

October 1994 • Volume 94 • Number 4

Complementary DNA cloning of the major allergen *Phl p I* from timothy grass (*Phleum pratense*); recombinant *Phl p I* inhibits IgE binding to group I allergens from eight different grass species

Sylvia Laffer MSc^a [\[MEDLINE LOOKUP\]](#)
Rudolf Valenta MD^a [\[MEDLINE LOOKUP\]](#)
Susanne Vrtala MSc^a [\[MEDLINE LOOKUP\]](#)
Markus Susani PhD^b [\[MEDLINE LOOKUP\]](#)
Ronald van Ree PhD^c [\[MEDLINE LOOKUP\]](#)
Dietrich Kraft MD^a [\[MEDLINE LOOKUP\]](#)
Otto Scheiner PhD^a [\[MEDLINE LOOKUP\]](#)
Michael Duchêne PhD^{a,d} [\[MEDLINE LOOKUP\]](#)

• Vienna and Salzburg, Austria, and Amsterdam, The Netherlands

Sections

- [Abstract](#)
- [Methods](#)
- [Results](#)
- [Discussion](#)
- [References](#)
- [Publishing and Reprint Information](#)
- [Articles with References to this Article](#)

Abstract

Background: Grass pollens, such as pollen from timothy grass (*Phleum pratense*), represent a major cause of type I allergy.

Objective: In this report we attempted to determine how cross-reactive allergenic components of grass pollens from different species can be represented by a minimum number of recombinant allergens.

Methods: We isolated and sequenced a timothy grass pollen cDNA coding for the major allergen *Phl p I*. A recombinant *Phl p I*- β -galactosidase fusion protein, which bound to IgE in 87% of patients with grass pollen allergy, was produced in *Escherichia coli*. Using recombinant *Phl p V* and *Phl p I*, we defined representative patients' sera that bound to group I but not to group V allergens, as well as sera with reactivity against group I and group V allergens. IgE immunoblot inhibition studies were done with nitrocellulose-blotted pollen extracts from eight grass species with different geographic distribution.

Results: Preadsorption of patients' sera with recombinant nonfusion *Phl p I* strongly reduced IgE binding to group I allergens from the eight grasses, showing extensive cross-reactivity between species.

Conclusion: A single recombinant group I allergen contains many of the IgE epitopes of group I isoallergens from a number of different grass species. (J ALLERGY CLIN IMMUNOL 1994;94:689-98.)

Abbreviations used

IPTG	Isopropyl- β -D-thiogalactoside
PAGE	Polyacrylamide gel electrophoresis
SDS	Sodium dodecylsulfate

Key words: [Timothy grass](#), [Phleum pratense](#), [allergen](#), [cDNA cloning](#), [IgE-immunoblot inhibition](#)

In the United States the prevalence of type I allergy to grass pollen, as determined by skin testing is 16%, which represents 75% of all persons with type I allergy. ¹ Group I grass pollen allergens, first described by Johnson and Marsh, ² are glycoproteins with a molecular weight of around 32 kd. For each grass species, the group I allergens exist as a number of cross-reactive isoforms of similar molecular masses but different isoelectric points. ³⁻⁵ *Lol p* I isoallergens from perennial rye grass (*Lolium perenne*) have been characterized by molecular cloning. ^{6, 7}

Until today, total extracts from pollens have been used for diagnosis of type I allergy by RAST and skin tests, as well as for treatment by hyposensitization. It is still difficult to biochemically standardize total extracts. Therefore the use of recombinant allergens may be suggested as an alternative. Recently, we showed, in the case of tree pollen allergy, that the cloned birch pollen allergens *Bet v* I ⁸ and birch profilin, *Bet v* II, ⁹ could be used for diagnosis of tree pollen allergy. ¹⁰ In the case of grass pollen allergy, recombinant timothy grass pollen allergens *Phl p* I, *Phl p* V, and timothy grass profilin were sufficient for diagnosis. ¹¹

In order to further test the hypothesis that recombinant allergens can be valuable for diagnosis and possibly therapy of type I allergy to grass pollens, we attempted to determine whether one recombinant group I allergen comprises most of the IgE epitopes of the isoforms in pollens from the same and from related grass species.

In this article we report the isolation of a cDNA coding for the *Phl p* I allergen from timothy grass (*Phleum pratense*) from an expression cDNA library by IgE immunoscreening. The cDNA coding for *Phl p* I was sequenced and expressed in *Escherichia coli* as an IgE-binding protein. Immunoblot inhibition experiments showed that the binding of IgE antibodies to the group I allergens from each of the eight grass species could be blocked with recombinant *Phl p* I. In conclusion, a very limited number of recombinant grass pollen allergens may contain the necessary structural elements for diagnosis, and possibly immunotherapy, of type I grass pollen allergy.

Methods

Biologic materials

Pollens from *Phleum pratense* (timothy grass), *Anthoxanthum odoratum* (sweet vernal grass), *Avena sativa* (cultivated oat), *Cynodon dactylon* (Bermuda grass), *Lolium perenne* (ryegrass), *Phragmites communis* (common reed), *Poa pratensis* (Kentucky bluegrass), and *Secale cereale* (cultivated rye) were obtained from Allergon AB (Engelholm, Sweden). The λ gt11 library was plated on a lawn of *E. coli* Y1090 cells. ¹² Lysogens were obtained from *E. coli* Y1089. ¹² Plasmid pUCBM20 (Boehringer Mannheim GmbH, Mannheim, Germany) was grown in *E. coli* XL1-Blue (Stratagene, La Jolla, Calif.). Recombinant nonfusion *Phl p* I was expressed in *E. coli* BL21 (DE3) (Novagen, Madison, Wis.).

Construction of a λ gt11 cDNA library of timothy grass pollen

PolyA⁺ RNA was isolated from timothy grass pollen by phenol–sodium dodecylsulfate extraction, ¹³ followed by oligo-dT cellulose chromatography. ¹⁴ Complementary DNA was synthesized (Amersham, London, U.K.) and methylated with *Eco*RI methylase (Promega, Madison, Wis.). After addition of *Eco*RI linkers (Boehringer Mannheim GmbH, Mannheim, Germany) and restriction with *Eco*RI, cDNA and linkers were separated by a

Nick column (Pharmacia, Uppsala, Sweden). After ligation into λ gt11 arms (Pharmacia) the cDNA was packaged in vitro (Amersham, London).

