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An immunoelectrophoretic analysis of the allergens of *Cynodon dactylon* (Bermuda grass) pollen

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A crude extract of Cynodon dactylon (Bermuda grass) pollen was obtained by extraction, centrifugation, dialysis, and lyophilization. The corresponding polyspecific rabbit antibodies were obtained by immunization, bleeding, and purification and were used for a cross immunoelectrophoretic analysis of the crude extract. At least 52 antigens (Ags), of which 47 migrated toward the anode, and five, toward the cathode, were revealed. Crossed radioimmuno-electrophoretic analysis performed with sera from 32 patients allergic to Bermuda grass and a pool from 1000 normal individuals revealed specific IgE binding to 13 of these Ags. The approximate molecular weights (MWs) for 10 of these IgE-binding Ags were determined by a combination of gel filtration and immunochemical analysis. These Ags had apparent MWs >14 kd. A combination of preparative sucrose gradient isoelectric focusing and immunochemical analysis was used to determine the approximate isoelectric point values of five of the IgE-binding Ags. Most precipitating Ags had isoelectric points between 4.5 and 5.5. Four of the Ags (Ag 24, MW, <65 kd; Ag 31, MW, 33 kd; Ag 33, MW, 20 kd; and Ag 34/35, MW, 32 kd) were classified as major allergens. (J ALLERGY CLIN IMMUNOL 1989;83:1124-34.)

Among pollen allergens, those derived from grasses are of prime importance. Antigenic relationships between the various grass pollens have been studied, and it has been revealed that allergens from many globally distributed grasses have IgE epitopes in common.¹⁻⁷ However, from several studies it also appeared

that *Cynodon dactylon* (Bermuda grass)-pollen allergens differed significantly from allergens of the common grasses of the temperate zones.^{1-3, 6, 8} Consequently, it was agreed internationally that Bermuda grass-pollen extract should be the second grass extract to be standardized, and with this purpose in view, a preliminary international study has been published.⁹

Characterization of grass-pollen Ags from several species, for example, *Lolium perenne*,¹⁰⁻¹⁶ *Phleum pratense*,^{17, 18} *Poa pratensis*,¹⁹⁻²¹ *Secale cereale*,^{22, 23} and *Dactylis glomerata*²⁴⁻²⁶ has been carried out, and several allergens have been isolated and characterized. The allergenic components of Bermuda-grass pollen

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Abbreviations used

Ag:	Antigen
MW _r :	Molecular weight
<i>Cynd 0481</i> :	<i>Cynodon dactylon</i> extract
<i>aCynd</i> :	Polyspecific antibodies against <i>Cynd 0481</i>
<i>aHorse</i> :	Polyspecific antibodies against horse dander
CIE:	Crossed immunoelectrophoresis
CLIE:	Crossed line immunoelectrophoresis
CRIE:	Crossed radioimmuno-electrophoresis
FRIE:	Fused-rocket immunoelectrophoresis
SRRID:	Single radial radioimmunodiffusion
IEF:	Isoelectric focusing
pI:	Isoelectric point
algE:	Monospecific antibodies against IgE

were described in a preliminary immunoelectrophoretic study some years ago,²⁷ and the allergenic composition has been studied by electroblotting techniques by Ford and Baldo²⁸ and recently by Shen et al.²⁹

The aim of this study was to investigate the antigenic composition of Bermuda-grass pollen with immunoelectrophoretic methods and also to identify those Ags that act as allergens in human beings. This information is a basic prerequisite for the future purification of the major allergens of Bermuda grass-pollen extracts.

MATERIAL AND METHODS

Extraction

Pollen from Bermuda grass was obtained from Allergon Inc., Joplin, Mo., as dried defatted material (batch 284210-2, sampled in 1980 in central United States). According to the manufacturer, the samples contained >90% pure Bermuda grass-pollen grains and approximately 10% nonpollen Bermuda-grass debris. Detectable foreign pollens constituted <0.5%, as stated in the certificate.

The pollen was stored at -20°C until it was used. Within 1 year of collection, the pollen was extracted. The extraction was performed at 5°C for 20 hours with a carbonate-buffer solution (1:10 wt/vol in 0.125 mol/L NH₄HCO₃ and 0.015 mol/L NaN₃, adjusted to pH 7.5), centrifuged (50,000 g for 1 hour at 0°C), dialyzed (one time against 0.125 mol/L NH₄HCO₃ and two times against water, each 24 hours at 5°C, with Visking dialysis tubing (Union Carbide Corp., Danbury, Conn.) with limiting pore size corresponding to approximately MW_r <5 kd), and lyophilized.

All immunochemical analyses were made within 2 years from the time of extraction. Solutions of the lyophilized material (denoted *Cynd 0481*, 10 mg/ml) were always freshly prepared in 0.15 mol/L of NaCl, 50% vol/vol of glycerol for use in analysis, or in 0.1 mol/L of NaHCO₃,

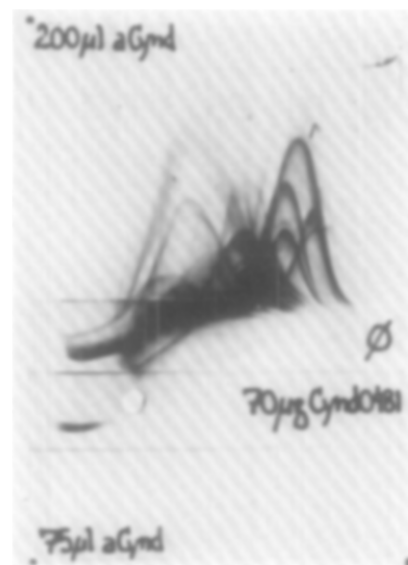


FIG. 1A. CIE reference precipitation pattern. *Cynd 0481* against *aCynd* on 5 by 7 cm² plates; 70 µg *Cynd 0481* is applied against 11.4 µl/cm² of *aCynd* in anodic gel and 10.0 µl/cm² of *aCynd* in cathodic gel. First-dimension electrophoresis for 30 minutes at 10 V/cm. Second-dimension electrophoresis for 16 hours at 2 V/cm.

