

Immunologic characterization of purified recombinant timothy grass pollen (*Phleum pratense*) allergens (Phl p 1, Phl p 2, Phl p 5)

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Background: Grass pollen allergens belong to the potent elicitors of type I allergy. Approximately 40% of allergic individuals display IgE reactivity with grass pollen allergens. In previous studies we have reported the complementary DNA cloning and expression in *Escherichia coli* of three of the most relevant timothy grass pollen allergens: Phl p 1, Phl p 2, and Phl p 5.

Objective: To achieve high level expression of immunologically active timothy grass pollen allergens in *E. coli*, the cDNAs were inserted into expression plasmids.

Methods: The three recombinant grass pollen allergens were expressed at high levels in *E. coli* as recombinant nonfusion proteins, purified by conventional protein chemical methods and tested for their IgE-binding capacity by immunoblot and ELISA, as well as in histamine release assays.

Results: Milligram amounts of pure recombinant allergens were obtained from cultured *E. coli*. IgE binding to purified recombinant Phl p 1, Phl p 2, and Phl p 5 could be demonstrated by immunoblot and ELISA. With ELISAs the percentage of grass pollen-specific IgE directed against the individual recombinant allergens could be estimated. In addition, the purified recombinant timothy grass pollen allergens induced dose-dependent and specific histamine release from patients' blood basophils.

Conclusion: Purified recombinant timothy grass pollen allergens represent useful tools for diagnosis and therapy of grass pollen allergy. (*J ALLERGY CLIN IMMUNOL* 1996;97:781-7.)

Key words: Recombinant timothy grass pollen allergens, Phl p 1, Phl p 2, Phl p 5, high level expression in *E. coli*, histamine release

Type I allergic reactions caused by grass pollen allergens occur worldwide. Groups of grass pollen allergens with extensive immunologic cross-reactivities have been described in various grass species.¹⁻³ We have previously isolated complementary DNAs coding for three major timothy grass pollen allergens representing group I (Phl p 1),⁴ group II (Phl p 2),⁵ and group V (Phl p 5).⁶ Phl p 1 is a target for IgE antibodies in more than 95% of patients with grass pollen allergy. Phl p 5, though bound by fewer

Abbreviations used

BSA: Bovine serum albumin
DFP: Diisopropyl fluorophosphate
PBS: Phosphate-buffered saline

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patients (80% of patients with grass pollen allergy) is of particular importance because of its extremely high IgE-binding capacity; and Phl p 2 represents a low molecular weight allergen (10 kd) for 60% of the patients, and although similar in sequence to Phl p 1, shows no relevant immunologic similarity to Phl p 1. In a plaque lift technique β -galactosidase fusion proteins of the above-mentioned grass pollen allergens were successfully used to diagnose grass pollen allergy in a representative number of individuals,⁷ and it was further demonstrated that recombinant Phl p 1, Phl p 2, and Phl p 5, as well as timothy grass profilin (Phl p 11),⁸ can be used to precisely determine the sensitization patterns of these patients with

allergy (allergogram).⁹ In addition, it was demonstrated that recombinant timothy grass pollen allergens bound a high proportion of grass pollen-specific IgE.⁹ However, the previous assays were done with recombinant β -galactosidase fusion allergens, which in addition to the mature protein contained the leader peptides and a large portion of β -galactosidase. Although the fused polypeptide did not significantly affect the allergens' IgE-binding capacity, the purification protocols had delivered rather small amounts of the recombinant allergens. In this study we have modified the allergen-encoding cDNAs so that milligram amounts of functional nonfusion allergens were expressed in *Escherichia coli*.¹⁰ The recombinant grass pollen allergens were tested for antibody binding and specific histamine release and were found to behave similarly to their natural counterparts. A considerable improvement of in vitro and in vivo diagnosis of grass pollen allergy is expected if recombinant allergens can be used for a routine determination of the sensitization patterns in patients with grass pollen allergy. Allergen-specific diagnosis may consecutively improve specific immunotherapy.

