strongly on the basis of RAST testing and immunoblotting with both ryegrass pollen and wheat endosperm proteins. Subject I presented with perennial allergic rhinitis with a seasonal exacerbation of symptoms and gave a positive skin-prick test with both ryegrass pollen extract and with a commercial (Dome/Hollister-

Stier, Spokane, U.S.A.) wheat endosperm skin-prick testing extract (albumins and globulins). When placed upon a wheat-free diet he experienced an improvement in his symptoms. Subject II presented with atopic eczema and was considered ryegrass pollen and wheat endosperm protein-sensitive on the basis of RAST and immunoblotting results. Subject II, whose symptoms were perennial but more pronounced in the pollen season, was placed upon a wheat-free diet and obtained significant remission of his symptoms. Subject III, considered from her seasonal symptoms to be pollen allergic, gave a positive skin-prick test with ryegrass pollen extract and the serum gave a high RAST value with ryegrass pollen extract but reacted only weakly with wheat endosperm proteins. Subject IV presented with perennial allergic rhinitis and had experienced an anaphylactic reaction after eating cooked rolled oats. He gave a substantial positive skin-test reaction to the commercial wheat protein extract and to a lesser extent with other cereal grain extracts and a doubtfully positive reaction to ryegrass pollen extract.

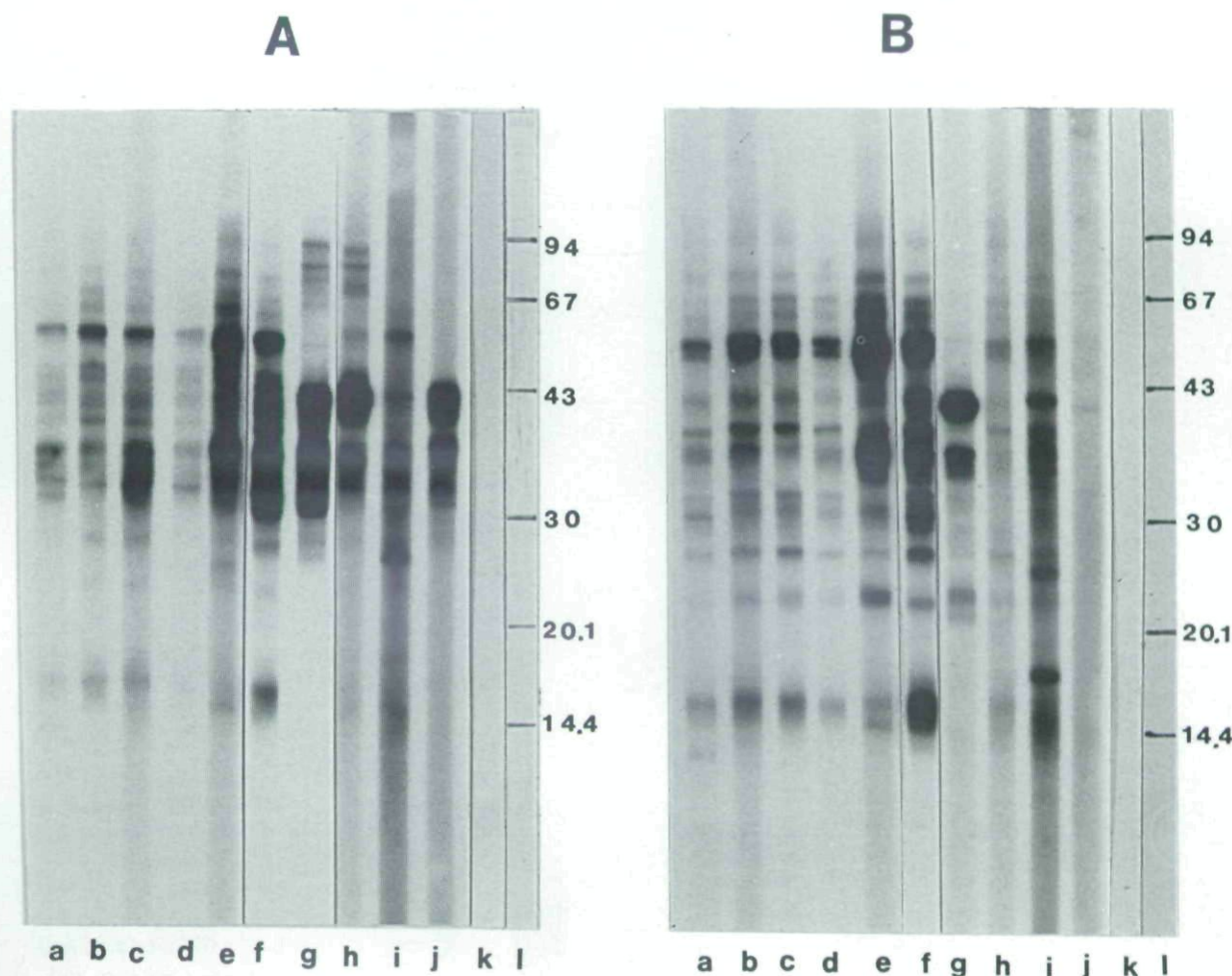


Fig. 2. IgE-binding to Western blots of wheat endosperm total (A) and albumin and globulin proteins (B). (a-j) Serum samples from ryegrass pollen-allergic subjects; (k) control strip incubated with [^{125} I] anti-human IgE only; (l) molecular weight markers (kD).

