

Crossed Radioimmuno-electrophoretic Analysis of Cultivated Rye (*Secale cereale*) Pollen Allergens

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Abstract. Cultivated rye pollen extracts were characterized by means of crossed immunoelectrophoresis (CIE) and crossed radioimmuno-electrophoresis (CRIE). 32 antigens were identified in CIE and among these 16 were radiostained in CRIE analysis of a rye pollen sensitive patient panel. Crossed line immunoelectrophoresis revealed that some of the rye pollen antigens were immunological partially identical with antigens of wheat flour and rye flour.

Introduction

Allergic reactions following inhalation of cereal proteins are well recognized [1–4]. Wheat and rye flour proteins are important allergens and particularly the flour dust may cause asthma and rhinitis in bakers [1, 4]. Extensive biochemical and immunochemical characterization of wheat and rye flour extracts have been reported [2, 3, 5, 6]. On the other hand relatively few studies have been performed on the characterization of cultivated rye pollen extracts. Kalveram et al. [7] have studied the antigenic relationship between grass and cultivated rye pollens by means of skin test and Rast.

In this study we report on the antigenic and allergenic composition of cultivated rye pollen extracts. Furthermore, the specificities of IgE antibodies reacting with individual rye pollen proteins were investigated in individuals with clinical symptoms related to rye pollen inhalation.

Materials and Methods

Allergen Extracts

Rye pollen (*Secale cereale*) was obtained from Allergon AB, Sweden, as dried nondefatted material. A crude extract of cultivated rye was prepared by extraction of rye pollen (extraction ratio 1:10, w/v) in PBS buffer (0.05 M phosphate, 0.075 M NaCl), pH 7.2, for 18 h in a cold room (4–8°C). After extraction the material was filtered to get rid of the debris, lyophilized and stored at –20°C until use.

Extraction of rye flour (fine rye flour; Kungsörnen, Sweden) and wheat flour (Kungsörnen, Sweden) were performed by shaking a 10% (w/v) suspension of each species in a cold room for 16 h in distilled water. The suspension was cleared by centrifugation (40,000 g for 20 min) and by passing the supernatant through a filter of 0.22 µm. The filtrates were then desalted on Sephadex G-25 (Pharmacia Fine Chemicals), lyophilized and stored at –20°C until use.

Rabbit Antisera

Antisera towards rye pollen proteins were prepared substantially as described by Harboe et al. [8] by immunization of 6 rabbits using 1,000 µg (d.w.) of rye allergenic extract per immunization and rabbit, in Freund's complete adjuvant. The rabbits were boosted every 6 weeks and bled 7 days after each immunization. The rabbit antisera used were pooled sera from many bleedings.

Patient Sera

Human patient sera were collected from patients allergic to cultivated rye pollen (from clinical history). The patients used in this study had not been subjected to immunotherapy.

IgE

Total and specific IgE were determined using Phadebas Prist® and Phadebas Rast® kits (Pharmacia Diagnostics, Uppsala, Sweden), respectively, according to the manufacturer's instructions.

Crossed Immunoelectrophoresis (CIE)

CIE was performed on 8 × 8 cm glass plates essentially in accordance with the previously described procedure [9, 10] using 0.025 M Tris-tricine buffer, pH 8.6, containing 0.0005 M calcium acetate in 1% Agarose (Agarose A; Pharmacia Fine Chemicals, Uppsala). Some components showed cathodic migration, therefore both an anodic and cathodic second dimension gel containing rabbit antisera were used. Samples of 5 µl of crude rye allergen extract (7.5 mg/ml) were run in the first dimension electrophoresis for 60 min with 10 V/cm at 15°C. The second dimension electrophore-

sis was performed for 20 h with 2 V/cm at 15°C, using 20 μ l rabbit antiserum/cm² gel.

Crossed-line immunoelectrophoresis (CLIE) was performed as previously described by Axelsen et al. [9].

Crossed Radioimmunoelectrophoresis (CRIE)

The washed CIE-plates were incubated with 0.74 ml of the patients' serum diluted to 13 ml with CRIE incubation buffer (0.05 mol/l phosphate buffer, pH 7.4, containing 0.9% sodium chloride, 3.2% Tween and 0.3% Dextran 10 for 18 h at 22°C in suitable plastic boxes. After washing the CIE-plates were incubated with ¹²⁵I-anti-IgE (Phadebas Rast Isotope Reagent) in 13 ml total volume (72,000 cpm/ml) for another 18 h at 22°C. After washing and drying the plates were placed on X-ray photographic film for autoradiographic exposure. All plates were exposed several times, using time periods varying from 1 to 27 h [11]. Each exposure corresponded to one particular CRIE class, using the CRIE classification system previously described [12, 13]. As a negative control, a patient serum (8369) not sensitive to rye pollen proteins was utilized (Phadebas Rast class 0, total IgE 16.3 kU/l).