0.015 mol/L of NaN₃ solution for immunization of rabbits. Analysis for protein content according to the method of Lowry et al.³⁰ with bovine serum albumin as a reference revealed around 60% wt/wt of *Cynd 0481* to be of protein nature.

Polyspecific antibodies

Anti-Bermuda-grass antibodies (*aCynd*) were raised by immunizing three rabbits with *Cynd 0481*. The immunization, bleeding, and subsequent isolation of the immunoglobulins by ammonium sulfate fractionation were performed according to the method of Harboe and Ingild.³¹ The immunoglobulins were isolated after 15 bleedings, and the antibodies, approximately 6.5 times concentrated relative to their concentration in serum, were stored at 5°C in a solution containing 0.015 mol/L of NaN₃ and 1% aprotinin (NOVO Industri A/S, Bagsvaerd, Denmark) to avoid proteolytic degradation of the immunoglobulins.³²

Immunoabsorption experiments were performed to determine the allergenic inhibitory capacity of *aCynd* compared to an irrelevant antibody against horse dander (*aHorse*). *Cynd 0481* (100 µg) was applied to columns with immobilized antibodies (700 µl antibodies to 1.0 gm of CNBr-activated Sepharose 4B, column volume 2.5 ml) and eluted with citrate-buffer (pH 6.2; I = 0.05) followed by citrate-buffer (pH 5.7; I = 0.5). Fractions from the *aHorse* column (contained Bermuda-grass antigens as determined by means of FRIE with *aCynd*) and the corresponding fractions from the *aCynd* column were collected, and serial dilutions were used to inhibit Bermuda-grass paper disks.