Table 1. IgE antibody reactivity* of the sera of four subjects to ryegrass pollen and wheat endosperm proteins

Serum	Ryegrass pollen	Wheat albumins + globulins	Wheat gluten (gliadin + glutenin)
I	29.7	16.0	13.5
II	18.0	14.1	14.4
III	43.8	3.6	1.7
IV	8.8	55.3	11.0
FCS	0.9	0.9	1.1

* RAST results with the sera of four ryegrass pollen and/or wheat endosperm protein-sensitive subjects. The RAST method used is described in Materials and methods.

Values given are the percentage uptakes of ^{125} I-labelled anti-human IgE used (20000 c.p.m.) in each assay.

FCS, foetal cord serum.

The serum from subject IV reacted strongly with wheat endosperm proteins and only moderately with ryegrass pollen in the RAST but failed to give IgE-binding bands with ryegrass pollen protein immunoblots. The subject was placed upon a wheat-free diet and his condition improved markedly.

These four sera (Fig. 3) were immunoadsorbed with either ryegrass pollen or wheat endosperm total proteins. The immunoadsorbed IgE antibodies were eluted from the nitrocellulose discs and used to probe blots of both ryegrass pollen and wheat endosperm total proteins. Although there were differences observed in the blot patterns after immunoadsorption of the crossreactive sera (I, II), immunoadsorption of these sera with either the ryegrass pollen or wheat endosperm total proteins significantly reduced the observed residual IgE-binding directed against both ryegrass pollen and wheat endo-