IgE Immunoscreening

λ gt11 phages of the unamplified timothy grass pollen library (1.7×10^5) were plated on a lawn of *E. coli* Y1090. Protein expression was induced with nitrocellulose filters (Schleicher & Schüll, Dassel, Germany) soaked in 10 mmol/L isopropyl- β -D-thiogalactoside (IPTG).¹² The filters were probed with serum IgE from a patient with grass pollen allergy with ¹²⁵I-labeled rabbit anti-human IgE (Pharmacia) as secondary antibody.⁸ The isolated immunopositive λ gt11 clones were tested with a serum pool from two patients with grass pollen allergy, reacting with group I but not with group V allergens. From lambda clones coding for group I allergens, the cDNA insert was excised with *EcoRI* and subcloned¹⁴ into pUCBM20 (Boehringer Mannheim GmbH). Both strands of the cDNA insert were sequenced¹⁵ with T7 polymerase by using M13 forward and reverse primers, as well as *Phl p I*-specific primers (Microsynth, Windisch, Switzerland).

Expression of *Phl p I* as fusion and nonfusion proteins in *E. coli*

For the expression of the recombinant *Phl p I* fusion protein, lysogenic *E. coli* Y1089 cells, which had been infected with the recombinant phage, were grown at 32° C to an optical density at 600 nm of 0.5. After 20 minutes at 42° C, the *Phl p I*- β -galactosidase fusion protein synthesis was induced by adding IPTG to a final concentration of 10 mmol/L. *E. coli* cells were grown at 37° C for 1 hour, harvested, and washed twice with 0.9% sodium chloride.

For the expression of a recombinant nonfusion protein, the cDNA coding for *Phl p I* was inserted downstream from a T7 promoter and expressed in *E. coli* BL21 (DE3), a strain that expresses IPTG-inducible T7 RNA polymerase (Susani et al. Unpublished data). Protein synthesis was induced for 4 hours at 37° C with 3 mmol/L IPTG, and cells were harvested and homogenized with an Ultraturrax homogenizer (IKA GmbH, Staufen, Germany) in the presence of buffer G (see below). Debris were precipitated by 15 minutes of centrifugation at 10,000g and 4° C.

Testing of the recombinant fusion protein with rabbit group I and group V specific antisera

Total proteins from *E. coli* Y1089 containing the *Phl p I*- β -galactosidase fusion protein were separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE)¹⁶ and blotted onto nitrocellulose.¹⁷ Nitrocellulose strips were incubated with either rabbit anti-group I or anti-group V antisera, diluted 1:1000 in buffer G (50 mmol/L sodium phosphate, pH 7.5, 0.5% Tween 20, 0.5% bovine serum albumin, 0.05% NaN₃). Bound rabbit antibodies were detected with ¹²⁵I-labeled donkey anti-rabbit antibody (1:2000 in buffer G) (Amersham, London). Protein extracts from *E. coli* Y1089 infected with λ gt11 phage without insert were used as negative controls.

Protein extraction from grass pollens

Two grams of pollen were shaken in 30 ml of distilled water for 2 hours at 4° C. The protein extracts were then centrifuged for 1 hour at 4° C and 80,000g, and the supernatants were dialyzed against distilled water at 4° C and lyophilized.

SDS-PAGE and immunoblot inhibition

Aqueous pollen extracts were separated by 12.5% SDS-PAGE¹⁶ with a preparative slot former and then blotted onto nitrocellulose.¹⁷ Strips of equal size were cut from the nitrocellulose sheets.

In order to determine whether sufficient pollen extract was loaded so that the amount of IgE-binding antigens would exceed the amount of patients' IgE, 1:10, 1:20, and 1:40 dilutions of patients' sera in buffer G (see above) were used for probing the strips. Bound IgE was detected as described above. In the whole range of dilutions, bound IgE was proportional to the amount of serum added.

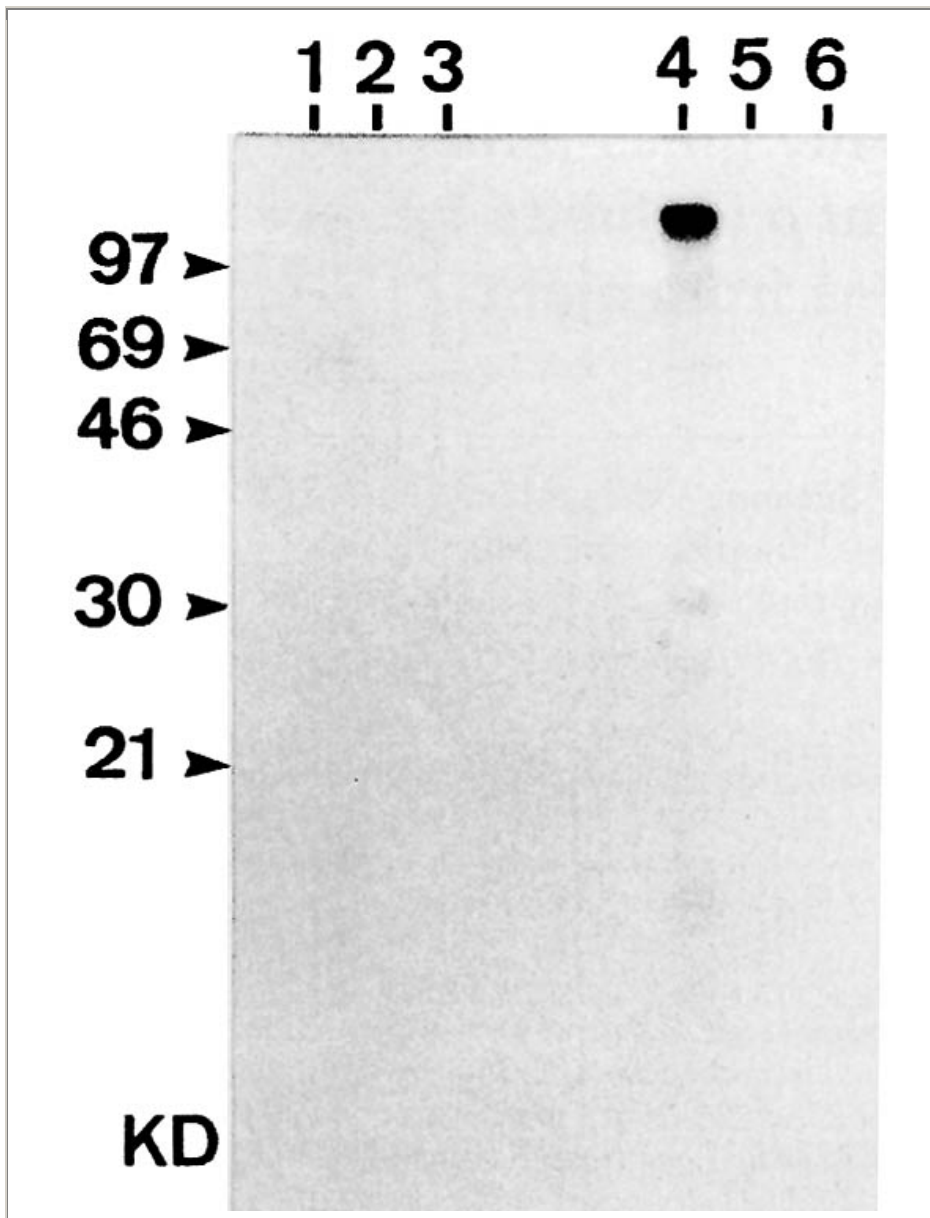
For the inhibition experiment, sera (1:10 in buffer G) were preadsorbed overnight at 4° C with 5% (vol/vol) of extracts from *E. coli* expressing the recombinant nonfusion *Phl p* I. Controls were preadsorbed with extract from nonrecombinant *E. coli*. Preincubated sera were used to probe the nitrocellulose strips containing total pollen extracts. Bound IgE was detected as described above. The autoradiograms were scanned with the EASY software of Herolab video-based gel analysis system (Herolab, Wiesloch, Germany).