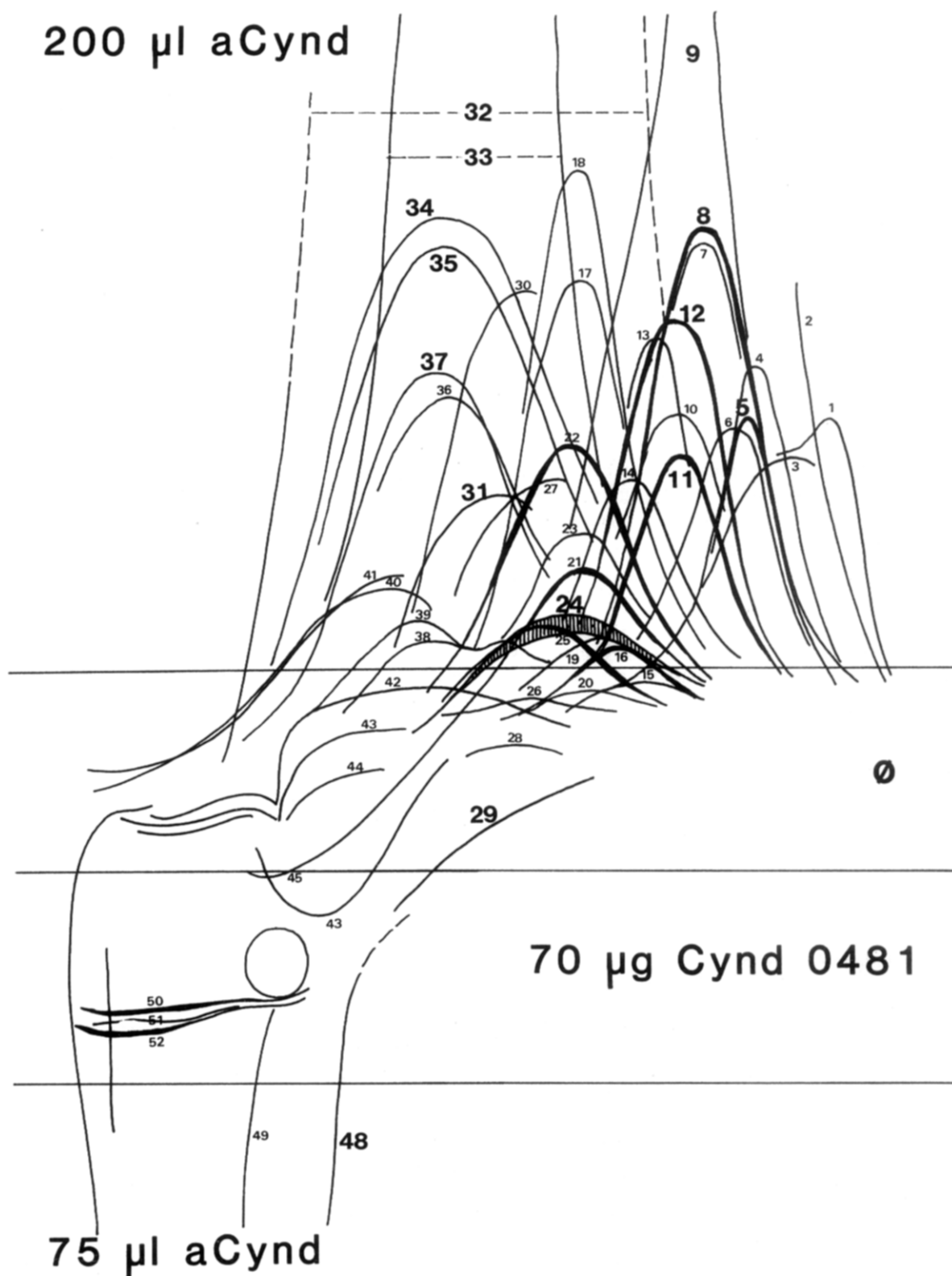


FIG. 1B. Reference drawing of the CIE precipitation pattern. Visible Ags have been numbered from *right to left*. *Dashed lines* indicate precipitation arcs only visible in CRIE.

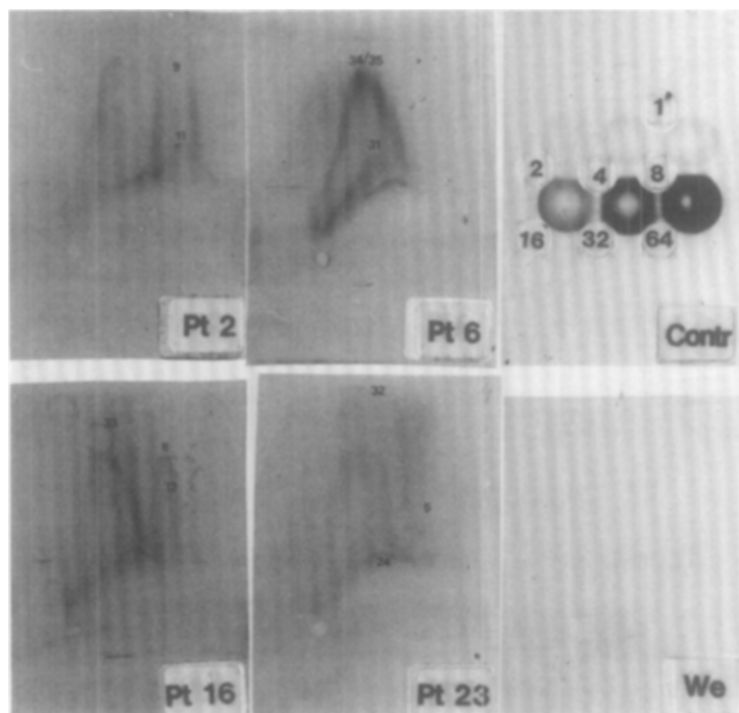


FIG. 2. CRIE. Representative allergoprints of sera from four patients (*Pt*), 2, 6, 16, and 23, together with the allergoprints of a negative control (*We*) and a SRRID score plate (*Contr*). The numbers of IgE-binding precipitates are presented.

The allergenic activity of the column eluates was estimated in a RAST-inhibition experiment with a serum pool composed of equal volumes of sera from the patients allergic to Bermuda grass.

Patient sera

Thirty-two patients who were residents of Tucson, Ariz., and who had allergic rhinitis during the grass season were investigated. All patients had skin test wheal responses to Bermuda grass–pollen extract of at least 7 mm in diameter. Nine patients had suffered from symptoms for four to 10 seasons and 23 patients for more than 10 seasons. Eighteen patients were women (age range 19 to 49 years) and 14 patients were men (age range 16 to 56 years). None of the patients had previously had specific immunotherapy.

In a preliminary RAST study, all the patients demonstrated a strongly positive response in Bermuda-grass RAST. Triplicate determination was performed for each serum with dialyzed Bermuda grass–pollen extract coupled to paper disks as allergosorbent and the Phadebas RAST isotope kit (Pharmacia, Uppsala, Sweden). RAST was performed according to the method of Ceska et al.³³

A serum pool of 1000 Danish individuals was used as a negative control, as described elsewhere.^{34, 35}

Qualitative immunoelectrophoresis

Analysis with CIE, CLIE, and FRIE was performed at pH 8.6 with 1% agarose (type HLA; Litex, Copenhagen,

Denmark) gels, as described earlier.³³ Details of the electrophoresis are presented in the legends to Figs. 1A, 1B, 5, and 6.

CRIE

A CRIE study with 32 sera from patients allergic to Bermuda grass was performed as follows. CRIE plates were prepared as described earlier.^{34, 35} The degree of radiostaining of individual precipitates in CRIE was evaluated by visual comparison of CRIE autoradiographs with autoradiographs of ¹²⁵I-labeled aIgE SRRID rings.

The control SRRID plates were prepared as follows: 50 μ l aliquots of twofold dilutions (nine in all) of ¹²⁵I-labeled aIgE were mixed with 5 μ l of rabbit IgG (Dakopatts A/S, Copenhagen, Denmark). SRRID was performed with 5 μ l of each dilution (7 to 0.03 nCi) for 48 hours on 5 by 5 cm² glass plates coated with agarose containing 3 μ l/cm² of swine antirabbit IgG (Dakopatts A/S). In each x-ray film cassette, one SRRID plate was autoradiographed together with 15 CRIE plates.

The radiostained precipitates were assigned a score N according to $N = 64/D$ where D is the dilution of ¹²⁵I-labeled aIgE in the SRRID ring with corresponding radiostaining intensity, for example, for a dilution of 1:15, $n = 4$. The degree of radiostaining of the precipitates was independently evaluated by two subjects who visually compared the intensity of the radiostained precipitation arcs with the intensity of the corresponding SRRID rings.