METHODS

Characterization of patients with timothy grass pollen allergy

Patients allergic to grass pollen in this study were evaluated by case history, RAST (Pharmacia, Uppsala, Sweden) and skin prick test. Sera from allergic individuals had been tested with natural and recombinant timothy grass pollen allergens and were selected from 100 tested sera to reflect a representative binding pattern.^{7,9}

Construction of the expression plasmids for Phl p 1, Phl p 2, and Phl p 5

The cDNA clones of Phl p 1, Phl p 2, and Phl p 5⁴⁻⁶ were transcribed by polymerase chain reaction to DNA fragments coding for the mature allergens. The signal peptides were replaced by a methionine by placing an *Nde* I (Phl p 1, Phl p 2) or an *Nco* I (Phl p 5) restriction site, both of which contain the ATG codon, in front of the coding region of the mature protein. An *Eco* R I site was introduced immediately after the stop codon. The genes were inserted as *Nde* I/*Eco* R I (Phl p 1, Phl p 2) or *Nco* I/*Eco* R I (Phl p 5) fragments into pMW 172¹¹ (Phl p 1, Phl p 2) a derivative of pRK 172¹² or pMW 175 (Phl p 5), a derivative of pMW 172 where the *Nde* I site is replaced by *Nco* I. The plasmids were transfected into *E. coli* BL 21 (DE3) with the calcium chloride method.¹³ BL 21 is derived from *E. coli* strain B.¹⁴

Expression of recombinant allergens in *E. coli*

Recombinant Phl p 1, rPhl p 2, and rPhl p 5 were expressed in *E. coli* BL21 (DE3) in liquid culture on

induction with isopropyl β -thiogalactoside (0.2 to 0.4 mmol/L) when cells were grown to an optical density of 0.8 to 1.2. Yields of 28 mg (Phl p 1), 3 mg (Phl p 2), and 14 mg (Phl p 5) per gram wet weight of cells were obtained in 150 ml cultures. The amount of proteins was estimated from band intensities of Coomassie blue-stained sodium dodecylsulfate polyacrylamide electrophoresis gels (Coomassie blue; Sigma Chemical Co., St. Louis, Mo.).

Purification of recombinant allergens

Phl p 1. Phl p 1 was expressed in inclusion bodies, which were isolated after cells were thawed and suspended in 10 mmol/L Tris (pH 8), 2 mmol/L β -mercaptoethanol, 0.1% vol/vol Triton X-100, 0.5 mmol/L ethylenediaminetetraacetic acid (5 ml buffer/gm cells). Cell lysis was performed by adding lysozyme (20 μ g/gm cells) for 30 minutes at room temperature. Ca^{2+} was then added to 3 mmol/L final concentration, and the mixture was then digested with deoxyribonuclease I (0.1 mg/gm) for an additional 30 minutes at room temperature. The volume was then doubled by addition of 20 mmol/L Tris pH 8, 1% wt/vol desoxycholate, 2 mmol/L ethyleneglycol-bis-(β -aminoethylether)-N,N,N',N'-tetraacetic acid, and 0.2 mol/L NaCl (buffer P). The lysate was then centrifuged (5 minutes, 8000 g) and the pellet washed four times with fourfold-diluted buffer P containing 0.05 mmol/L diisopropyl fluorophosphate (DFP) by centrifugation. The resulting pellet was finally washed with 10 mmol/L Tris pH 8, 3% vol/vol isopropanol to remove residual detergents.