Results

cDNA cloning of the major allergen *Phl p* I of timothy grass

A full-length λ gt11 cDNA clone, designated λ 55, was obtained from a timothy grass pollen cDNA library by immunoscreening of 1.7×10^5 phages with patients' IgE as described in the Methods section. Lysogens of λ 55 and of λ gt11 phage without insert were prepared, subjected to electrophoresis, blotted, and probed with rabbit antisera directed against group I and group V allergens (■Fig. 1). The *Phl p* I– β -galactosidase fusion protein expressed in *E. coli* infected with the clone λ 55 produced a positive signal with rabbit anti-group I antiserum.

FIG. 1. IgG immunoblot. Lanes 1-3, λ gt11 lysate without insert; lanes 4-6, λ 55 *Phl p* I– β -galactosidase fusion protein; lanes 1 and 4, probed with rabbit anti-group I antiserum; lanes 2 and 5, probed with rabbit anti-group V antiserum; lanes 3 and 6, buffer control without addition of rabbit antiserum.



Sequence analysis of the *Phl p I* allergen

The cDNA insert from clone λ 55 was subcloned into a pUCBM20 plasmid, and the complete sequence was obtained. The open reading frame of the complete *Phl p I* allergen (■Fig. 2) has a length of 263 amino acid residues. The first 23 N-terminal residues represent a typical hydrophobic signal sequence with a high degree of similarity to *Lol p I* around the signal peptide cleavage site. ^{6,7} The start of the mature protein is predicted by homology to be the isoleucine in the same position as the isoleucine that forms the start of the mature *Lol p I*. The predicted mature polypeptide has a length of 240 amino acid residues like the isoallergens *Lol p I* (1A) and *Lol p I* (5A) that were described by Perez et al. ⁶ and the *Lol p I* (13R) sequence, which was published by Griffith et al. ⁷ The deduced molecular weight of the mature *Phl p I* polypeptide was calculated as 26.1 kd. The alignment of the *Lol p I* isoallergen sequences with *Phl p I* is shown in ■Fig. 3. The closest homolog is the *Lol p I* (13R) ⁷ sequence, which has 91% amino acid sequence identity with *Phl p I*. The *Lol p I* (5A) ⁶ and *Lol p I* (1A) ⁶ sequences have 89% and 90% sequence identity with *Phl p I*. Other proteins with significant homology are *Lol p II* ¹⁸ and *Lol p III*, ¹⁹ which can be aligned with the C-terminal 97 amino acid residues of *Phl p I* and have 35% (*Lol p II*) and 30% (*Lol p III*) amino acid identity with *Phl p I*. Both the *Phl p I* and *Lol p I* sequences contain one possible N-linked glycosylation site at asparagine 9, which is part of the consensus sequence asparagine-X-threonine.

FIG. 2 Nucleotide and deduced amino acid sequence, displayed in the one-letter code, of the *Phl*

p I allergen of *P. pratense*. The sequence of the putative signal peptide is underlined. Numbering of amino acid starts with the first amino acid of the mature polypeptide.

```

1  ATGGCTTCTTCTCTGCTGGTTCTGCTGGTGGTGGCTTTTGGCCGCTCTTCTGGGCTCTGCTATGGCATCCCCAAGGTTCCCCCGGGT
   M A S S S S V L L V V V L F A V F L G S A Y G I P K V P P G
91  COGAACATCAAGGGAAGTCAAGGGAAGTGGCTGGAGCGGGAAGGACATGGTAAGGCAAGCGGACCGGGCGCGGTCCCAAGGACAAC
   8  P N I T A T Y G D K W L D A K S T W Y G K P T G A G P K D N
181 GGCGGCGCTTGGGGTACAAGGATGTGGACAAGCCCCGTTCAAGCGCATGACCGGCTGGGCAACACCCCATCTTCAAGTCCGAGCGC
   38  G G A C G Y K D V D K P P F S G M T G C G N T P I F K S G R
271 GGCTGGGCTCTCTCTTGGATCAAGTGCACCAAGCCGAGGCGCTCTCTGGGAGCCGTTGGTAGTCCACATCAAGGACGACAAAGAG
   68  G C G S C F E I K C T K P E A C S G E P V V V H I T D D N E
361 GAGCCCATGCCCCCTACCACTTGAAGTCTCCGCGCAAGCGTTCCGGGGGATGGCCAGAAGGGGATGAGCAGAAGCTGGCCAGCGGC
   98  E P I A P Y H F D L S G H A F G A M A K K G D E Q K L R S A
451 GGCGAGCTGGAGCTCAGTTCCGGGGCGTCAAGTGCAGTACCCGAGGGGCAACCAAGGTGAAGCTTCCAGCTGGAGAAGGGGTCCAAACCC
   128  G E L E L Q F R R V K C K Y P E G T K V T F H V E K G S N P
541 AACTAAGTGGGCTGCTTGTGAAGTACGTTAAAGGGAAGGAGAGCTGGTGGGGTGGACATCAAGGAGAAGGGCAAGGACAAGTGGATC
   158  N Y L A L L V K Y V N G D G D V V A V D I K E K G K D K W I
631 GAGCTCAAGGAGTCTGGGGGAGCATCTGGAGGATGGACACTCCCGACAAGCTCAAGGGCCCCCTTCAAGCTCCGCTACACCAAGAGGGC
   188  E L K E S W G A I W R I D T P D K L T G P F T V R Y T T E G
721 GGCACCAAGAACGAGCGAGGAGCTCATCCCTGAGGGCTGGAAGGCGGACACCAAGCTACGAGTCCAAAGTGAACAAGGAGAAGTGAATCT
   218  G T K T E A E D V I P E G W K A D T S Y E S K *
811 CTGCTTCTCTTCCGGCCCCCTGCTTAATTAATTTTGGCTCAAGGTTACCAATAAAACCGGCCACAGGAGAGAGCAACCAAGCTTCCG
   901  GATGCATGAGCATATTCTATGCTGATACGAGAATAATAGTTTGGAGTGGAGATCTTGTGAGGTGAATTATGTAGGAGACAACCAATGAAG
991 AATTGGCTGGCCCTCCCGGCCACTCCCGAGCGCTTGTTCATGTATCTAAAAACAGTTCATGATTATGAAGATATATT

```

FIG. 3. Alignment of the deduced *Phl p* I sequence with the sequences of *Lol p* I clones 13R⁷, as well as 1A and 5A.⁸ The 13R clone, which is closest to *Phl p* I, is aligned directly with vertical bars indicating sequence identity. For the other two *Lol p* I sequences, only differing amino acids are shown. Numbering starts with the first amino acid of the mature polypeptide.