TABLE I. Patient responses in RAST and CRIE

Pt No.	RAST binding (%)	CRIE scores												
		Antigen No.												
		5	8	9	11	12	24	29	31	32	33	34/35	37	48
1	26	—	2	2	1	2	8	—	2	2	1	4	4	—
2	35	—	—	8	4	—	16	—	—	2	—	4	—	—
3	35	—	—	—	—	—	8	8	8	4	—	4	—	4
4	36	—	—	—	—	—	8	—	—	—	4	2	2	—
5	34	—	—	1	—	—	16	—	8	—	—	8	—	—
6	34	—	—	4	—	—	16	—	16	—	—	16	—	—
7	15	—	—	4	4	4	8	—	2	—	—	2	—	—
8	30	—	—	—	—	—	8	2	—	2	—	8	8	—
9	24	—	—	—	—	—	8	—	—	—	1	4	1	—
10	35	—	2	—	—	—	16	4	4	4	—	8	—	—
11	13	—	—	—	—	—	4	—	—	1	4	1	—	—
12	14	—	—	—	—	—	4	—	—	—	—	—	—	—
13	32	—	—	—	—	—	8	—	2	2	—	4	—	—
14	36	—	—	—	—	—	16	—	4	4	8	16	—	—
15	39	—	—	—	—	—	8	2	1	4	—	4	—	—
16	33	—	8	—	—	2	16	—	2	—	4	8	2	—
17	34	—	—	2	—	—	8	—	—	—	4	8	8	—
18	35	—	—	—	—	—	8	1	2	4	4	8	—	—
19	39	—	—	—	—	—	8	1	2	—	4	4	—	—
20	44	—	—	—	—	4	8	1	—	—	4	4	—	—
21	30	—	—	—	—	—	2	—	—	—	2	2	—	—
22	24	—	—	—	—	—	2	—	—	—	4	2	—	—
23	43	2	—	—	2	2	8	—	2	8	—	4	—	1
24	39	—	—	4	—	—	8	—	1	—	2	4	—	—
25	42	—	—	1	—	—	8	—	—	1	2	4	—	—
26	21	—	4	—	—	—	16	—	2	—	2	2	—	—
27	41	—	—	—	—	—	4	—	1	—	4	4	—	—
28	33	—	—	—	—	—	2	—	1	—	2	2	1	—
29	34	—	—	1	—	—	4	—	1	—	4	2	—	—
30	27	—	—	1	—	—	4	—	1	—	4	2	—	—
31	41	—	—	—	—	—	8	—	—	—	—	16	—	—
32	ND	—	—	2	—	—	2	—	—	2	—	1	—	—
Nonsp	2	—	—	—	—	—	2	—	—	—	—	—	—	—

Pt, Patient; *nonsp*, nonspecific binding; *ND*, not determined.
CRIE scores were determined as described in text.

MW determinations

MW determinations associated with a successive immunochemical analysis of Bermuda grass-pollen antigens were performed as follows: a Sephadex G75 column (Pharmacia type C100/16, gel volume 190 ml, flow rate 5.3 ml/hr) was equilibrated and run at 4° C with an 0.125 mol/L of NH_4HCO_3 buffer solution. The column was calibrated with 1.0 ml of a solution containing 1 mg of blue dextran and 2 mg of each of the following marker proteins (Sigma Chemical Co., St. Louis, Mo.): equine heart cytochrome c (MW_r , 12 kd), equine skeletal muscle myoglobin (MW_r , 17.5 kd), bovine pancreas α -trypsinogen A (MW_r , 24 kd), ovalbumin (MW_r , 43.5 kd), and human serum al-

bumin (MW_r , 68 kd). The elution volume for the serum proteins was determined by FRIE with monospecific antibodies against human serum albumin (Dakopatts A/S) and ovalbumin (Behringwerke AG, Marburg, West Germany), and for blue dextran and the rest of the markers by ultraviolet absorption. A solution of *Cynd 0481* (30 mg in 1 ml of buffer) was then fractionated on the column, and a gel-filtration profile of the Bermuda grass-pollen antigens was obtained by means of FRIE. Individual antigens were identified by CLIE with *Cynd 0481* as the reference antigenic mixture. The distribution coefficient for the markers and the antigens were calculated, and the MWs for the antigens interpolated from the calibration curve.

