

Rationale for Specific Immunotherapy of Grass Pollen Allergy with Extracts of Rye Pollen

Skin Test Reactivity and Immunochemical Relationship between Pollen Allergens from Rye and Other Common Grasses

H. LØWENSTEIN,¹ J.-Å. WIHL,² K. BACHE BILLESBØLLE¹ and H. BØWADT³

¹The Protein Laboratory, University of Copenhagen, Denmark, ²E.N.T. Clinic, Malmö General Hospital, University of Lund, Malmö, Sweden, and ³ALK Research Group, Copenhagen, Denmark

In immunotherapy of grass pollen allergy, an extract of rye (*Secale cerebale*) is often included. The aim of this study was to investigate by skin prick test (SPT) and immunochemical methods whether rye pollen contains specific allergens justifying the use of this extract separately. Twenty grass pollen allergic patients were skin prick tested with a dialysed freeze-dried raw extract of rye pollen (Sc), timothy extract (Soluprick® SQ, 1 HEP) and two other rye extracts (Soluprick®). Sera from the patients were RAST-tested using Sc and timothy (Pp). CRIE was performed using Sc and rabbit-anti grass (aNG) antibodies. The antigenic relations between rye and common grasses were investigated by CLIE using Sc and aNG as references, and by RAST inhibition. The ability of aNG to absorb the allergen activity of Sc was also tested. Significant correlations were found between timothy and rye when compared by means of SPT and RAST. The immunochemical analyses did not reveal any rye antigens containing rye epitopes only. However, the possibility of rye antigens with several epitopes, of which at least one is specific for rye, could not be excluded. Clinical symptoms supposedly elicited by rye alone can be explained quantitatively by the strongly time-limited and concentrated natural exposition. Diagnosis and treatment can, however, be performed with extracts of common grasses.

Key words: rye, grass pollen; immunotherapy.

Accepted for publication 7 January 1984

Grass pollen allergy is the most common reason for immunotherapy in northern Europe. Already in the early years of this century it was obvious that a high degree of cross-reactivity existed between different grasses (6). In this part of the world there are around 100 species of wild and cultivated grasses.

During the years, timothy (*Phleum pratense*) has been used for immunotherapy as it was supposed to have a broad allergen content sufficient for a good therapy result against most grasses. In an attempt to improve the efficacy of immunotherapy a mixture of five to six common grasses, including timothy, came into

common use in the middle of this century. A further step was the recommended use of tailor-made extracts containing 10 to 13 grasses from different species depending on whether to be used in northern or southern Sweden (9).

During recent years the pendulum seems to swing back again to the use of timothy alone. The reason for this is the growing demand for partially purified allergen extracts containing mainly antigens of major allergenic importance (7, 24). Modern separation and immunochemical methods have made this possible, but the techniques are time-consuming and too expensive to be used on a greater number of



Fig. 1. A Scandinavian rye field with a cloud of pollen above. (Photo: S. O. Strandhede).

grasses. Further, the increasing knowledge of patients' individual response towards allergenic components has shown that "the general optimal allergenic extract" does not exist (14), thus limiting this development.

In Germany, Denmark and southern Sweden it has for many years been a tradition to supplement grass immunotherapy by giving an extract of rye (*Secale cereale*) separately. There are several reasons for this. Rye is the only truly wind-pollinating species of the four commonly grown cereals in this part of the world. The other three, wheat (*Triticum sativum*), oats (*Avena sativa*) and barley (*Hordeum* spp.), are all to a large extent self-pollinating, leaving most of their pollen inside the flower. Close vicinity to a flowering field of rye is without doubt a situation in which the heaviest natural exposition to grass pollen occurs (Fig. 1). In Denmark and Sweden rye is cultivated yearly on approximately 84,000 and 80,000 hectares, respectively. This is about 3% of the total arable land. In the middle of June the rye fields present a

substantial risk of exposure to large amounts of rye pollen.

Another reason for using rye pollen extract separately has been the belief that it contains specific allergenic epitopes not present in pollen from other grasses. This was reported from Holland using a method developed by Dale whereby a sensitized guinea pig uterus showed contractions when exposed to allergen extracts (2). In a more recent study from Western Germany the existence of a cereal-specific antigen determinant is discussed (19).

The aim of this study is to investigate whether rye pollen contains specific allergens justifying the use of rye pollen extracts separately in immunotherapy of grass sensitive patients.

This will include a study of the immunochemical relationship between the antigens from rye and other grass pollens, and of the response of a selected patient population to these allergen sources. Furthermore, it will involve an identification of the major allergens in a standard rye pollen extract by means of CRIE (crossed radio

immunoelectrophoresis) as well as a definition of the 1 HEP (histamine equivalent prick) value for our defined standard rye pollen extract.

MATERIAL AND METHODS

Twenty patients with a convincing history of allergic rhinitis during the grass season were investigated. They also fulfilled the inclusion criteria proposed by Eriksson (5). Eleven had suffered from symptoms for 3–10 seasons and nine for more than 10 seasons. Eight were women, mean age 27.3 years (range 14–50), and 12 men, mean age 22.6 years (range 15–34). None of the patients had previously undergone specific immunotherapy. All the selected patients showed a positive nasal provocation test towards rye pollen (20).

In a preliminary RAST study all had a positive timothy-RAST (results given in Phadebas RAST class): Fourteen patients were in class 4, five patients in class 3, and one in class 2. Thirteen patients had a PRIST® value higher than 100 ku/l (range 150–1200), six patients had PRIST values under 100 (range 40–100). For one patient the PRIST value is missing. Eighteen of the patients were also sensitized to other kinds of pollen such as birch (*Betula* spp.) and mugwort (*Artemisia vulgaris*) which was shown by prick test or RAST, and five were sensitized to cat and/or dog epithelium.