	1
<i>Phl p I</i>	MASSSSVLLVVVLFVAVFLGSAYGIPKVPPGPNITATYGDK
<i>Lol p I</i> (13R)	MASSSSVLLVVALFVAVFLGSAHGIKAVPPGPNITAEYGDK
<i>Lol p I</i> (1A)	
<i>Lol p I</i> (5A)	
	18
<i>Phl p I</i>	WLDKSTWYGKPTGAGPKDNGGACGYKDVKPFPSGMTGC
<i>Lol p I</i> (13R)	WLDKSTWYGKPTGAGPKDNGGACGYKDVKAPFNGMTGC
<i>Lol p I</i> (1A)	
<i>Lol p I</i> (5A)	N.....
	58
<i>Phl p I</i>	GNTPIFKSGRGCSCFEIKCTKPEACSGEPVVVHITDDNE
<i>Lol p I</i> (13R)	GNTPIFKDGRGCSCFEIKCTKPESCSGEAVTVTITDDNE
<i>Lol p I</i> (1A)	
<i>Lol p I</i> (5A)	
	98
<i>Phl p I</i>	EPIAPYHFDLSGHAFGAMAKKGDEQKLSAGELELQFRRV
<i>Lol p I</i> (13R)	EPIAPYHFDLSGHAFGSMAKKGEEQKLSAGELELQFRRV
<i>Lol p I</i> (1A)	NV.....
<i>Lol p I</i> (5A)	NV.....
	138
<i>Phl p I</i>	KCKYPEGTVTFHVEKGSNPNYLALLVKYVNGDGDVVAVD
<i>Lol p I</i> (13R)	KCKYPDGTKPTTFHVEKASNPNYLAILVKYVDGDGDVVAVD
<i>Lol p I</i> (1A)	D.....G.....
<i>Lol p I</i> (5A)	D.....A.....
	178
<i>Phl p I</i>	IKEKGDKWIELKESWGAIWRIDTPDKLTGPFTVRYTTEG
<i>Lol p I</i> (13R)	IKEKGDKWIELKESWGAVWRIDTPDKLTGPFTVRYTTEG
<i>Lol p I</i> (1A)	
<i>Lol p I</i> (5A)	T.....
	218 240
<i>Phl p I</i>	GTKTEAEDVIPEGWKADTSYESK
<i>Lol p I</i> (13R)	GTKSEVEDVIPEGWKADTSYSK
<i>Lol p I</i> (1A)	
<i>Lol p I</i> (5A)	F.....

Inhibition of IgE binding to group I allergens in pollen extracts from eight grass species by recombinant *Phl p I*

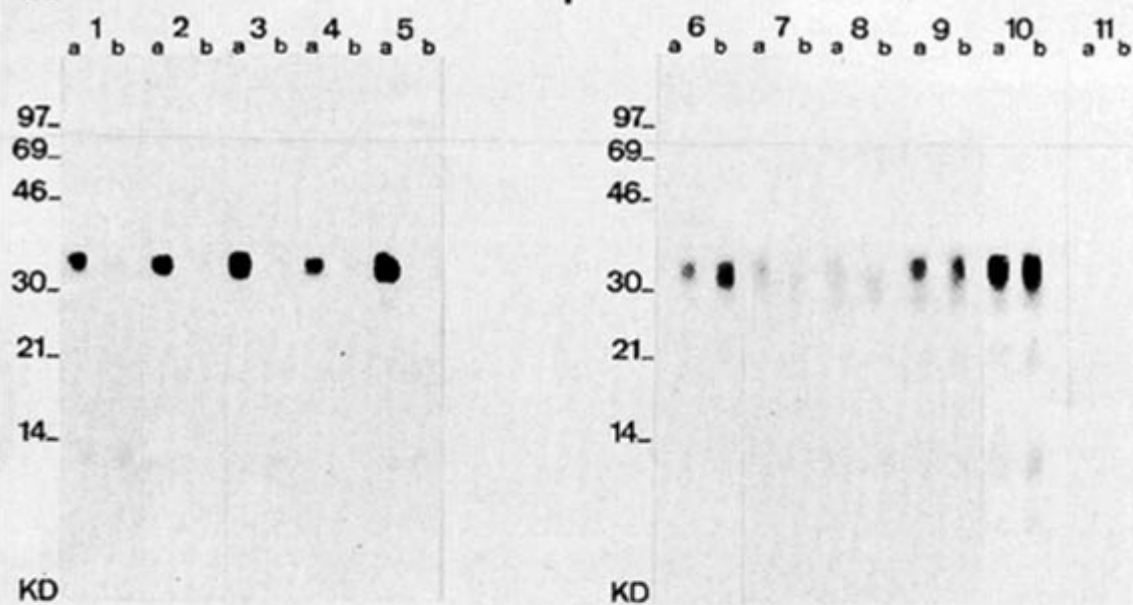
Proteins were extracted from pollens of *P. pratense* (timothy grass), *A. odoratum* (sweet vernal grass), *A. sativa* (cultivated oat), *C. dactylon* (Bermuda grass), *L. perenne* (rye grass), *P. communis* (common reed), *P. pratensis* (Kentucky bluegrass), and *S. cereale* (cultivated rye). These grass species were selected for maximum variation in geographic distribution.

The extracts were separated by SDS-PAGE and blotted onto nitrocellulose. Five well-defined patient serum samples with IgE reactivity toward group I but not group V grass pollen allergens and five patient serum samples with reactivity toward group I and group V were selected from 100 serum samples, which had been tested with recombinant *Phl p* I, *Phl p* V, and timothy grass profilin.¹¹ Nitrocellulose strips from each of the eight grass species were probed with the serum samples. In a preliminary experiment described in the Methods section, we attempted to determine whether the antigen on the blots was in excess of the IgE antibodies at the serum dilution used (1:10). The serum samples from the 10 patients and a nonallergic control subject were preincubated with extract containing the recombinant *Phl p* I or nonrecombinant *E. coli* extract. Immunoblot strips from each of the eight grass species were probed with the preadsorbed sera. Bound IgE was detected by ¹²⁵I-labeled rabbit anti-human IgE.