Inclusion bodies were then solubilized with 8 mol/L urea, 10 mmol/L Tris pH 8, 1 mmol/L ethylenediaminetetraacetic acid, 5 mmol/L β -mercaptoethanol, 0.05 mmol/L DFP (5 ml/gm cells) and dialyzed against the same buffer without urea. The dialyzed sample was then centrifuged for 10 minutes at 8000 g to remove precipitated material (containing approximately 20% to 30% of the recombinant Phl p 1), and the supernatant was then adjusted to 150 mmol/L NaCl and dialyzed against 100 mmol/L NaCl, 20 mmol/L NaH_2PO_4 , 0.05 mmol/L DFP without adjustment of pH. After dialysis, the supernatant was extracted with 0.5% vol/vol of chloroform, and the aqueous phase was precipitated with ammonium sulfate 60% wt/vol. A final purification step with a diethylaminoethyl cellulose column (M. Susani, et al. Unpublished data) yielded a soluble protein that was more than 95% pure.

Phl p 2. Phl p 2 was expressed as soluble protein. Cells were lysed as described above and centrifuged for 15 minutes at 14,000 g; the supernatant was brought to 0.05 mmol/L DFP. The supernatant was then dialyzed against 50 mmol/L phosphate buffer, pH 4.5, overnight. After centrifugation at 14,000 g for 15 minutes, the supernatant was brought to pH 5.5 with NaOH, and ammonium sulfate was added to 65% wt/vol. After another centrifugation (14,000 g, 15 minutes), the supernatant was applied to a phenyl-Sepharose column (Pharmacia), and the protein was eluted with 1 mol/L ammonium sulfate. The protein was then dialyzed against 25 mmol/L imidazole, pH 7.4, and rechromatographed on a diethylami-

noethyl cellulose–Sephacrose column to yield a protein more than 98% pure.

Phl p 5. *E. coli* cells were lysed as described above. After centrifugation (15 minutes, 14,000 g), the supernatant was adjusted to pH 6.0 with HCl. Material that was precipitated by addition of ammonium sulfate between 30% and 50% wt/vol was used for further purification. The precipitate was dissolved in 10 mmol/L phosphate, 0.1 mmol/L dithiothreitol, and 0.1% Triton X-100, pH 5.0, then dialyzed against the same buffer without Triton X-100 and applied to SP-Sephacrose. Fractions containing more than 90% pure Phl p 5 were concentrated by ammonium sulfate precipitation, dissolved in 100 mmol/L phosphate, pH 5.3, brought to 0.7 mol/L ammonium sulfate and chromatographed on a PS-Sephacrose column to yield a protein of more than 99% purity.

IgE binding to natural timothy grass pollen extracts and purified recombinant timothy grass pollen allergens (Phl p 1, Phl p 2, and Phl p 5)

IgE binding to timothy grass pollen allergens was measured by immunoblotting and ELISA. Nitrocellulose strips, containing total timothy grass pollen proteins, purified rPhl p 1, purified rPhl p 2, or purified rPhl p 5 were incubated with sera from 13 different representative patients with timothy grass pollen allergy. Patients' sera were diluted 1:10 in buffer G (50 mmol/L sodium phosphate, pH 7.5, 0.5% Tween-20, 0.5% bovine serum albumin [BSA], 0.05% NaN₃), and bound IgE was detected with iodine 125-labeled anti-human IgE (Pharmacia) as described by Valenta et al.¹⁵ For semiquantitative estimation of specific IgE against total timothy grass pollen proteins and purified recombinant timothy grass pollen allergens (rPhl p 1, rPhl p 2, and rPhl p 5) an ELISA was used. Amounts of allergens and serum dilutions were used so that antigens were in excess of IgE and IgG antibodies to allow the determination of the relative percentage of grass pollen-specific antibodies directed against the recombinant allergens. The optimal amounts of allergens and serum dilutions were determined in pilot experiments.