Allergen extracts

Rye (*Secale cereale*) pollen samples were obtained from Allergon, Engelholm, Sweden, as dry non-defatted material which had been stored at 4°C in tightly sealed containers. After delivery, the samples were stored in their original containers at –20°C until used. According to the manufacturers, the samples were >95% pure rye pollen grains plus approximately 5% non-pollen fragments of rye; other pollen species were approximately 0.1%.

Dialyzed freeze-dried extract from a 1975 collection of this pollen, denoted Sc, was prepared using a procedure similar to that previously described for other grass pollens (12, 18). Briefly, the method consisted of extracting

the pollen (1:10 w/v) at 5°C for 20 h with a buffer solution (0.125 M NH_4HCO_3 and 0.015 M NaN_3 , adjusted to pH 7.5), centrifugation (50,000 $\times$ g, 1 h, 0°C), dialysis (3 $\times$ 24 h, 5°C, using Union Carbide (Wisking) dialysis tubing with limiting pore size corresponding to approximately molecular weight <5,000) and freeze-drying. Solutions (1–20 mg freeze-dried material/ml) were freshly prepared in 0.030 M NaHCO_3 , 0.085 M NaCl /glycerol (50%, v/v) for use in QIE (quantitative immunoelectrophoresis) technique and RAST or RAST inhibition. NaHCO_3 (0.1 M), NaN_3 (0.015 M) solution was used for immunization of rabbits. Similarly prepared pollen extracts from other grasses: false oat (*Avena elatior*), orchard grass (*Dactylis glomerata*), meadow fescue (*Festuca pratensis*), rye grass (*Lolium perenne*), common reed (*Phragmites communis*), and timothy (*Phleum pratense*) were produced on earlier occasions in our lab (11, 16, and unpublished results) and used for comparative studies. Timothy Ag 19/25 was prepared as described recently (12).

Skin prick tests (SPT)

SPT were performed with five concentrations (half decade serial dilutions from 0.01 to 1 mg/ml) of the above mentioned standard Sc as well as two rye pollen extracts (Soluprick) and a standardized timothy pollen extract (Soluprick SQ, 1 HEP) from ALK, Copenhagen, Denmark). These were all dissolved in 0.030 M NaHCO_3 , 0.085 M NaCl /glycerol (50%, v/v) which was also used as a negative control. Positive control: 1 mg/ml histamine dihydrochloride in the same solvent.

SPT were performed 2–3 months after the pollen season. The tests were performed in duplicate with a conventional blood lancet using both lower arms and making an acute angle of 45° between lancet and skin. After 15 min the weals were outlined and, by means of a transparent tape, transferred to mm²-squared paper. Calculations of the results of the skin prick tests were obtained by cutting out a magnified area of the skin weal area and weighing and plotting the mean sum of the log to the skin indices:

$\frac{\sum \log SI}{n}$, where

$$SI = \text{skin index} = \frac{\text{skin reaction to extracts}}{\text{skin reactions to histamine (1 mg/ml)}}$$

for both left and right arm reactions versus the various concentrations of the standard Sc-preparations used. From this curve both the 1 HEP concentration for the standard (given in $\mu\text{g/ml}$) and the HEP concentrations for the three Solu-prick pollen extracts were calculated.

Antibodies

Anti-Sc antibodies were raised by immunizing three rabbits with Sc. The immunization and subsequent isolation of the immunoglobulins were performed according to Harboe & Ingild (8). During isolation the concentration of the immunoglobulins was increased about five-fold. The antibodies were stored at 4°C in a solution containing 0.015 M NaN_3 and 1% (v/v) aprotinin (NOVO, Mainz, F.R.G.) to avoid proteolytic degradation of the antigens in the second dimension gel immunoelectrophoresis (3). Anti-timothy, -meadow fescue, -orchard, -ryegrass, -false oat, and -common reed had all been obtained by a similar procedure on earlier occasions (11, 16). Anti-Ng was a mixture of anti-timothy, -orchard, -false oat, and -timothy Ag 19/25 (12) representing a total of 15 immunized rabbits.

Quantitative immunoelectrophoresis (QIE)

QIE methods including crossed-, crossed-line-, crossed- with intermediate gel-, and crossed radio-immunoelectrophoresis were performed as described recently (13) and as specified in the figures. In brief, crossed immunoelectrophoresis (CIE) was performed as follows: Electrophoresis of the antigen was carried out in agarose gel at pH 8.6, followed by a two-dimensional electrophoresis into a gel containing a purified IgG fraction of the relevant antibodies.

Crossed-line immunoelectrophoresis (CLIE) was performed in order to identify antigens specific for various grass pollens as compared with antigens from the rye pollen extract. The procedure was like CIE for a grass/anti-grass system except that in the second dimension run a gel containing rye pollen extract was introduced between the first dimension gel and the antibody-containing gel. The precipitated antigens from the investigated grass pollen extracts were then identified as specific for the pollen as compared with rye pollen antigens.

Crossed immunoelectrophoresis in the Sc/anti-Sc system with intermediate gel containing rabbit anti-NG antibodies was performed in order to study the capacity of the latter antibodies to precipitate rye antigens as compared with that of anti-Sc.