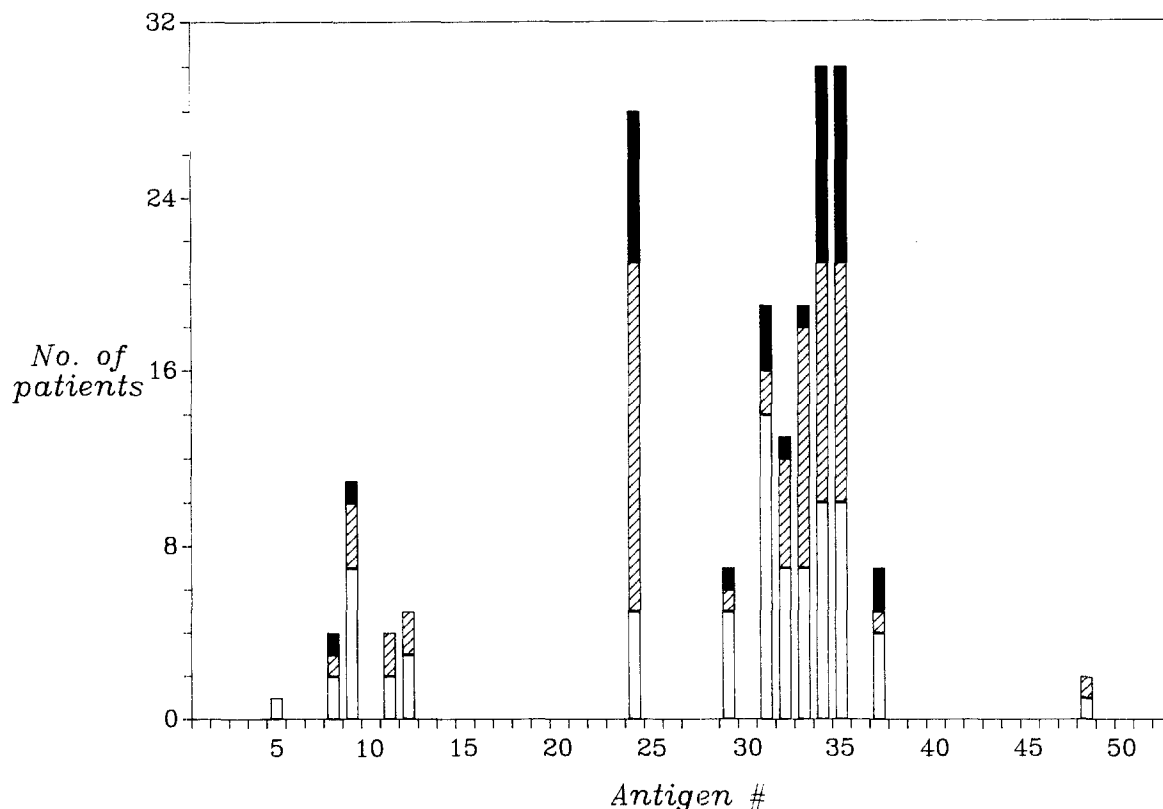


FIG. 3. Allergogram. Number of patients' sera demonstrating specific IgE binding to each of the 52 recognized Ags. CRIE score was found, as explained in the text: scores >7 (■); scores 4 to 6 (▨); scores <4 (□). Thirty-two patient's sera (100%) were investigated. Major allergens bind IgE from >50% of the patients' sera and strongly bind IgE from >25% of these sera. The scores of Ag 24 have been adjusted for nonspecific binding.

PI determinations

Determination of the pI values of Bermuda grass-pollen Ags was performed as follows: a sample containing 30 mg of *Cynd 0481* in 1 ml of water was subjected to IEF on an Ampholine 8101 column (LKB Instruments, Bromma, Sweden). A pH gradient 3.5 to 9.5 was distributed in a sucrose gradient (0% to 50% wt/vol) at 15° C during a period of 48 hours with a potential of 200 V (current at start 2.2 mA, falling to 0 mA); pH values of the collected fractions were measured, and those corresponding to Ags in the immunochemical elution profile of the fractions were determined by FRIE. Individual Ags were identified in CLIE.

RESULTS

Determination of the allergenic composition

The CIE precipitation pattern obtained with *Cynd 0481* against *aCynd* is illustrated in Fig. 1A. A reference drawing of the precipitation pattern is illustrated in Fig. 1B. Very weak precipitates were detected either by increasing the amounts of Ag and/or antibody or after performance of radiostaining in CRIE.

Precipitates only visible in CRIE are indicated by *dashed lines* in the drawing.

A total of 52 antigenic components were revealed. Five of these components migrated toward the cathode; the remaining components migrated against the anode. The precipitation pattern for most Ags was symmetric. The precipitation patterns for Ag 5, 8, 11, 12, 21, 22, 24, 34/35, 37, 50, 51, and 52 were dense and well stained. In contrast, Ag 32 was only visible after performance of CRIE. Ag 34/35 precipitated as a double arc; it was not possible to distinguish between the two precipitation arcs in CLIE or CRIE.

CRIE was performed on 32 sera from Bermuda grass-allergic patients as well as on a negative control serum pool. Four representative allergoprints, together with the allergoprint of the negative control, are illustrated in Fig. 2. The control plate (*We*) reveals only a faint radiostaining corresponding to Ag 24. The four chosen allergoprints differ both qualitatively and quantitatively in their binding of IgE to the various