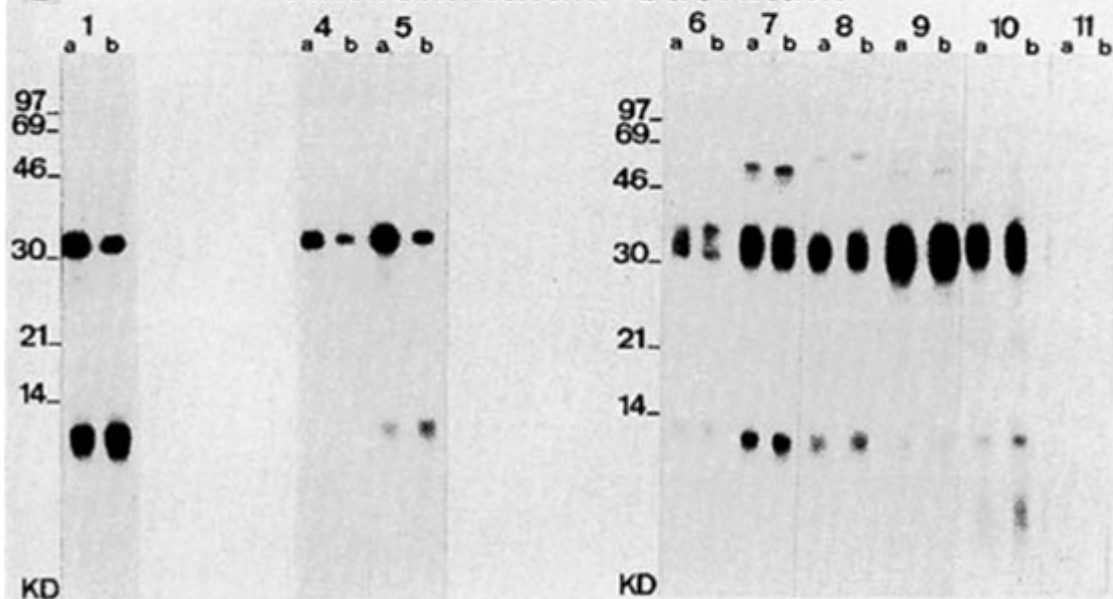
■Fig. 4 (A-G) shows the results. The five patients who were allergic to group I but not group V allergens (patients 1 to 5; in the case of *A. odoratum*, only patients 1, 4, and 5) all had IgE against proteins of around 30 kd in each of the extracts. In *Poa pratensis* and possibly in *Phleum pratense* and *C. dactylon*, double bands were observed. IgE binding to all these putative grass pollen group I allergens was clearly inhibited by preincubation with the recombinant *Phl p* I protein. The bands were scanned with a video-based gel analysis system to quantify the percentage of inhibition. The average was 100% for *A. sativa*, 94% for *P. pratense*, 88% for *S. cereale*, 67% for *L. perenne*, 64% for *P. pratensis*, 60% for *C. dactylon*, 59% for *A. odoratum*, and 56% for *P. communis*, with an average of 76%. Different results were obtained for the sera that reacted with group I and group V grass allergens (patients 6 to 10). The gels did not resolve groups I and V, except in the case of *S. cereale* pollen. Therefore the group I inhibition is less obvious, and the group I-specific IgE comprises a smaller share of the total anti-pollen IgE.

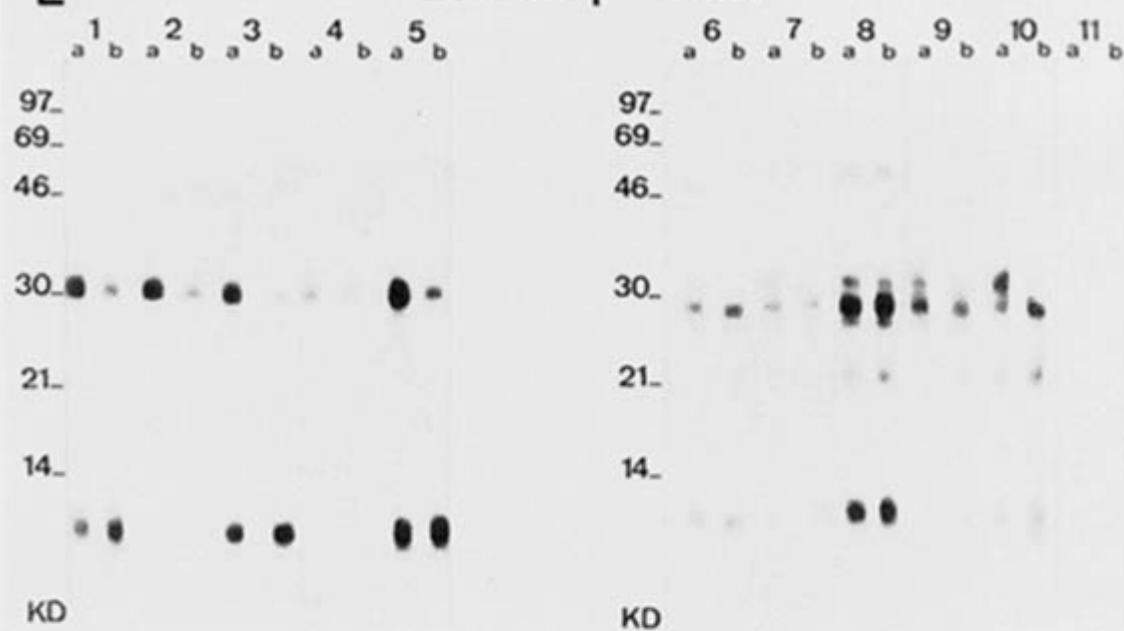
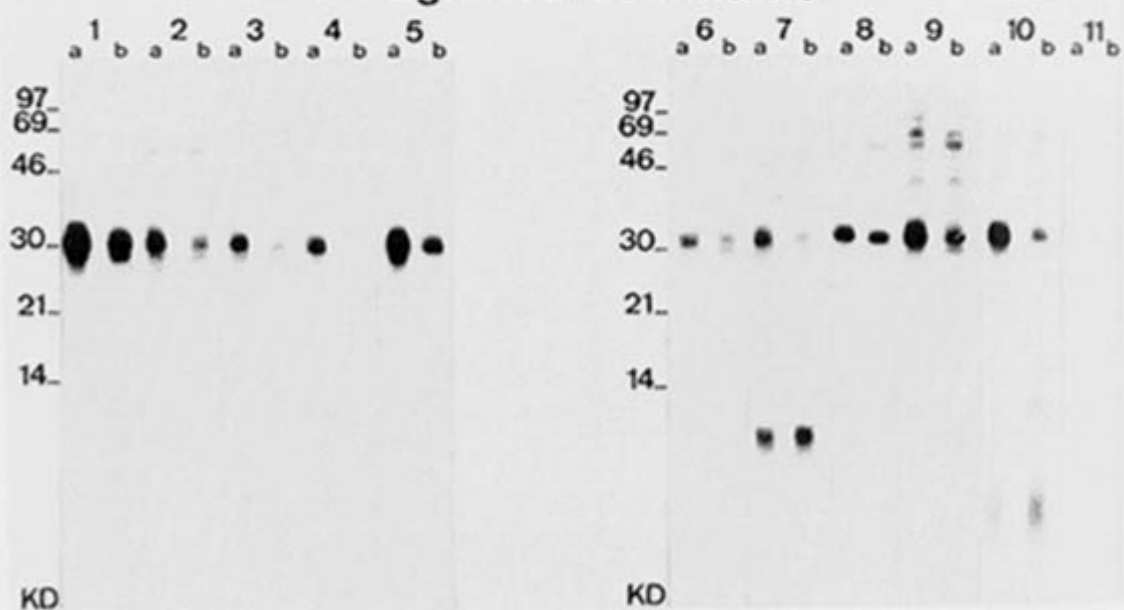
FIG. 4. IgE immunoblot inhibition experiment. Aqueous pollen extracts of *P. pratense* (A) and *A. odoratum* (B), *A. sativa* (C), *C. dactylon* (D), *L. perenne* (E), *P. communis* (F), *P. pratensis* (G), and *S. cereale* (H) were separated by SDS-PAGE and blotted onto nitrocellulose. Lanes 1 to 5, Sera from selected patients with grass pollen allergy with IgE against group I but not group V allergens; lanes 6 to 10, sera from patients with grass pollen allergy with IgE against group I and group V allergens; lane 11, control serum from a nonallergic individual; a, Patients' sera preadsorbed with lysates from nonrecombinant *E. coli*; b, patients' sera preadsorbed with lysates from *E. coli* expressing recombinant *Phl p* I.