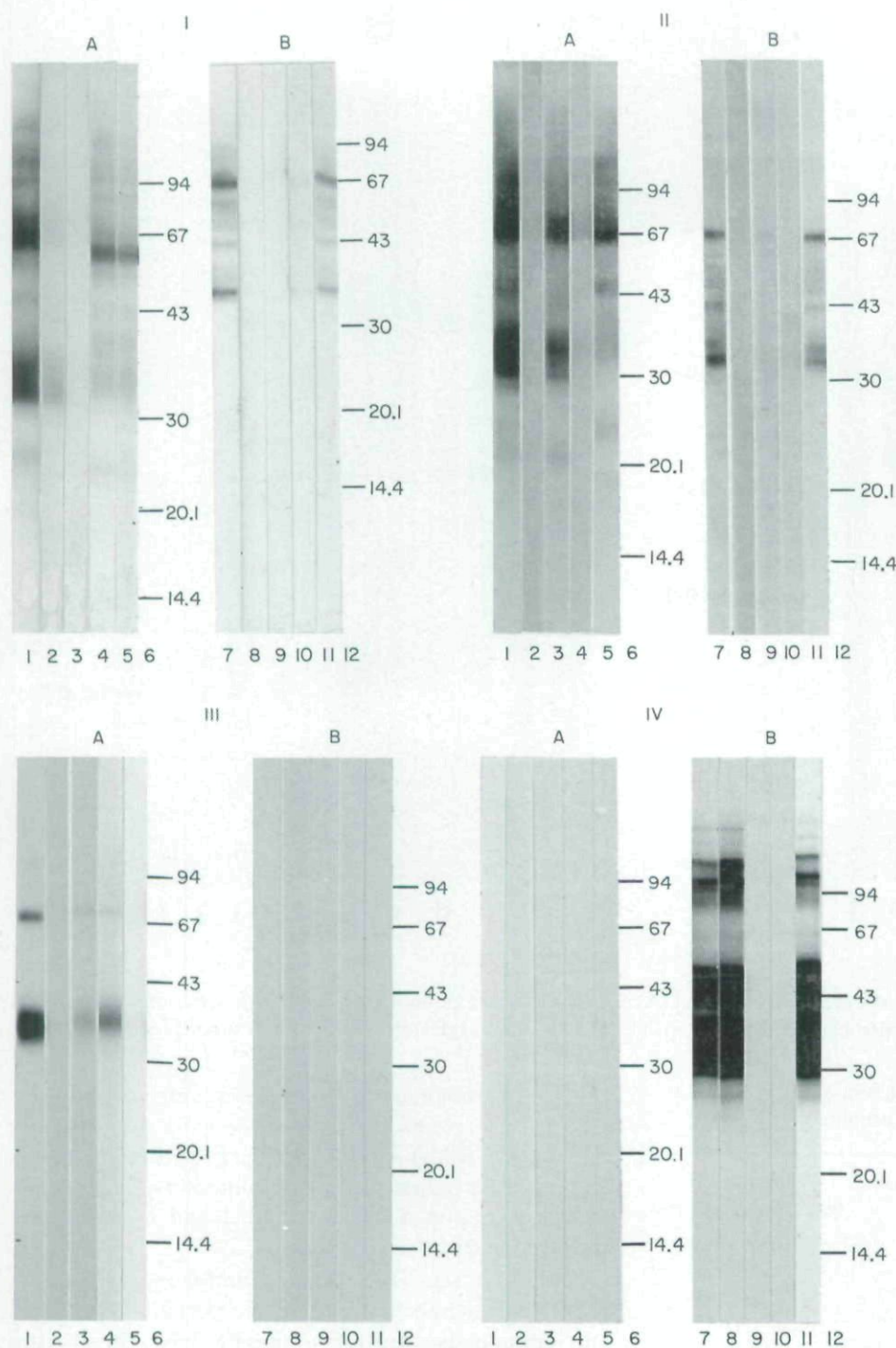


Fig. 3. IgE antibody-binding profiles on Western blots of ryegrass pollen (A) and wheat endosperm (B) proteins after probing with untreated and immunoadsorbed sera and IgE eluates recovered after immunoadsorption. (I-IV) Sera I-IV: (lanes 1 and 7) normal serum; (lanes 2 and 8) ryegrass pollen immunoadsorbed; (lanes 3 and 9) wheat total protein immunoadsorbed; (lanes 4 and 10) eluate from ryegrass pollen immunoadsorption; (lanes 5 and 11) eluate from wheat total protein immunoadsorption; (lanes 6 and 12) molecular weight markers (kD).

sperm total proteins. The IgE-binding patterns of the immunoadsorbed sera are shown in lanes 2 and 3, and lanes 8 and 9. In all cases the immunoadsorption with ryegrass pollen protein is shown in lanes 2 and 8 and the immunoadsorption with wheat endosperm protein is shown in lanes 3 and 9. The IgE-binding patterns of the eluted IgE were quite variable. This was thought to be due to differences in the ease of elution of the adsorbed IgE

antibodies and a reflection of their relative affinity for the different proteins contained in the whole extract. In all cases the IgE elution profiles from the ryegrass disc are shown in lanes 4 and 10 and from the wheat disc in lanes 5 and 11.