ELISA plates were coated with 50 µg/ml total timothy grass pollen proteins or purified, recombinant allergens (2 µg/ml). The plates were washed two times with phosphate-buffered saline (PBS), 0.05% Tween-20, blocked for 2.5 hours with PBS, 1% BSA, 0.05% Tween-20 at room temperature and incubated overnight at 4° C with sera from patients with grass pollen allergy. Patients' sera were diluted 1:20 in PBS, 0.5% BSA, 0.05% Tween-20 for the detection of specific IgE and 1:100 for detection of IgG₁, IgG₂, IgG₃, and IgG₄. The plates were washed five times with PBS, 0.05% Tween-20, and bound immunoglobulins were detected with monoclonal mouse anti-human Ig antibodies (PharMingen, San Diego, Calif.), diluted 1:1000 in PBS, 0.5% BSA, 0.05% Tween-20 overnight at 4° C. The plates were washed five times with PBS, 0.05% Tween-20 and

incubated for 30 minutes at 37° C and 30 minutes at 4° C with a horseradish-peroxidase-coupled sheep anti-mouse antiserum (Amersham, Buckinghamshire, U.K.), diluted 1:2000 in PBS, 0.5% BSA, 0.05% Tween-20. Plates were again washed five times with PBS, 0.05% Tween-20, and ABTS (60 mmol/L citric acid, 77 mmol/L Na₂HPO₄·2H₂O, 1.7 mmol/L ABTS [Sigma, St. Louis, Mo.], 3 mmol/L H₂O₂) was added. Plates were incubated in the dark for 30 minutes at room temperature, and the color reaction was stopped by addition of 100 µl/well 0.32% NaF. Extinctions (optical density 450 to 405 nm) were determined with an ELISA reader (Dynatech, Denkendorf, Germany).

In vitro histamine release assays with purified recombinant allergens

Heparinized blood samples were obtained from patients with grass pollen allergy after informed consent had been given. Granulocytes were prepared by dextran sedimentation and washed and incubated with various doses of recombinant allergens, anti-human IgE, or buffer as previously described.¹⁶ Liberated histamine was measured in the cell-free supernatants by radioimmunoassay (Immunotech, Marseille, France).

RESULTS

Expression in *E. coli* and purification of recombinant timothy grass pollen allergens

In a previous study recombinant timothy grass pollen allergens were expressed as β-galactosidase fusion proteins.⁹ In an attempt to obtain recombinant nonfusion allergens, which are similar to the natural allergens, polymerase chain reaction primers were synthesized according to the Phl p 1, Phl p 2, and Phl p 5 cDNA sequences to allow the amplification of cDNAs without the hydrophobic leader sequences that are absent in the mature proteins. The cDNAs coding for the mature allergens were amplified from the allergen cDNAs and subcloned into plasmid pET. Recombinant nonfusion proteins were expressed in *E. coli* BL21 (DE3) on induction with IPTG and purified. Fig. 1 shows a Coomassie blue-stained protein gel containing total timothy grass pollen extract in lane P and purified recombinant timothy grass pollen allergens (lanes I, II, and V). Milligram amounts of more than 90% pure and water-soluble recombinant Phl p 1, Phl p 2, and Phl p 5 were obtained for subsequent immunologic testing.

IgE-binding capacity of recombinant timothy grass pollen allergens

Purified recombinant timothy grass pollen allergens were tested for their antibody-binding capacity in denaturing and nondenaturing assays. Nitrocellulose-blotted natural timothy grass pollen

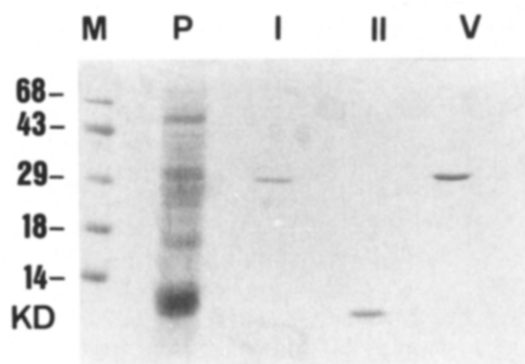


FIG. 1. Coomassie brilliant blue-stained polyacrylamide gel. Lane *M* represents a molecular weight marker; lane *P* shows a protein extract from timothy grass pollen; lane *I*, purified rPhl p 1; lane *II*, purified rPhl p 2; and lane *V*, purified rPhl p 5.