A *Phleum pratense*

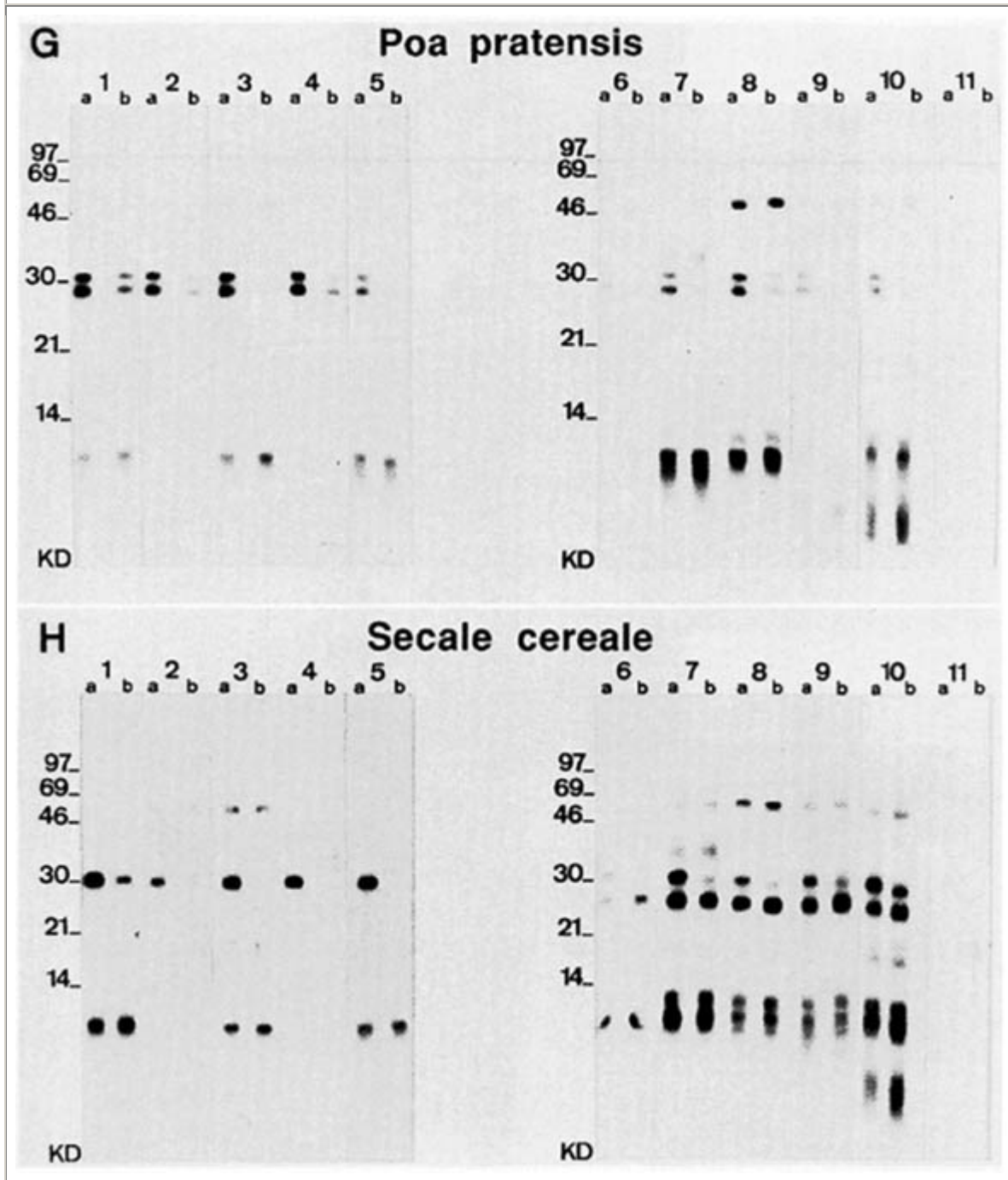


B *Anthoxanthum odoratum*



E**Lolium perenne****F****Phragmites communis**

No image



Discussion

In this article, the cDNA cloning and expression in *E. coli* of *Phl p I* of timothy grass (*P. pratense*), an IgE-binding protein, are reported. The recombinant *Phl p I* molecule was found to contain the majority of the relevant IgE epitopes of group I allergens from eight grasses.

Timothy grass pollen was chosen in this study because of its worldwide occurrence and particular importance as a source of grass pollen allergy. A λ gt11 cDNA library was constructed from pollen mRNA as starting material. Screening the library with serum IgE from a selected patient with IgE antibodies against a number of allergens provided a spectrum of allergen-encoding clones. A complete cDNA coding for *Phl p I* was isolated, subcloned, and sequenced.

The complete clone λ 55 coded for a polypeptide with end-to-end homology with the *Lol p I* allergen from *L. perenne*.^{6,7} The recombinant β -galactosidase fusion protein produced in *E. coli* was bound by specific rabbit antibodies raised against group I grass pollen allergens. The *Lol p I* (13R)⁷ and *Phl p I* sequences shared 91% amino acid sequence identity, which correlates well with the known cross-reactivity of the group I grass pollen allergens. Several features are conserved between *Phl p I* and *Lol p I*: the N-linked putative glycosylation site at asparagine 9; all seven cysteine residues in positions 41, 57, 69, 72, 77, 83, and 139; a hydrophobic signal sequence of 23 amino acid residues; and start of the predicted mature protein with an isoleucine. These data identified the cloned allergen unambiguously as *Phl p I*.