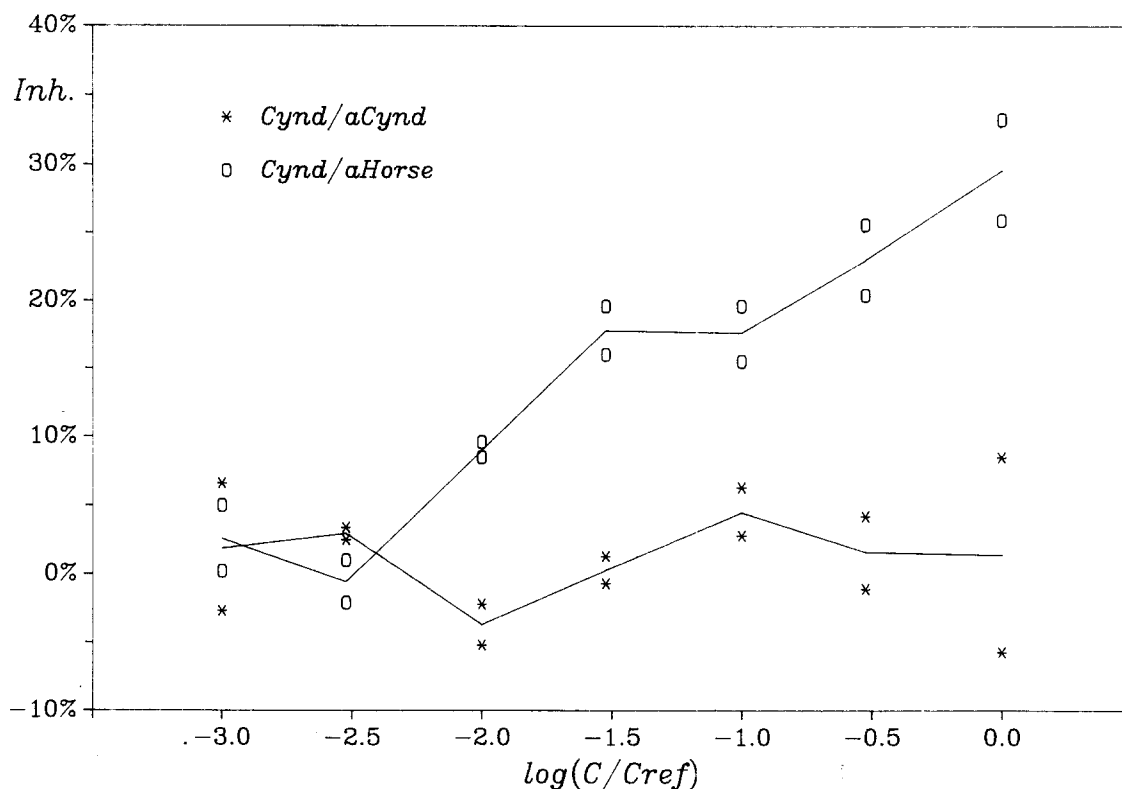


FIG. 4. Immunoabsorption experiment. RAST-inhibition test on eluates from CNBr-activated Sepharose 4B column coupled with *aCynd* and *aHorse*, on which *Cynd 0481* has been applied. The mean of two determinations is illustrated. *Cref*, nondiluted serum.

Bermuda grass Ags present in the CIE immunoprecipitates.

The radiostained precipitates were roughly quantified, as presented in Table I. An allergogram, which summarizes the results in relation to the IgE-binding Ags, is illustrated in Fig. 3. Ag 34/35 was able to bind IgE in sera from 31 of 32 patients. An additional three Ags (Ags 24, 31, and 33) bound IgE from more than half of the patients.

RAST was performed on 31 of the sera. The results are presented in Table I and are presented in percentage of total activity added. In RAST, 20 sera bound 30% to 44% of total activity added; eight sera bound 20% to 30%, and three sera bound 10% to 20%. The degree of nonspecific RAST binding was 2%.

To check the ability of *aCynd* to precipitate all important allergens of the extract, an immunoabsorption experiment was performed. The RAST-inhibitory capability of *aCynd* absorbed *Cynd 0481* was compared with that of *aHorse* absorbed *Cynd 0481*. No material capable of inhibiting RAST was eluted from the *aCynd* column in contrast to that eluted from the *aHorse* column, as illustrated in Fig. 4.

Determination of allergen characteristics

The physicochemical analyses of Bermuda grass-pollen Ags by gel filtration and preparative IEF were performed on the crude extract *Cynd 0481*, and the antigenic composition of the individual fractions was evaluated mainly by CIE.

There were about 40 distinctly visible precipitates that were obtained by FRIE of the fractions from a Sephadex G75 gel-permeation experiment. Ten Ags could be identified in the CIE reference pattern and could be characterized in terms of apparent MWs. In Fig. 5, A, the immunochemical elution profile is illustrated, as revealed in FRIE. In Fig. 5, B, the method of CLIE and an example of Ag identification is illustrated, indicating that the Ag 34/35 has an MW of 32 kd.

Similarly, the pI values of some Ags in fractions obtained after preparative IEF of *Cynd 0481* were estimated. The approximate pIs were estimated as the measured pH of the fractions in which the maximum concentration of the Ag was eluted from the column. About 25 detectable Ags were distributed over the pH range of 4 to 9, the major part having pIs between 4.5 and 5.5. Some of the 13 IgE-binding Ags were