Immunoadsorption of serum I with ryegrass pollen and wheat endosperm protein discs efficiently removed virtually all the IgE directed against ryegrass pollen (lanes 2

and 3) and wheat endosperm proteins (lanes 8 and 9). The IgE-binding profiles of the eluted IgE from both ryegrass pollen and wheat endosperm immunoabsorption discs against both ryegrass pollen (lanes 4 and 5) and wheat endosperm proteins (lanes 9 and 10) indicated that elution was not fully efficient. Although the ryegrass pollen protein profiles (lanes 4 and 5) were reasonably representative of the major bands in the untreated serum (lane 1) only the band centred around 50 kD was strong. The strong major band in the 30–35 kD region of the untreated serum was only weakly represented in the elution profiles from either the ryegrass pollen (lane 4) or the wheat endosperm protein (lane 5) discs. The profiles of the eluates with wheat total protein strips (lanes 10 and 11) were less satisfactory than those obtained with the ryegrass pollen strips. IgE-binding of the eluate from the ryegrass pollen disc was barely discernible (lane 10) whereas the profile obtained with the wheat protein disc eluate (lane 11) was qualitatively comparable with that obtained with the untreated serum (lane 7).

Immunoabsorption of serum II with the ryegrass pollen disc was completely efficient in removing all the IgE directed against both ryegrass pollen (lane 2) and wheat proteins (lane 8). However, immunoabsorption with the wheat disc was only partially efficient in removing the IgE directed against ryegrass pollen (lane 3) although it was almost completely efficient in removal of IgE directed against wheat proteins (lane 9). The IgE-binding profile of the eluate from the ryegrass disc, although weak, was qualitatively similar to the untreated serum with the ryegrass strip (lane 4), however, the eluate from the wheat disc (lane 5) gave a profile qualitatively similar to the untreated serum but gave strong bands for proteins greater than 60 kD and for the major band centred around 35 kD in the untreated serum. The IgE-binding profile of the eluate from the ryegrass disc with the wheat blot strip (lane 10) was weak but showed limited qualitative similarity to the untreated serum. The eluate from the wheat disc (lane 11) gave both good quantitative recovery of IgE antibodies and a profile similar to the untreated serum.

No IgE-binding to wheat protein blot strips was observed with serum III, although it gave a low RAST value of 3.6% to wheat endosperm albumins and globulins which was fourfold greater than background (see Table 1). Some low molecular weight albumins below 10 kD exist in wheat endosperm and might bind IgE and account for this low RAST. Proteins of this molecular weight, however, would run off the bottom of the gel during electrophoresis and therefore would not be represented on the blot but would probably be present on the immunoabsorption disc. Immunoabsorption of serum III with the wheat protein disc reduced the IgE-binding of the

treated serum. This was surprising since it did not give an IgE-binding profile with a wheat blot strip. Apart from the possible presence of low molecular weight IgE-binding proteins on the immunoabsorption disc, carbohydrate might account for the reduction in IgE-binding. A number of complex carbohydrates, including starch and several pentosans are found in wheat endosperm and one of these might also account for the reduction in IgE binding. Despite the reduction in IgE binding as a result of immunoabsorption with the wheat disc, no elution profile was obtained with the ryegrass blot strip (lane 5). Immunoabsorption of this serum with the ryegrass disc was totally efficient (lane 2) and the elution profile (lane 4) was qualitatively similar to the untreated serum.

No IgE-binding profile was obtained with serum IV with ryegrass blot strips despite this serum (undiluted) giving a RAST value of 8.8%. This IgE-binding might well be associated with a low molecular protein or a carbohydrate moiety and may not be present on the Western blot. No obvious reduction in IgE binding was observed with ryegrass pollen immunoabsorption (lane 8) and no IgE binding to a wheat blot strip could be detected in the eluate from the disc (lane 10). Immunoabsorption of serum IV with the wheat disc was almost complete (lane 9) and the elution of the adsorbed IgE was efficient and gave a profile qualitatively and quantitatively equivalent (lane 11) to the untreated serum (lane 7).