Protein Determination

The protein contents of rye pollen extract, rye flour extract and wheat flour extract were determined by a modified Lowry procedure [14].

Results and Discussion

Figure 1a demonstrates the CIE pattern of cultivated rye pollen extract (*Secale cereale*) against hyperimmune rabbit antisera after protein staining with Coomassie brilliant blue. Many well-resolved components can be detected after protein staining of the CIE-plate. In figure 1b the CIE-reference pattern of rye allergen extract (a schematic drawing) is demonstrated with arbitrarily numbered immunoprecipitates from anode to the cathode. The presence of 32 different components is illustrated in the CIE-reference pattern of cultivated rye. Two immunoprecipitates (Ag-22 and Ag-32) were not visible when protein stained and therefore could only be detected after incubation with patient serum in CRIE. Most of the components in the studied rye extract are acidic in nature and migrate against the anode, while some are neutral and alkaline. Analysis by CRIE of 14 clinically rye pollen positive patients' sera was performed by incubating the CIE-plate with patient serum for specific IgE binding followed by incubation with isotope. The CRIE data together with Rast data and total IgE levels are summarized in fig. 3. The background radiostaining (unspecific IgE binding) was very low. By incubating CIE-plates with negative patient serum (8369) or saline followed by incubation with isotope

1a

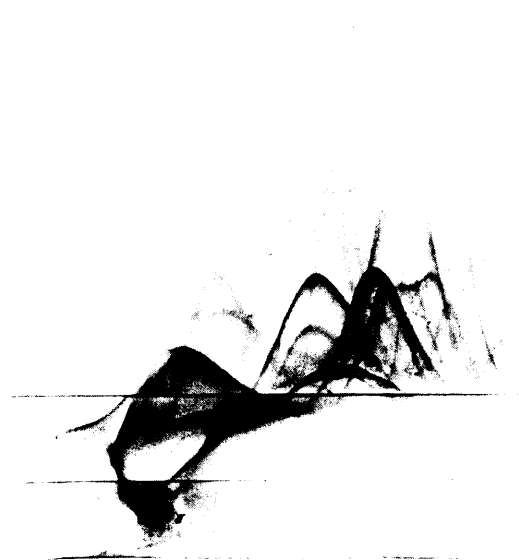


Fig. 1. a Crossed immunoelectrophoresis of cultivated rye pollen extract against hyperimmune rabbit antisera. 5 μ l of rye pollen crude extract (7.5 mg/ml) electrophoresed (pH 8.6) with 10 V/cm, 15°C for 20 h in agarose containing rabbit anti-rye pollen protein serum (20 μ l antiserum/cm² gel). Anode to the right in first dimension and at top in second dimension. **b** CIE reference pattern of cultivated rye extract, a drawing with arbitrary numbers assigned to the precipitates. Two antigens (dotted lines) could only be detected by means of CRIE radiostaining.

solution no radiostaining at all was detected after exposure even in CRIE class G. For that reason radiostaining corresponding to CRIE class G may be used as specific for evaluation of data in fig. 3. As shown in fig. 3 it was evident that 16 different immunoprecipitates gave specific IgE binding.

Some patients responded to many different antigens, whilst others gave radiostaining to only a few individual antigens. One immunoprecipitate (Ag-3) was detected in just one particular patient serum (K.S.), and this patient serum exhibited a very dense radiostaining (CRIE class A) against this particular antigen. Many of the resolved protein components did not bind specific IgE from any of the studied patient sera and must consequently be regarded to be unrelated to allergenic activity in rye pollen extracts.