Crossed radiomunoelectrophoresis (CRIE) was performed in order to identify rye pollen allergens, and the technique was carried out as described in detail elsewhere (21). The procedure is a combination of an ordinary CIE and binding of allergic patients' specific IgE to native crossed immunoprecipitates (13). The native crossed immunoprecipitates (Sc versus anti-NG) were incubated with ^{125}I -anti-IgE, washed, followed by autoradiography. The autoradiography was performed from day zero to day 1, day 1 to day 4, and day 4 to day 14, corresponding to 1, 3, and 10 days, respectively. The degree of radiostaining of individual precipitates was evaluated by visual comparison of CRIE autoradiographies (140 nCi, ^{125}I -anti-IgE) with autoradiographies of ^{125}I -anti-IgE Mancini rings (SRRID, 25) (standard internal reference for CRIE studies performed at the Protein Laboratory). For each CRIE autoradiography, the score for the individual precipitates was evaluated independently by at least two persons.

RAST techniques

RAST was performed as described elsewhere (4, 22) on serum samples from blood drawn from each skin-prick-tested patient. A standard timothy pollen extract and Sc coupled to paper discs (coupling conditions: 1 mg/ml) were used as allergosorbent. RAST inhibition experiments

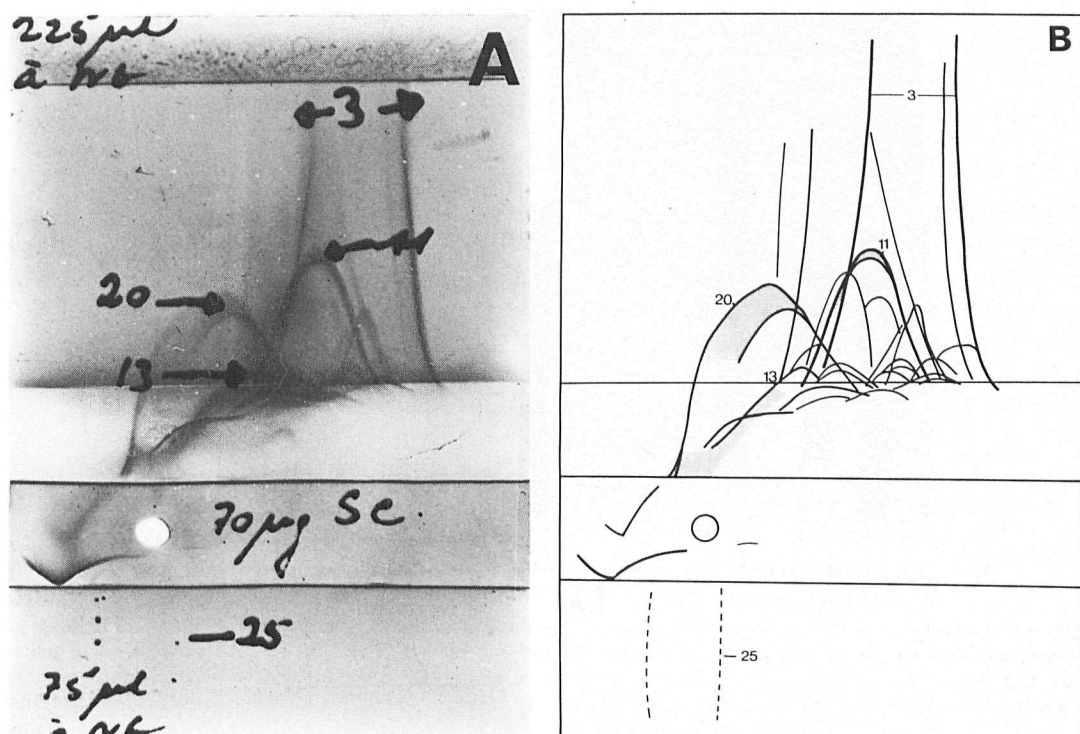


Fig. 2. Crossed immunoelectrophoretic (CIE) analysis of the reference rye pollen extract, Sc, against anti-NG. CIE was performed in 1% agarose gel containing 0.073 M Tris, 0.024 M barbital, 0.0006 M calcium lactate, and 0.0003 M sodium azide (pH 8.6, 25°C) at 15°C using 10 V/cm for 30 min in the first dimension and 2 V/cm for 15 h in the second dimension. Anodes to the right for the first dimension and at the top for the second dimension. An intermediate gel containing no antigens or antibodies was placed between the first dimension gel and the anodic antibody-containing gel in order to improve the resolution in the second dimension. Amounts of antigens and antibodies are indicated in the figure. B is a drawing of the reference precipitation pattern (Sc vs. anti-NG) with classification numbers for the precipitates and corresponding antigens.

were performed as described elsewhere (23) using the above-mentioned allergosorbents. Inhibitors were Sc, a standard timothy pollen extract, and a standard horse dander extract (17) in half decade serial dilutions from 1–0.001 mg/ml. The inhibition was performed using a pool of sera from six rye pollen-allergic patients (Nos. 5, 6, 7, 8, 9, and 11).

Immunoabsorption experiments were performed in order to determine the allergenic inhibitory capacity of anti-Sc antibodies as compared with a nonsense antibody (anti-horse dander). Cyanogen bromide-activated Sepharose 4B-coupled-anti-Sc and -anti-horse dander antibodies were used as column material (3 ml) and 0.5 mg Sc dissolved in 100 µl equilibration buffer (0.05 M sodium citrate buffer, pH 6.2) was applied to both columns. The columns were

eluted with subsequent application of 1 column volume of the following buffers: Equilibration buffer, 0.05 M sodium citrate buffer, pH 6.2, 0.5 M sodium chloride; 0.05 M citrate buffer, pH 6.2, 1.0 M sodium chloride. The Sc-containing fractions from the anti-horse column (as determined by means of fused rocket immunoelectrophoresis using anti-Sc (13)) and the corresponding fractions from the anti-Sc column were collected. Serial dilutions (half decade from undiluted to 10^{-3}) were used to inhibit Sc-paper discs, and RAST performed as described above.