Discussion

The occurrence of 19 subjects having positive RAST uptakes (in excess of 5% uptake) for wheat endosperm proteins out of a total of 78 subjects who had both a positive skin-prick test and RAST uptakes (>5%) to ryegrass pollen proteins is clear evidence that wheat endosperm proteins are potentially important allergens.

The observations of crossreaction of serum IgE antibodies on blots of proteins from distantly related sources can be explained by either two populations of antibodies separately recognizing the proteins for each source, or by the two sources of proteins sharing common structural determinants to which the same IgE antibodies bind. The results of immunoabsorption of the serum IgE molecules by a form of affinity chromatography in which the allergens were attached to nitrocellulose discs, as reported in this paper, provide strong evidence that both ryegrass pollen and wheat endosperm allergens share common structurally related determinants. In particular, the adsorption/elution results reported in this study with IgE antibodies from the crossreactive sera of subjects I and II confirm this claim and the observation correlates well with their clinical symptoms.

Affinity chromatography of antibodies using antigens

attached to solid supports for the purification of antibodies is a standard method in the preparation of monospecific antibodies from serum. This technique has its parallel in this study by the use of allergen proteins attached to nitrocellulose membranes to both immunoadsorb and to affinity purify IgE antibodies from sera of subjects exhibiting crossreactivity to both ryegrass pollen and wheat endosperm proteins. The antibody specificity of this technique can be illustrated by a recent study on the house dust mite allergens [16]. Using an excised band from a Western blot of a cloned house dust mite IgE-binding protein as the immunoadsorbant, it was shown that the immunoadsorbed and eluted IgE antibodies faithfully bound not only to the cloned protein, but also to the same allergen band of the whole mite extract. An additional argument to support the antibody specificity of the methods used in this study is the very low concentration of IgE antibodies in serum relative to the other immunoglobulin classes. Their further dilution in this study would be expected to improve their specificity.

Immunoadsorption greatly reduced the residual IgE antibody levels in treated serum samples, but it was not always successful in removing all of the IgE. The efficiency of immunoadsorption of IgE antibodies from diluted serum will depend to a great extent on the IgE antibody concentration in the serum. To some extent the apparent inability of immunoadsorption to remove all of the IgE antibodies totally may have been due to high antibody levels for which there was insufficient immunoadsorbant surface to remove all of the IgE antibodies from the sample. The two examples of strong crossreactivity on the basis of RAST testing (sera I and II) in which immunoadsorption with either ryegrass pollen or wheat endosperm proteins reduced the observed IgE binding to wheat and ryegrass pollen protein blot strips showed differences which might in part be accounted for by these factors. Although it was possible to immunoadsorb the IgE antibodies efficiently using the two different protein extracts as immunoadsorbents, they were difficult to elute efficiently as judged by their IgE-binding profiles. These were in most cases weaker than the untreated serum, probably requiring longer autoradiographic exposure and in some cases, qualitatively different. Nonetheless the IgE-binding profiles of the eluates in most instances complemented the immunoadsorption and indicated the relative affinity of the IgE antibodies for the different allergenic proteins on the basis of the ease of elution.

The pattern of crossreactivity based upon related allergenic determinants in allergenic proteins from different sources has implications in the clinical management of these patients. Where IgE antibodies are directed against the same or similar determinants in the different protein sources, both must be regarded as potentially involved in

the sensitization of the subject and avoidance of both sources might be considered a good therapeutic measure, especially when looked at in conjunction with the clinical histories of the allergic subjects. Desensitization with one of the allergenic proteins may result in desensitization to the other. The crossreactivity studies here suggest that the seasonal pollinosis is likely to be exacerbated by a diet of wheaten products and may provide continuous sensitization during the remainder of the year.

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