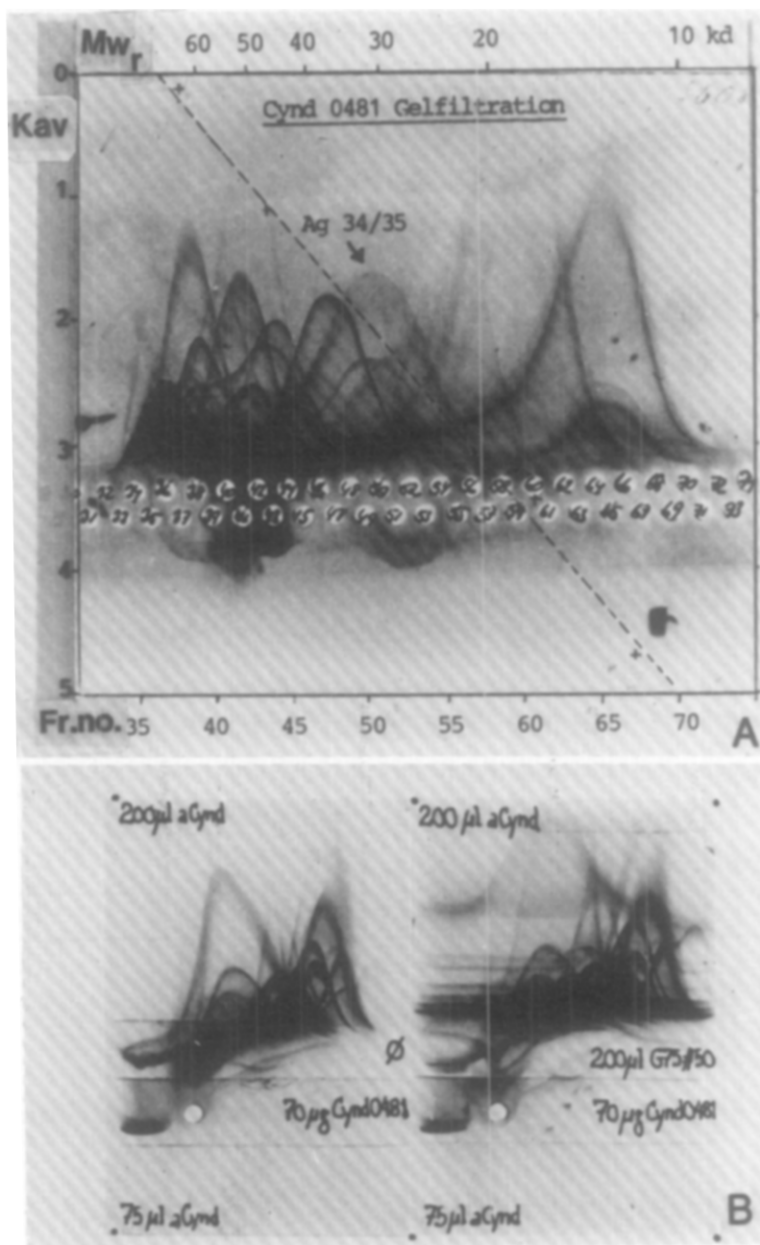


FIG. 5. Gel-filtration experiment. Analysis of 45 fractions (31 to 75) from a Sephadex G75 superfine column; 30 mg of *Cynd 0481* was applied on the column. **A**, The immunochemical elution profile as revealed in FRIE; 14 µl of each fraction was applied in the wells against 7.0 µl/cm² of *aCynd* in the anodic and cathodic gel. Electrophoresis for 16 hours at 2 V/cm after allowing the fractions to fuse for 1 hour. **B**, Identification of Ags in a fraction from the elution profile by CLIE. Ags from the fraction are distributed in the intermediary gel and raises their corresponding precipitates in CIE. Here an Ag in fraction 50 (corresponding to a MW, of 32 kD) raises the precipitate of Ag 34/35. Experimental conditions, as stated in Fig. 1A, except that the *right-hand* intermediate gel contains 200 µl of the analyzed fraction.

identified by means of CLIE as components of the CIE reference pattern. The immunochemical elution profile is illustrated in Fig. 6, as revealed in FRIE. It appears that some of the Ags are broadly distributed over the pI range, indicating heterogeneity of pIs; for

example, the major allergen Ag 34/35 is distributed over the pI range of 6.0 to 6.8.

Electrophoretic mobilities of allergens (at pH 8.6 relative to Ag 34/35) and their determined physico-chemical characteristics are presented in Table II.

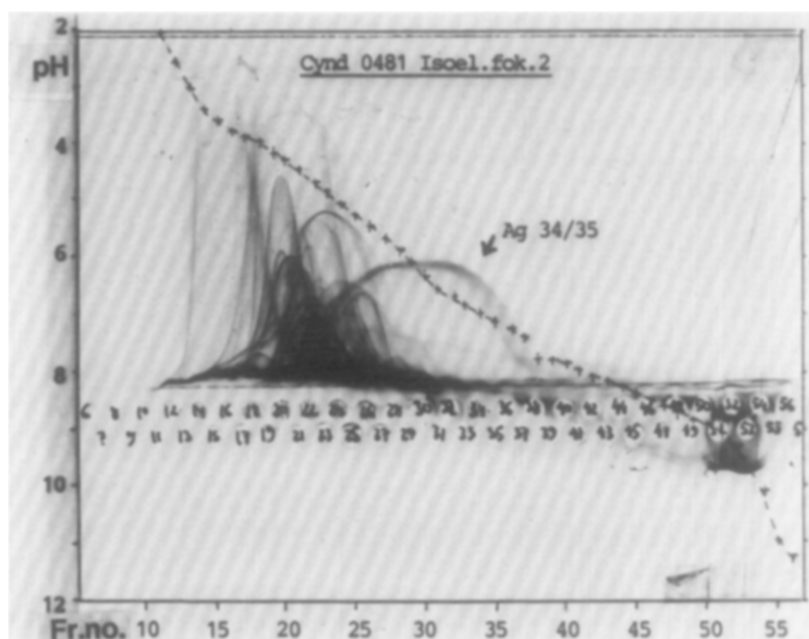


FIG. 6. FRIE on fractions from a preparative IEF experiment. Analysis of 52 fractions (5 to 57) from an Ampholine 8101 column IEF; 30 mg of *Cynd 0481* was applied on the column; 14 μ l of each fraction was applied in the wells against 7.0 μ l/cm² of *aCynd* in the anodic and cathodic gel; electrophoresis for 16 hours at 2 V/cm after allowing the fractions to fuse for 1 hour.