RESULTS

Figs. 2 A and B show examples of the CIE reference precipitation pattern obtained with water-extractable, non-dialysable rye pollen

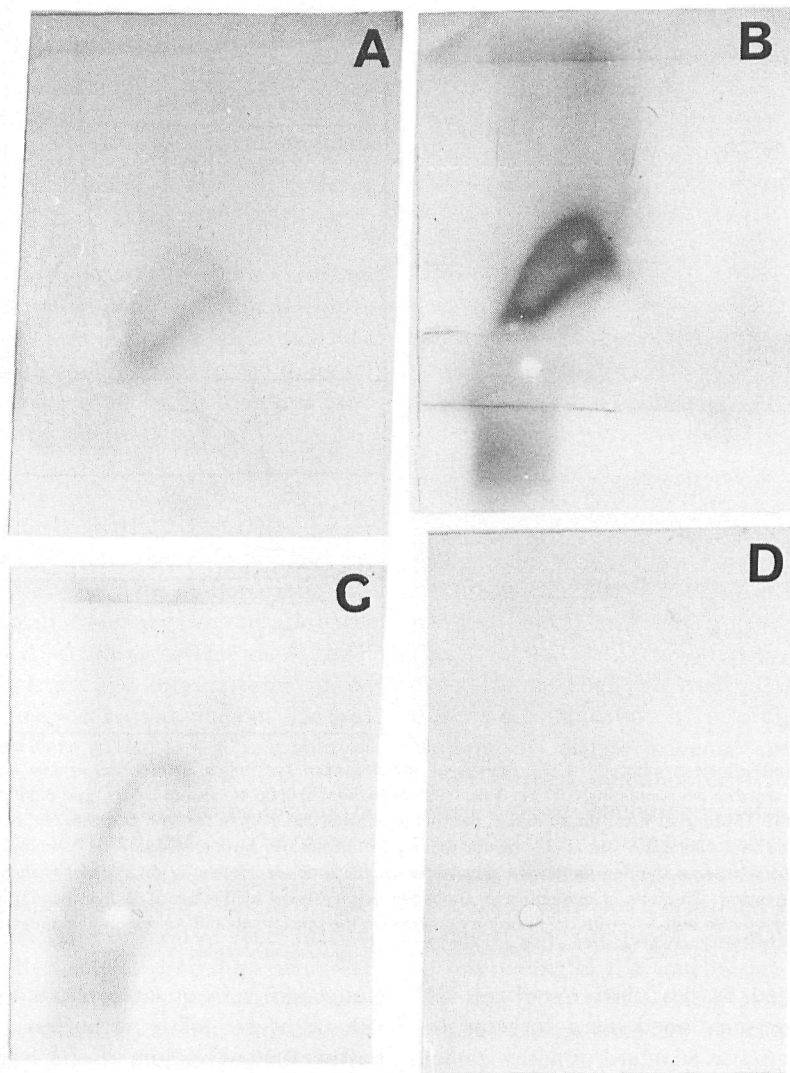


Fig. 3. CRIE performed by using the sera of three different rye pollen allergic patients and the control serum (D) from a pool of 1000 normal individuals. The autoradiography was carried out for 10 days. The Coomassie Blue-stained CIE reference pattern is shown in Fig. 2.

preparation Sc against anti-NG. Appropriate separation of the antigens was obtained by varying the time and position of the application well for electrophoresis in the first dimension, and optimal antigen-antibody balance was attained by varying the proportions of the reactants. By fixing and staining the first dimension gel, it was confirmed that all protein-staining components were included in the portion of the gel transferred to the second dimension

run. Very weak precipitates were detected only after performance of CRIE and are indicated in the drawing (Fig. 2 B). The analysis revealed a total of 25 antigens of which all but three moved against the anode.

Fig. 3 shows examples of CRIE analyses performed with sera from three patients. Among the 25 antigens, a total of seven were able to bind allergen-specific IgE from the 20 individual sera investigated. The most intense IgE