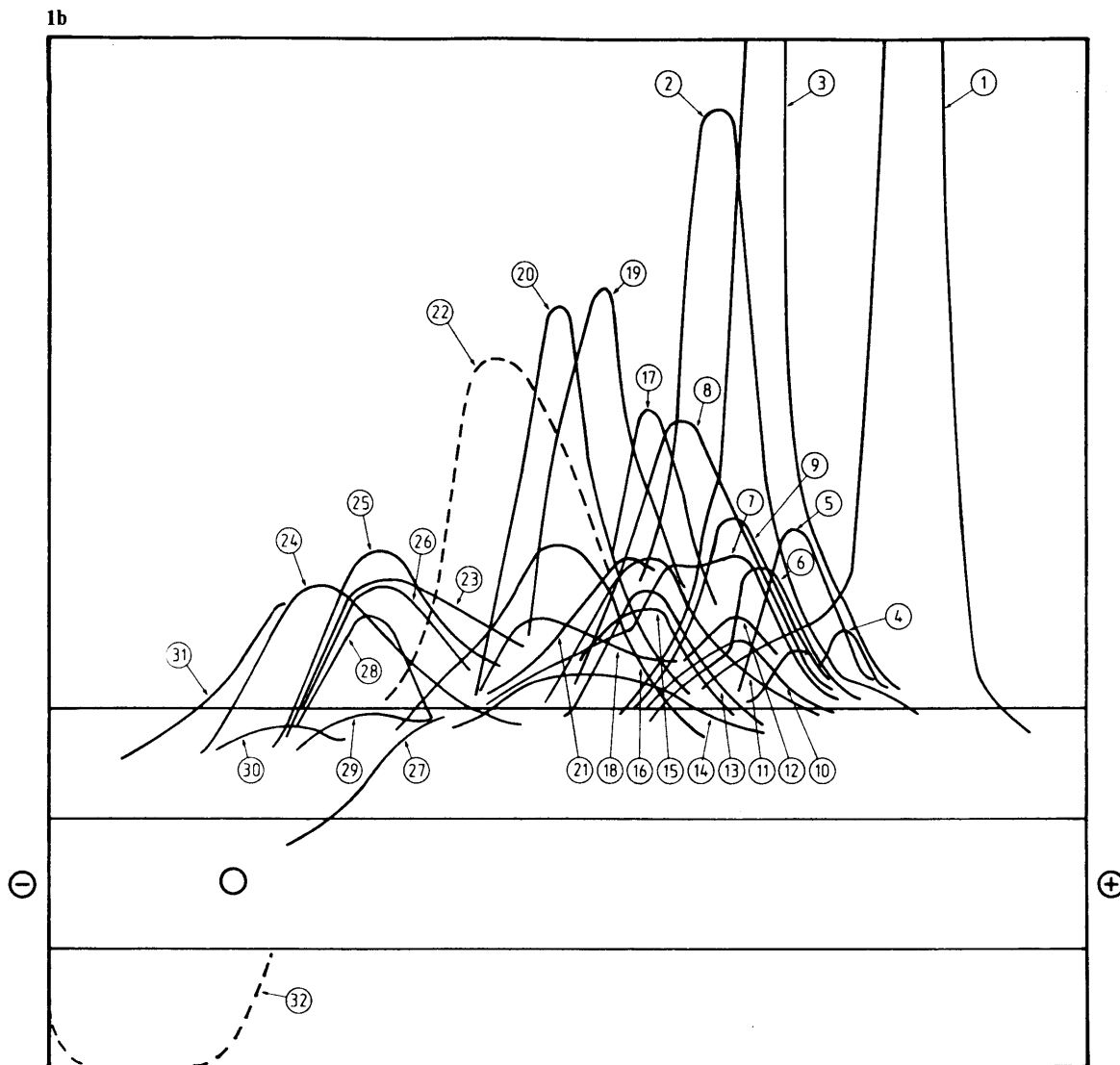


Figure 2 demonstrates some examples of developed CRIE films on patient sera presented in figure 3.

Based upon the frequency and density of radiostaining of individual components (degree of specific IgE binding) in the patient panel (fig. 3), a more precise definition of major intermediate and minor allergens [11, 12] in rye pollen extract was established. 5 antigens are considered as major allergens (Ag-24, Ag-26, Ag-27, Ag-29 and Ag-30), 5 as intermediate allergens (Ag-16, Ag-18, Ag-22, Ag-31 and Ag-32) and at least 6 as minor allergens (Ag-3, Ag-11, Ag-14, Ag-15, Ag-20 and Ag-28). When trying to relate the CRIE pattern for individual patients to the total IgE levels (fig. 3) no definite pattern could be established. On the other hand, the Rast-values and the intensities of CRIE radiostaining seem to correlate very well.

Immunochemical cross-reactivity between cultivated rye pollen antigens, rye flour antigens and wheat flour antigens were analysed directly without any prior separation by means of CLIE. It must be observed, however, that the proteins extracted from rye flour and wheat flour are the water-soluble fraction only. Rye pollen proteins were electrophoresed in the first dimension and subsequently rye flour proteins or wheat flour proteins were applied in the line well prior to starting the second dimension electrophoresis, into the agarose gel containing rabbit antisera raised against rye pollen proteins. Antigens No. 7, 11, 14 and 15 of cultivated rye pollen extract were partially identical to antigens in rye flour, while antigens No. 12 and 14 were partially identical to antigens of wheat flour.