Secondary structure prediction according to the algorithm of Chou and Fasman²⁰ produced analogous results; similarly, the regions with a high antigenic index²¹ were the same for both polypeptides (not shown).

In a recent report²² the isolation of a cDNA coding for the *Phl p V* allergen is described. Recombinant *Phl p V*, as well as recombinant *Phl p I* described in this study, strongly bound patients' IgE. Therefore these molecules were useful for the identification of patients' sera with IgE antibodies against group I or group V allergens. Recombinant *Phl p I*, produced as a nonfusion protein, was used to deplete from these sera IgE binding to group I allergens from eight different grass species. The sera with IgE against *Phl p I* and not *Phl p V* allowed an estimation of the group I cross-reactivity. On average, 76% of the group I-specific reactivity could be extinguished with the recombinant *Phl p I*. The degree of inhibition varied with some relation to the evolutionary distances between the different grass species, but even in the case of the distantly related grasses, such as *P. communis* and *C. dactylon*, IgE binding could be inhibited by more than 50%. This supports the theory that different group I isoallergens share highly cross-reactive protein epitopes.

All of the group I allergens from the eight grass species, except for the lower molecular weight isoform from *P. pratense*, produced signals at or above 30 kd. This observation shows that the mature polypeptide encoded by the cDNA (26.1 kd) is posttranslationally modified. One site for modification could be the putative N-linked glycosylation site at asparagine 9, which is conserved between *Phl p I* and *Lol p I*.⁶ Some of the differences in the IgE binding capacity of the different isoforms could be attributed to protein glycosylation.

IgE binding to the group II/III isoallergens seen in the region around 10 to 12 kd was not visibly inhibited by preincubation of patients' sera with recombinant *Phl p I*, although a sequence identity of 30% to 35% could be sufficient for IgE cross-reactivity, as shown in the case of profilins,^{9,23} and although a cross-reactive B-cell epitope was detected close to the C-terminal end of group I grass pollen allergens.^{5,24} However, this epitope was recognized by IgE in the serum samples of only a very small percentage of patients with grass pollen allergy²⁴ (and unpublished results). No IgG cross-reactivity between group I and group II/III allergens had been detected by double-antibody radioimmunoassay methods.²⁵ Although the major B-cell epitopes seem to be different between group I and group II/III grass pollen allergens, isolation of T-cell clones cross-reactive between these allergens was reported recently.²⁶

In conclusion, the cloning and expression of *Phl p I* as an IgE-binding protein are reported. With the recombinant allergen, it was demonstrated that one recombinant allergen, *Phl p I*, shared IgE epitopes with the group I allergens of each of the eight species investigated. Although on the average 76% of the group I reactivity could be blocked with the recombinant *Phl p I* fusion protein, no complete inhibition was obtained. The extensive cross-reactivity makes single recombinant grass allergens prime candidates for allergen-specific diagnosis of type I hypersensitivity. The usefulness of each recombinant allergen as a representative of a group of isoallergens may also depend on its contribution to patients' total IgE reactivity. A first attempt to measure these contributions has recently been undertaken for recombinant grass pollen allergens.²⁷ Specific in vivo models of allergy will have to show whether the structural features of recombinant molecules may also be sufficient for immunotherapy.

References

1. Freidhoff LR, Ehrlich-Kautzky E, Grant JH, Meyers DA, Marsh DG. A study of the human immune response to *Lolium perenne* (rye) pollen and its components, *Lol p I* and *Lol p II* (rye I and rye II). J ALLERGY CLIN IMMUNOL 1986;78:1190-1201.
2. Johnson P, Marsh DG. "Isoallergens" from rye grass pollen. Nature 1965;206:935-7.
3. Standring R, Spackman V, Porter SJ. Distribution of a major allergen of rye grass (*Lolium perenne*) pollen between other grass species. Int Arch Allergy Appl Immunol 1987;83:96-103.

4. Esch RE, Klapper DG. Identification and localization of allergenic determinants on grass group I antigens using monoclonal antibodies. *J Immunol* 1989;142:179-84.
5. Esch RE, Klapper DG. Isolation and characterization of a major cross-reactive grass group I allergenic determinant. *Mol Immunol* 1989;26:557-61.
6. Perez M, Ishioka GY, Walker LE, Chesnut RW. cDNA cloning and immunological characterization of the rye grass allergen *Lol p I*. *J Biol Chem* 1990;265:16210-5.
7. Griffith IJ, Smith PM, Pollock J, et al. Cloning and sequencing of *Lol p I*, the major allergenic protein of rye-grass pollen. *FEBS Lett* 1991;279:210-5.
8. Breiteneder H, Pettenburger K, Bito A, et al. The gene coding for the major birch pollen allergen *Bet v I*, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;8:1935-8.
9. Valenta R, Duchêne M, Pettenburger K, et al. Identification of profilin as a novel pollen allergen: IgE autoreactivity in sensitized individuals. *Science* 1991;253:557-60.
10. Valenta R, Duchêne M, Vrtala S, et al. Recombinant allergens for immunoblot diagnosis of tree-pollen allergy. *J ALLERGY CLIN IMMUNOL* 1991;88:889-94.
11. Valenta R, Vrtala S, Ebner C, Kraft D, Scheiner O. Diagnosis of grass pollen allergy with recombinant timothy grass (*Phleum pratense*) pollen allergens. *Int Arch Allergy Immunol* 1992;97:287-94.
12. Huynh TH, Young RA, Davis RW. Construction and screening of cDNA libraries in λ gt10 and λ gt11. In: Glover DM, ed. *DNA cloning. A practical approach*. Vol. I. Oxford: IRL Press, 1985:49-78.
13. Ausubel FM. Phenol/SDS method for plant RNA preparation. In: Ausubel FM, Brent R, Kingston RE, et al., eds. *Current protocols in molecular biology*. Suppl. 9. New York: Wiley, 1990:4.3.1-4.3.4.
14. Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: laboratory manual*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989.
15. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A* 1977;74:5463-7.
16. Fling SP, Gregerson DS. Peptide and protein molecular weight determination by electrophoresis using a high-molarity Tris buffer system without urea. *Anal Biochem* 1986;155:83-8.
17. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A* 1979;76:4350-4.
18. Ansari AA, Shenbagamurthi P, Marsh DG. Complete amino acid sequence of a *Lolium perenne* (perennial rye grass) pollen allergen, *Lol p II*. *J Biol Chem* 1989;264:11181-5.
19. Ansari AA, Shenbagamurthi P, Marsh DG. Complete primary structure of a *Lolium perenne* (perennial rye grass) pollen allergen, *Lol p III*: comparison with known *Lol p I* and *II* sequences. *Biochemistry* 1989;28:8665-70.
20. Chou PY, Fasman GD. Prediction of the secondary structure of proteins from their amino acid sequence. *Adv Enzymol* 1978;47:45-148.
21. Jameson BA, Wolf H. The antigenic index: a novel algorithm for predicting antigenic determinants. *CABIOS* 1988;4:181-6.
22. Vrtala S, Sperr WR, Reimitzer I, et al. cDNA cloning of a major allergen from timothy grass (*Phleum pratense*) pollen: characterization of the recombinant *Phl p V* allergen. *J Immunol* 1993;151:4773-81.