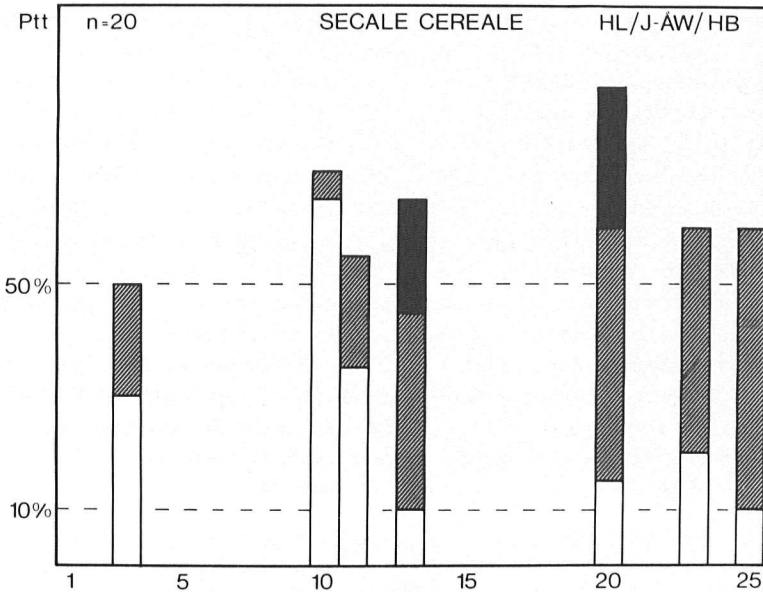


Fig. 4. Allergogram for antigens of rye (*Secale cereale*) pollen based on sera from 20 patients positive to *Sc* pollens. Specific IgE binding to the individual antigens (abscissae) was obtained by means of CRIE. The extent of IgE binding was evaluated visually from the autoradiographs of the CRIE plates. The height of the column indicates the percentage of patients reacting to the individual allergens. Radiostaining visible after 1 day: black column; after 3 days: hatched column; and 10 days; open column. Antigens were corrected for unspecific IgE binding (13).

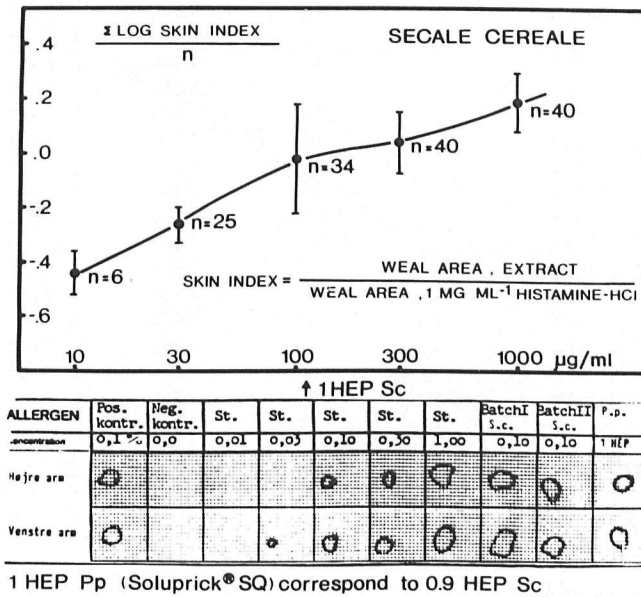


Fig. 5. Dosage/skin prick test response curve for the *Sc* reference preparation. *n* = the number of measurable prick tests at the various extract concentrations. An arrow indicates the estimated 1 HEP *Sc*-concentration. Below is shown an example of a patient's skin prick test response from right and left arms for positive and negative controls, serial dilutions of the reference (St), two commercial batches of rye as well as a HEP standardized timothy pollen extract batch.

binding was obtained with antigens 13 and 20 which, with some of the sera, were radiostained after 1 day of autoradiography. The other five IgE-binding antigens all showed examples of radiostaining after three days of autoradiography, but, frequently, particularly for antigens 3, 10, and 11, radiostaining was not obtained until after 10 days of exposure, if at all.

The results of CRIE of 20 sera from rye pollen-allergic individuals are summarized in an allergogram (Fig. 4). From this it appears that at least 50% of the patients react to all of the seven identified allergens. However, in four cases (antigens 13, 20, 23, and 25) a strong IgE binding visible after 3 days of autoradiography

was obtained for most of the positive patients, thus defining these as major allergens (1, 15).

Fig. 5 shows the dose response curve for SPT performed with Sc. By plotting the geometric mean of the obtained skin indices against the concentration of the standard, a curve was obtained for which the HEP value can be read. The 1 HEP value for Sc corresponds to a concentration of 115 µg/ml. From this curve the 1 HEP value for the rye pollen preparation (Soluprick) was calculated to correspond to 14.2 µg/ml. Calculation of the HEP value for the tested timothy preparation (Soluprick SQ, 1 HEP) from the Sc calibration curve revealed a concentration of 0.9 HEP.

Table 1
Results of *in vitro* and *in vivo* investigations in the individual patients

Pt.	Sc ¹ (%)	RAST Pp ¹ (%)	Pp ² (class)	Σ CRIE ³	SPT		PRIST (Ku/l)
					Timothy ⁴ (SI)	Rye ⁵ (SI)	
1	15.6	14.5	4	20	0.886	1.652	200
2	13.2	20.0	4	74	1.038	1.888	225
3	4.2	4.1	3	0	0.941	1.216	80
4	10.1	10.3	3	22	0.584	3.129	50
5	11.4	12.9	4	30	1.134	2.677	80
6	5.1	6.4	3	0	0.685	1.948	150
7	18.1	14.2	4	96	1.976	4.120	40
8	15.2	13.5	4	65	1.216	2.832	> 800
9	19.8	16.8	4	196	2.040	3.151	n.d.
10	11.6	12.5	4	63	0.888	1.417	250
11	18.9	20.2	4	213	1.504	2.644	250
12	15.9	15.4	4	203	1.562	2.203	800
13	6.3	5.9	3	8	0.279	0.524	450
14	21.3	16.9	4	113	1.176	2.733	375
15	16.1	9.1	3	24	0.612	0.645	325
16	14.0	14.2	4	110	0.375	0.589	50
17	10.3	16.3	4	66	0.637	1.120	65
18	18.8	22.8	4	173	0.760	2.468	180
19	12.7	17.0	4	42	1.006	1.177	500
20	14.3	6.7	2	48	0.995	1.085	150