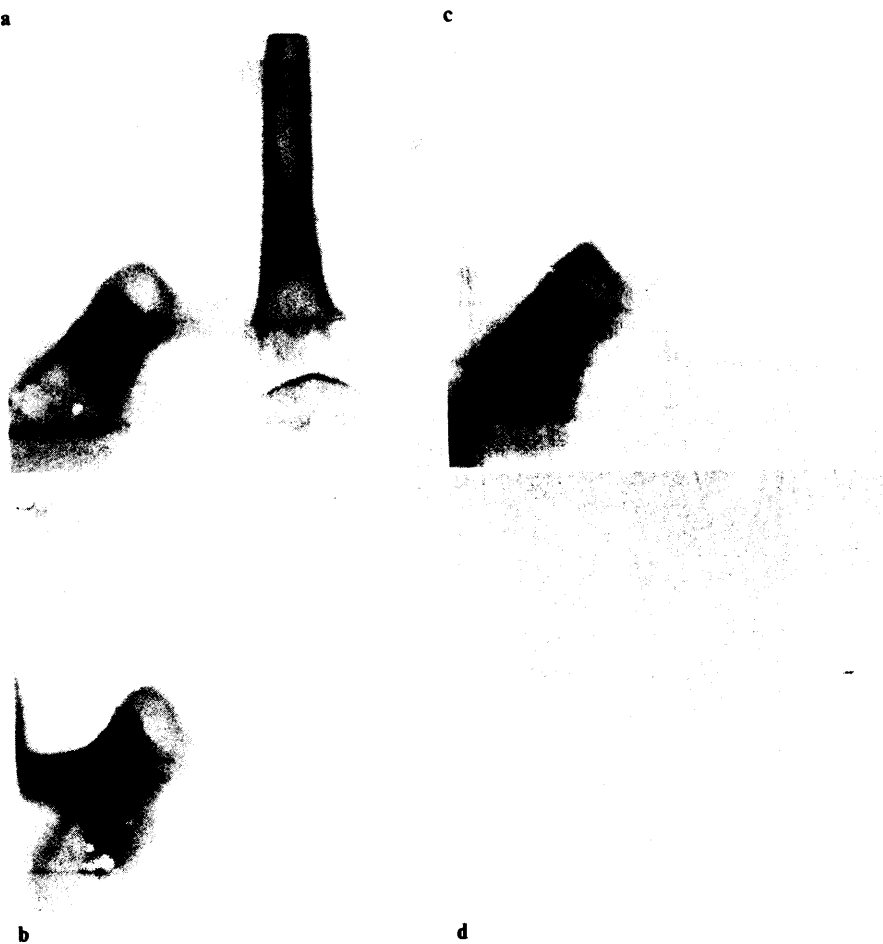


Fig. 2. X-ray films from CRIE analysed patients in figure 1. Radiostaining corresponding to CRIE class G is shown for 3 different patients, patient K.S. a; P.J. b; S.N. c, and a control patient serum 8369 d.

Patient	IgE U/ml	Antigen																																			
		RAST PRU/ml	No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32		
B.A.	7.6	60.5																																			
P.J.	172.5	1300.0																																			
K.P.	25.5	188.5																																			
K.S.	46.0	720.0																																			
S.N.	24.2	88.0																																			
H.A.	1.4	212.5																																			
H.A.	31.0	2513.0																																			
M.H.	2.6	105.5																																			
L.N.	20.5	595.0																																			
S.M.	12.5	138.0																																			
R.J.	10.0	81.5																																			
A.M.	19.3	122.0																																			
G.T.	16.8	340.0																																			
PO.W.	6.4	23.0																																			

The intensity of radiostaining is shown as hatched squares symbolizing the CRIE classes: ■ = A; ▤ = B; ▥ = C; ▦ = D; ▧ = E; ▨ = F; ▩ = G.

Fig. 3. Total and specific IgE against cultivated rye and CRIE radiostaining of the various precipitates in a CIE reference pattern of cultivated rye pollen extract.

A quite different approach was used by *Blands et al.* [5] when they studied immunological cross-reactivity between cereal antigens and grass pollens. Using rabbit antisera against wheat flour proteins they found that between 2 and 7 grass pollen antigens, depending on species, showed partial identity to wheat flour antigens. Our findings, in which we used rabbit antisera against rye pollen extracts, were similar further demonstrating the existence of the high degree of immunological cross-reactivity between different grass pollens [15].

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