TABLE II. Characterization of the IgE-binding Ags found in this study

Ag No.*	Migr†	MW (kd)‡	pI§	Prev No. pt	Prev %	Comments¶
5	3.1	—	—	1	3	—
8	2.8	14	—	4	13	—
9	2.7	20	4.5	11	28	—
11	2.6	50	—	3	9	—
12	2.5	38	4.5	4	13	—
24	1.8	>65	—	32	100	Putative aggregate of antigens?
29	(1.5)	—	—	7	22	—
31	1.4	—	5.5	19	59	—
32	1.4	15	—	13	41	—
33	1.3	20	4.5	19	59	—
34/35	1	32	6.0-6.8	31	97	Ag 24 ²⁷ , Fr9 ²⁸ , Fr9 ²⁹
37	0.9	25	—	6	19	—
48	-0.5	—	—	1	3	—

*Antigen number according to CIE reference pattern (Fig. 1B).

†Antigen migration in CIE relative to the major allergenic component Ag 34/35.

‡Molecular weight as determined on a Sephadex G75 column by size-exclusion chromatography.

§pI, as determined in a preparative IEF experiment.

||Prevalence: number and percentage of patients' sera revealing specific IgE against the antigen.

¶Comments on possible earlier reported identifications of the allergen. Fr fraction according to Ford and Baldo²⁸ and Shen et al.²⁹

DISCUSSION

The analysis of a dialyzed and lyophilized extract of Bermuda-grass pollen by means of CIE and CRIE with sera from 32 allergic patients has demonstrated that 13 of the found Ags are able to bind IgE. Four of the Ags bound IgE from >50% of the sera. Three

of these Ags strongly bound >25% of the sera (scores >7) and might then be regarded as major allergens.³⁵ One allergen (Ag 34/35) bound IgE from 31 of the 32 sera and must be paid special attention in further purification procedures.

The number of allergens detected resembles the

number detected in other investigations on the allergenic composition of Bermuda grass. Fourteen allergens were identified in CRIE in the study of the proposed international reference,⁹ 17 allergens were identified in the immunoblot study of Ford and Baldo,²⁸ and 14 allergens were identified in the immunoblot study of Shen et al.²⁹

Some of this observed IgE binding might be due to coprecipitation because of allergens binding to other comparatively "sticky" precipitates that therefore appear to bind IgE.^{34,35} However, none of the Ags demonstrated any clear coprecipitation pattern. All Ags demonstrated some individual IgE-binding pattern, and therefore, they might all be considered as allergens, binding IgE specifically.

Ag 24 demonstrated some nonspecific IgE binding, as revealed in CRIE with a negative control serum. The IgE binding observed might partly be due to coprecipitation, but all sera appear to reveal specific IgE against the precipitate, indicating that the precipitate contain one or more allergenic components. We assume that this high MW Ag (MW >65 kd) could be an aggregate of many Ags (including allergens), specifically and nonspecifically binding human IgE and/or rabbit anti-IgE.

The 52 Ags probably do not represent all the antigenic (and then potentially allergenic) components of the extract that can be extracted with an aqueous buffer. Although the CRIE method is very sensitive, the technique may still not detect antigenic components present in small amounts against which the immunized rabbits made little or no antibody. However, the CRIE identification of Bermuda grass-pollen allergens probably accounts for all the important allergen molecules in this extract, since the rabbit antibodies, covalently bound to Sepharose 4B, were unable to absorb all the RAST-inhibitory capacity of the extract.

The physicochemical parameters of some of the 13 IgE-binding Ags were determined by gel filtration and preparative IEF followed by FRIE and CLIE.

All 10 allergens for which a molecular size has been determined (including the four major allergens) had MWs >14 kd. Only one Ag (the possible aggregate of Ag 24) had an MW >50 kd.

The MW of the major allergenic component Ag 34/35 was determined as 32 kd. Ford and Baldo,²⁸ and recently also Shen et al.,²⁹ determined the size of the major allergen by electroblotting techniques as 34 kd and 32 kd, respectively. There appears to be no doubt that these major allergens (which were revealed by two different techniques, CRIE and immunoblotting) are the same (Table II).

Most Ags had pI values between 4.5 and 5.5. The major allergenic component Ag 34/35 was distributed

over a pI interval from 6.0 to 6.8, indicating that the component consisted of at least two immunochemically similar allergens with slightly different pI values. Ag 34/35 has a higher pI value than most of the other revealed Bermuda-grass Ags. This might be important in purification procedures.

Other major grass allergens studied in CIE and CRIE demonstrate similar precipitates and IgE-binding characteristics as the major Bermuda-grass allergen,^{4, 5, 17, 22, 23} but generally, most other major grass allergens demonstrate lower pI values than Ag 34/35. The size and allergenicity of this component indicate, however, that it possibly constitutes a Bermuda-grass homologue to the well-described group I allergens of other grasses.^{7, 12-14, 21} Like *Lol p I* this allergen appears to consist of two or more allergenically isomeric components with slightly different pIs.

The results from several RAST-inhibition experiments performed with extracts from temperate and subtropical grasses have indicated that the Bermuda-grass allergens and allergens from these grasses do not reveal any strong cross-reactivity.^{2, 3, 6, 8} This allergenic difference between Bermuda grass and the common temperate grasses may be due to a relatively high genetic divergence (prompting taxonomists to place Bermuda grass in another grass subfamily). Many epitopes, but perhaps not the overall biologic function of the Ags, might have been changed during evolution. These possible differences call for further immunochemical comparisons of the Bermuda-grass major allergens (especially Ag 34/35) with other grass major allergens to study possible IgE epitope difference.

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