¹ Percentage of total activity (¹²⁵I-aIgE) added to discs.
² Phadebas RAST.
³ Summation of individuals' CRIE-responses to various allergenic molecules.
⁴ Skin index =
$$\frac{\text{Weal area (allergen: Timothy Soluprick SQ, 1 HEP)}}{\text{Weal area (Histamine hydrochloride 1 mg/ml)}}$$

⁵ Skin index =
$$\frac{\text{Weal area (allergen: Rye Soluprick, 100 µg/ml)}}{\text{Weal area (Histamine hydrochloride 1 mg/ml)}}$$

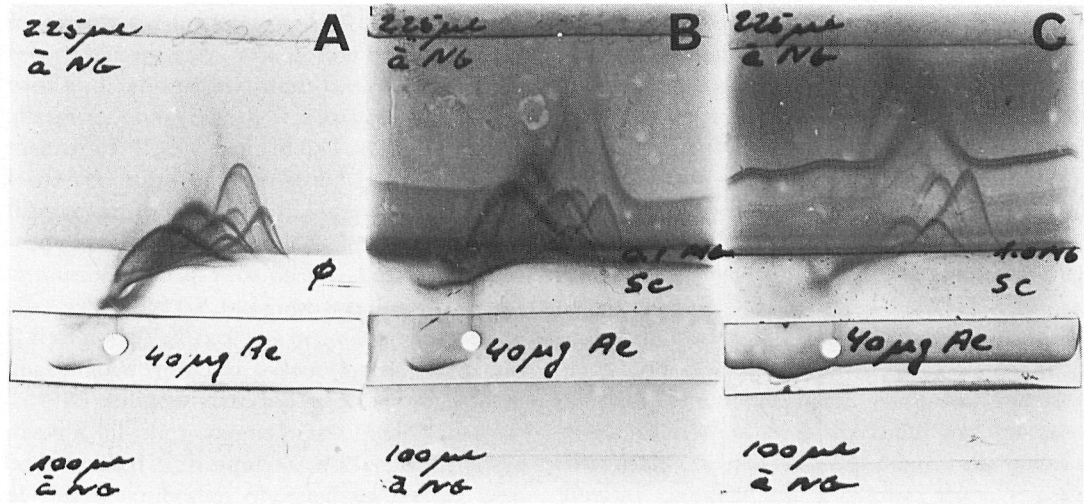


Fig. 6. *In situ* absorption by means of crossed line immunoelectrophoresis to illustrate the immunological (partial) identity between pollen antigens from a grass (*Avena elatior*) and *Secale cereale*. None, 0.1 mg, and 1.0 mg of Sc were incorporated in the line gel in A, B, and C, respectively. Only very few Ae antigens were not influenced by the Sc antigens, thus illustrating a high degree of partial identity.

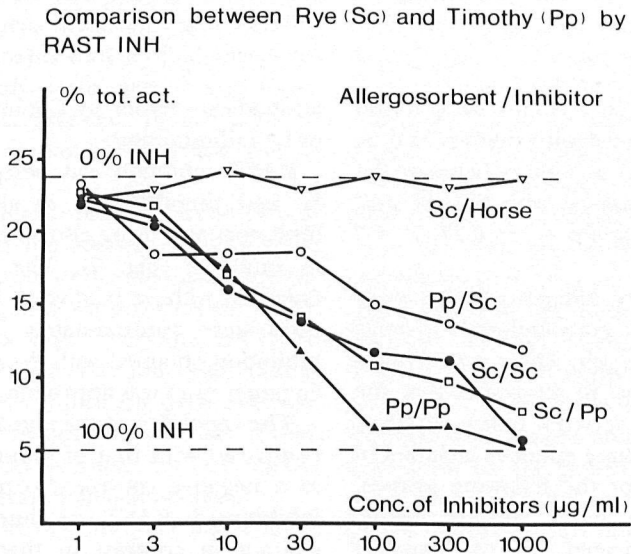


Fig. 7. The ordinate gives the percentage of the added ^{125}I -anti IgE which was bound to the paper discs. 0% inhibition was defined by the inhibition of Sc by an extract of horse hair and dander and 100% as the activity remaining after having inhibited Sc or Pp by themselves.

The results of the *in vivo* and *in vitro* investigations of the individual patients included RAST performed with timothy and Sc, the summed CRIE for Sc, and skin index for ti-

mothy (1 HEP) and rye I (100 µg/ml), timothy Phadebas RAST, total IgE by the PRIST method as well as allergy towards other allergen sources (Table 1). Using Spearman rank corre-

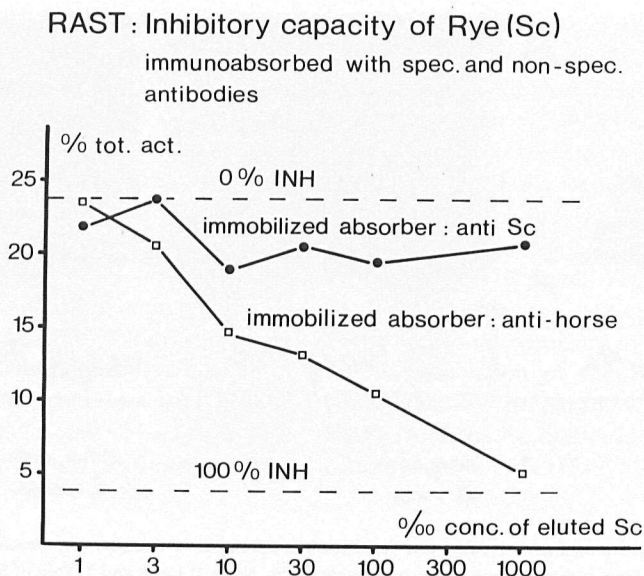


Fig. 8. The ability of anti-Sc to absorb the allergenic molecules of Sc as compared with a nonsense antibody (anti-horse dander) was determined indirectly by performing RAST inhibition using the desorbed antigenic material as inhibitors. The ordinate gives the percentage of total activity (^{125}I -anti IgE) bound to the paper disc. 0% inhibition is defined as the percent activity obtained by non-inhibited Sc paper discs. 100% inhibition is the activity measured after inhibition with non-diluted Sc.

lation test, significant correlations were found between RAST performed with timothy and Sc ($r_s = 0.64$, $P < 0.01$) as well as between the results of the SPT obtained with the rye and timothy pollen preparations ($r_s = 0.77$, $P < 0.001$).