23. Valenta R, Duchêne M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377-85.
24. van Ree R, van Leeuwen A, Aalberse RC. A synthetic peptide from the *Lol p I* C-terminus is recognized by human IgE and IgG and rabbit IgG antibodies. Presented at Eighth International Congress of Immunology; August 24-28, 1992; Budapest, Hungary.
25. Ansari AA, Kihara TK, Marsh DG. Immunochemical studies of *Lolium perenne* (rye grass) pollen allergens, *Lol p I*, II, and III. *J Immunol* 1987;139:4034-41.
26. Baskar S, Parronchi P, Mohapatra S, Romagnani S, Ansari AA. Human T cell responses to purified pollen allergens of the grass, *Lolium perenne*. *J Immunol* 1992;148:2378-83.
27. Laffer S, Vrtala S, Duchêne M, et al. IgE-binding capacity of recombinant timothy grass (*Phleum pratense*) pollen allergens. *J ALLERGY CLIN IMMUNOL* 1994 (in press).

Publishing and Reprint Information

- From^aInstitute of General and Experimental Pathology, Vienna; ^bAcademy of Sciences, Salzburg, Austria; ^cCentral Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands; and ^dInstitute of Specific Prophylaxis and Tropical Medicine, University of Vienna.
- Supported by grant 3/8167 of the "Fonds zur Förderung der gewerblichen Wirtschaft," Vienna, and grants S6002-BIO and S06703-MED of the "Fonds zur Förderung der wissenschaftlichen Forschung," Vienna.
- Received for publication July 7, 1993.
- Revised Apr. 11, 1994.
- Accepted for publication Apr. 19, 1994.
- Reprint requests: Michael Duchêne, PhD, Institute of General and Experimental Pathology, AKH, Währingergürtel 18-20, A-1090 Vienna, Austria.
- Copyright © 1994 by Mosby-Year Book, Inc.
- 0091-6749/94 \$3.00 + 0 1/1/56974

Articles with References to this Article

This article is referenced by these articles:

Exposure to a farming environment has allergen-specific protective effects on TH2-dependent isotype switching in response to common inhalants

Journal of Allergy and Clinical Immunology, The
February 2007 • Volume 119 • Number 2

Debra A. Stern, Josef Riedler, Dennis Nowak, Charlotte Braun-Fahrlander, Ines Swoboda, Nadja Balic, Kuan-Wei Chen, Susanne Vrtala, Hans Grönlund, Marianne van Hage, Rudolf Valenta, Susanne Spitzauer, Erika Von Mutius, Donata Vercelli

Spatial clustering of the IgE epitopes on the major timothy grass pollen allergen Phl p 1: Importance for allergenic activity

Journal of Allergy and Clinical Immunology, The
June 2006 • Volume 117 • Number 6

Sabine Flicker, Peter Steinberger, Tanja Ball, Maria-Theresa Krauth, Petra Verdino, Peter Valent, Steve Almo, Rudolf Valenta

Sublingual immunotherapy with once-daily grass allergen tablets: A randomized controlled trial in seasonal allergic rhinoconjunctivitis

Journal of Allergy and Clinical Immunology, The
April 2006 • Volume 117 • Number 4

Stephen R. Durham, William H. Yang, Martin R. Pedersen, Niels Johansen, Sabina Rak

Intranasal tolerance induction with polypeptides derived from 3 noncross-reactive major aeroallergens prevents allergic polysensitization in mice

Journal of Allergy and Clinical Immunology, The

August 2005 • Volume 116 • Number 2

Karin Hufnagl, Birgit Winkler, Margit Focke, Rudolf Valenta, Otto Scheiner, Harald Renz, Ursula Wiedermann

A hybrid molecule resembling the epitope spectrum of grass pollen for allergy vaccination

Journal of Allergy and Clinical Immunology, The

May 2005 • Volume 115 • Number 5

Birgit Linhart, Arnulf Hartl, Beatrice Jahn-Schmid, Petra Verdino, Walter Keller, Maria-Theresa Krauth, Peter Valent, Friedrich Horak, Ursula Wiedermann, Josef Thalhammer, Christof Ebner, Dietrich Kraft, Rudolf Valenta

Characterization of natural Dac g 1 variants: An alternative to recombinant group 1 allergens

Journal of Allergy and Clinical Immunology, The

November 2004 • Volume 114 • Number 5

Erica van Oort, Pleuni G. de Heer, Miranda Dieker, Astrid W. van Leeuwen, Rob C. Aalberse, Ronald van Ree

Human monoclonal antibody-based quantification of group 2 grass pollen allergens

Journal of Allergy and Clinical Immunology, The

March 2004 • Volume 113 • Number 3

Katharina Marth, MD, Margarete Focke, PhD, Sabine Flicker, PhD, Rudolf Valenta, MD*

Patterns of pollen cross-allergenicity

Journal of Allergy and Clinical Immunology, The

August 2003 • Volume 112 • Number 2

Richard W. Weber, MD

Dissociation of allergen-specific IgE and IgA responses in sera and tears of pollen-allergic patients: A study performed with purified recombinant pollen allergens

Journal of Allergy and Clinical Immunology, The

April 2000 • Volume 105 • Number 4

Rousan Aghayan-Ugurluoglu, MD^a, Tanja Ball, PhD^a, Susanne Vrtala, PhD^a, Christian Schweiger, MD^b, Dietrich Kraft, MD^a, Rudolf Valenta, MD^a

How far can we simplify in vitro diagnostics for grass pollen allergy?: A study with 17 whole pollen extracts and purified natural and recombinant major allergens

Journal of Allergy and Clinical Immunology, The

August 1998 • Volume 102 • Number 2

Ronald van Ree, PhD, W. Astrid van Leeuwen, Rob C. Aalberse, PhD
Amsterdam, The Netherlands

IgE antibodies to recombinant pollen allergens (Phl p 1, Phl p 2, Phl p 5, and Bet v 2) account for a high percentage of grass pollen-specific IgE

Journal of Allergy and Clinical Immunology, The

February 1998, Part 1 • Volume 101 • Number 2

Verena Niederberger, MD^a, Sylvia Laffer, PhD^b, Renate Fröschl^a, Dietrich Kraft, MD^b, Helmut Rumpold, MD^a, Sonja Kapiotis^a, Rudolf Valenta, MD^a, Susanne Spitzauer, MD^a
Vienna, Austria