In order to compare antigens, CLIEs were performed for various grass/anti-grass systems including Sc in the line gels. These experiments (example shown in Fig. 6) illustrated that the grasses contained only very few pollen antigens, if any, which did not share epitopes included in Sc. This was shown for the following grasses: Timothy, orchard, rye grass, meadow fescue, false oat, and common reed. CIE of Sc against anti-Ng, including purified timothy antigen 19/25 in a line gel, identified the major allergen of Sc (antigen 20) as partially identical to antigen 25 of timothy.

For three patients (Nos. 9, 11, and 12) CRIE was performed in the Sc anti-Sc system including anti-NG in the intermediate gel. We detected no precipitates by means of anti-Sc which had not been detected by the anti-NG

antibodies – either by Coomassie Blue staining or by radiostaining.

RAST inhibition was performed using both Sc and timothy (Pp) as allergosorbents and inhibitors and using also a horse dander extract as inhibitor (Fig. 7). The RAST inhibitions obtained with Sc relative to Pp using Pp paper discs were approximately 70%, whereas the inhibition obtained with Pp relative to Sc using Sc paper discs was approximately 90%.

The capability of the anti-Sc to absorb Sc was compared with that of anti-horse dander used as a negative control. No material capable of inhibiting Sc-RAST was eluted from the anti-Sc column in contrast to that eluted from the anti-horse dander column. The results are shown in Fig. 8.

DISCUSSION

Although a high degree of immunological, partial identity between grasses has been well known for many years, extracts of pollen from cultivated rye are still in many places used

separately for immunotherapy of summer hay fever. The question whether or not this is relevant and rational has often been raised among allergists, and recently it has been studied by means of RAST and RAST inhibition (10). However, until now no study has given a definitive answer to this question. The present study was therefore undertaken to reveal the immunochemical relationship between pollen allergens from rye and other grasses.

Rye pollen extract has been characterized by quantitative immunoelectrophoretic technique. The 25 antigens detected probably do not represent all the antigens of rye pollen that can be extracted with aqueous buffer, but the use of rabbit antibodies obtained by mixing antibodies from several grass pollens revealed a number of antigens comparable with that of other grasses studied by these methods (16) where the number of antigens varied between 25 and 40.

The allergens were defined by means of CRIE as those antigens which were able to bind serum IgE from patients with verified allergy to rye pollen. The study performed on sera from 20 selected patients revealed 10 allergens, a number which is comparable with that found for other grasses (16).

This immunochemical characterization of rye pollen antigens and the allergen identification by means of CRIE were performed using mixed antibodies raised against various grasses. Some unique rye antigens might therefore not have been detected. However, rye antibodies raised in rabbits were able to absorb any detectable rye pollen allergenic activities and did not reveal any CIE precipitates which had not been developed by using the grass pollen antibodies.

The 20 patients in this study all had a convincing history of hay fever, they all showed strong positive skin- and RAST reactions to extracts of timothy as well as rye pollen. Furthermore, they also all had positive nasal provocation with native rye pollen. Skin prick test titration on the 20 selected patients revealed the dose response curve for Sc. The 1 HEP value for Sc is comparable with that for other aqueous extracts of various grass pollens, although great variation may be found due to selection of pa-

tients, technique in performing SPT, and the regions involved. It is, however, notable that a previously standardized timothy pollen extract (with a nominated activity of 1 HEP) from this dose response curve obtained the value 0.9 HEP. Correspondingly, statistically significant correlations were obtained between rye and timothy pollen extracts when testing the 20 selected patients with these extracts using either SPT or RAST.

The immunological partial identity with antigens from other grass pollens was shown directly with immunochemical methods, and the major allergen of Sc (antigen 20) was immunochemically related to the major allergen of timothy (antigen 25). However, these experiments revealed no antigens from Sc that did not have some epitopes in common with the antigens they were compared with. Consequently, we compared rye and timothy pollen antigens by RAST inhibition using both preparations as allergosorbents and inhibitors. Provided that the serum pool used includes IgEs with specificities against all common and unique epitopes in the rye and timothy pollen extracts, we can conclude that no unique rye antigens exist but unique timothy antigens might exist. However, it cannot be excluded that rye antigens with common as well as specific epitopes do exist.

On the basis of this study, we consider the probability of finding patients with specific IgE against unique rye pollen epitopes to be low and not clinically relevant. Case histories describing extensive symptoms caused by rye is probably a quantitative question owing to the strongly time-limited and concentrated natural exposition of rye pollen. Extract of rye does not seem to add any further specificities to the diagnosis and therapy of grass allergies.

REFERENCES

1. Aukrust, L. & Borch, S. M.: Purification of allergens in *Cladosporium herbarum*. Proc. of the Annual Meeting of the European Academy of Allergology and Clinical Immunology. *Allergy* 35, 206-207, 1980.
2. Bejnamins, C. G. & Vermeulen, B. S.: Recherches sur les substances sensibilisantes qui provoquent le rhume des foines, au moyen de la methode de Dale. *Acta Otolaryngol.* 15, 121-138, 1931.

3. Bjerrum, O. J., Ramlau, J., Clemmensen, I., Ingild, A. & Bøg-Hansen, T. C.: An artifact in quantitative immunoelectrophoresis of spectrin caused by proteolytic activity in antibody preparations. *Scand. J. Immunol.* 4, Suppl. 2, 81, 1975.
4. Ceska, M., Eriksson, R. & Varga, J. M.: Characterization of allergen extracts by polyacrylamide gel isoelectrofocusing and radioimmunosorbent allergen assay. I. Distribution of allergenic components in birch pollen extracts. *Int. Arch. Allergy Appl. Immunol.* 42, 430–437, 1972.
5. Eriksson, N. E.: Diagnostic methods in reaginic allergy. A clinical evaluation in patients with bronchial asthma and allergic rhinitis. Thesis, University of Göteborg, Göteborg, Sweden, 1977.
6. Freeman, J.: Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. *Lancet* ii, 814–817, 1911.
7. Frostad, A. B., Bolle, R., Grimmer, Ø. & Aas, K.: A new, well-characterized, purified allergen preparation from timothy pollen. II. Allergenic in vivo and in vitro properties. *Int. Arch. Allergy Appl. Immunol.* 55, 35–40, 1978.
8. Harboe, N. & Ingild, A.: Immunization, isolation of immunoglobulins, estimation of antibody titre. *Scand. J. Immunol.* 2, Suppl. 1, 161–164, 1973.
9. Heijer, A. & Göransson, K.: The significance of testing and hyposensitization with several grass pollens for hay fever. *Acta Allergol.* 23, 146–160, 1968.
10. Kalveram, K.-J. & Forck, G.: Studies on antigenic relationship between grass- and cult. rye pollens by skin test, RAST and RAST-inhibition test in patients with pollinosis. *Arch. Derm. Res.* 260, 17–27, 1977.
11. Løwenstein, H.: Immunological partial identity and in vitro inhibitory effect of two major allergens of timothy pollen to whole pollen extracts of four grasses. *Int. Arch. Allergy Appl. Immunol.* 57, 379–383, 1978.
12. Løwenstein, H.: Isolation and partial characterization of three allergens of timothy pollen. *Allergy* 33, 30–41, 1978.
13. Løwenstein, H.: Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergen. *Progr. Allergy* 25, 1–62, 1978.
14. Løwenstein, H.: Technique for definition and identification of allergenic molecules. Possible use for predicting optimal diagnostic and therapeutic extracts. In Steffen, C. & Ludwig, H. (eds.): *Clinical immunology and allergology*, pp. 265–274. Elsevier Biomedical, Amsterdam, 1981.
15. Løwenstein, H.: Timothy pollen allergens. *Proc. of the Annual Meeting of the European Academy of Allergy and Clinical Immunology. Allergy* 35, 188–191, 1980.
16. Løwenstein, H.: Fractionation and purification of allergens from grass pollens. *Proc. of the 10th International Congress of Allergology*. In Oehling, A. (ed.): *Allergology and immunology*, pp. 505–511. Pergamon Press, Oxford, 1980.
17. Markussen, B., Løwenstein, H. & Weeke, B.: Immunochemical characterization of an extract of allergens from horse hair and dandruff. *Int. Arch. Allergy* 51, 25–37, 1976.
18. Marsh, D. G., Haddad, Z. H. & Campbell, D. H.: A new method for determining the distribution of allergenic fractions in biological materials: its application to grass pollen extract. *J. Allergy* 46, 107, 1970.
19. Rudolph, R., Kunkel, G., Staud, R.-D., Baumgarten, C. & Kossack, G.: Rhino-manometric-thermometric studies concerning the skin-mucosa-relation of rye, wheat, barley and oat pollen in patients in Rhinitis pollinosa. *Allergologie* 1, 27–34, 1978.
20. Wihl, J.-Å.: Methods for assessing nasal reactivity. *Eur. J. Respir. Dis.* 64, Suppl. 128, 175–179, 1983.
21. Weeke, B., Søndergaard, I., Lind, P., Aukrust, L. & Løwenstein, H.: Crossed radio-immunoelectrophoresis (CRIE) for identification of allergens and determination of the antigenic specificities of patients IgE. *Scand. J. Immunol.* 17, Suppl. 10, 265–272, 1983.
22. Wide, L., Bennich, H. & Johansson, S. G. O.: Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* ii, 1105–1107, 1967.
23. Yman, L., Ponterius, G. & Brandt, R.: RAST-based allergen assay methods. *Dev. Biol. Stand.* 29, 151–165, 1975.
24. Østerballe, O., Løwenstein, H., Prahl, P., Skov, P. & Weeke, B.: Immunotherapy in hay fever with two major allergens 19, 25 and partially purified extract of timothy grass pollen. A controlled double blind study. In vitro variables, season I. *Allergy* 36, 183–199, 1981.
25. Østerballe, O., Ipsen, H., Weeke, B. & Løwenstein, H.: Specific IgE response toward allergenic molecules during perennial hyposensitization: a three-year prospective double-blind study. *J. Allergy Clin. Immunol.* 71, 40–46, 1983.

Address:

Henning Løwenstein
The Protein Laboratory
Sigurdsgade 34
DK-2200 Copenhagen N
Denmark

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.