allergens and purified recombinant timothy grass pollen allergens were compared for IgE binding by using sera from 13 patients with grass pollen allergy. The sera were selected to represent the typical IgE-binding pattern to timothy grass pollen allergens from 100 individuals with grass pollen allergy as determined by case history, RAST, and skin prick testing.^{7,9} With the exception of patient 6 (Fig. 2), all patients showed IgE reactivity with nitrocellulose-blotted natural timothy grass pollen allergens. IgE binding was observed in the 30 kd range with group I and group V allergens and at around 10 kd with group II allergens. Two patients (nos. 2 and 4) showed weak reactivity to group IV allergens at 54 kd. All patients, including patient 6 who had no detectable IgE against natural timothy grass pollen allergens, displayed IgE reactivity to recombinant Phl p 1. Twelve of 13 patients reacted with recombinant Phl p 5, and 10 patients had IgE against recombinant Phl p 2. Recombinant timothy grass pollen allergens thus allowed differentiation among IgE reactivity to group I, group II, and group V allergens, which cannot be distinguished when nitrocellulose-blotted natural grass pollen extracts are used.

The ELISA results (Fig. 3) showed that in most of the patients high levels of timothy-specific IgE were directed against Phl p 5 and Phl p 1. Phl p 2 bound rather low levels of specific IgE. The reactivity in the different IgG subclasses with a given allergen was not always associated with its IgE-binding capacity (Fig. 3).

Recombinant timothy grass pollen allergens induce specific and dose-dependent histamine release

To demonstrate specific histamine release capacity of recombinant timothy grass pollen aller-

gens, basophils from patients with grass pollen allergy were incubated with recombinant Phl p 1, Phl p 2, or Phl p 5. Figs. 4 and 5 show that purified recombinant timothy grass pollen allergens induced specific and dose-dependent histamine release in patients with grass pollen allergy. To ensure that specific IgE antibodies were present in the patients' sera at the time when the histamine release assays were done, supernatants obtained during the enrichment of basophils were probed for IgE reactivity with natural and recombinant timothy grass pollen allergens (Figs. 2 and 3). Comparing the IgE reactivity of the patients displayed in the immunoblots in Fig. 2 with the histamine release capacity of recombinant allergens, we found that all patients with IgE antibodies specific for the recombinant allergen tested also had positive histamine release results. The optimal dose of recombinant timothy grass pollen allergens for inducing maximal histamine release was usually approximately 1 μ g/ml. As in previous studies performed with recombinant fusion allergens and natural allergens, histamine release was observed at doses as low as 10 ng/ml allergen.⁶ The recombinant allergens were also tested with basophils from patients without specific IgE antibodies and were found to induce no histamine release up to concentrations of 10 μ g/ml recombinant allergen (data not shown). Negative controls performed without addition of recombinant allergens or recombinant allergens against which patients were not sensitized did not lead to histamine release, whereas by using a monoclonal anti-human IgE antibody (positive control), histamine release could be induced in all patients tested as previously described.¹⁶

DISCUSSION

A number of cDNAs coding for plant, mite, animal, and fungal allergens have been characterized.^{17,18} Many of these cDNAs were obtained by IgE immunoscreening of expression cDNA libraries,¹⁸ and it was thus possible to test the bacterially expressed allergens for their IgE-binding capacity with sera from allergic patients. In previous studies the molecular cloning and expression of cDNAs coding for three major timothy grass pollen allergens (Phl p 1, Phl p 2, and Phl p 5) has been reported.⁴⁻⁶ With β -galactosidase-fused recombinant timothy grass pollen allergens it was shown that a panel of only three to four recombinant timothy grass pollen allergens can be sufficient to diagnose grass pollen allergy.⁷ In another study it has been demonstrated that a combination of these

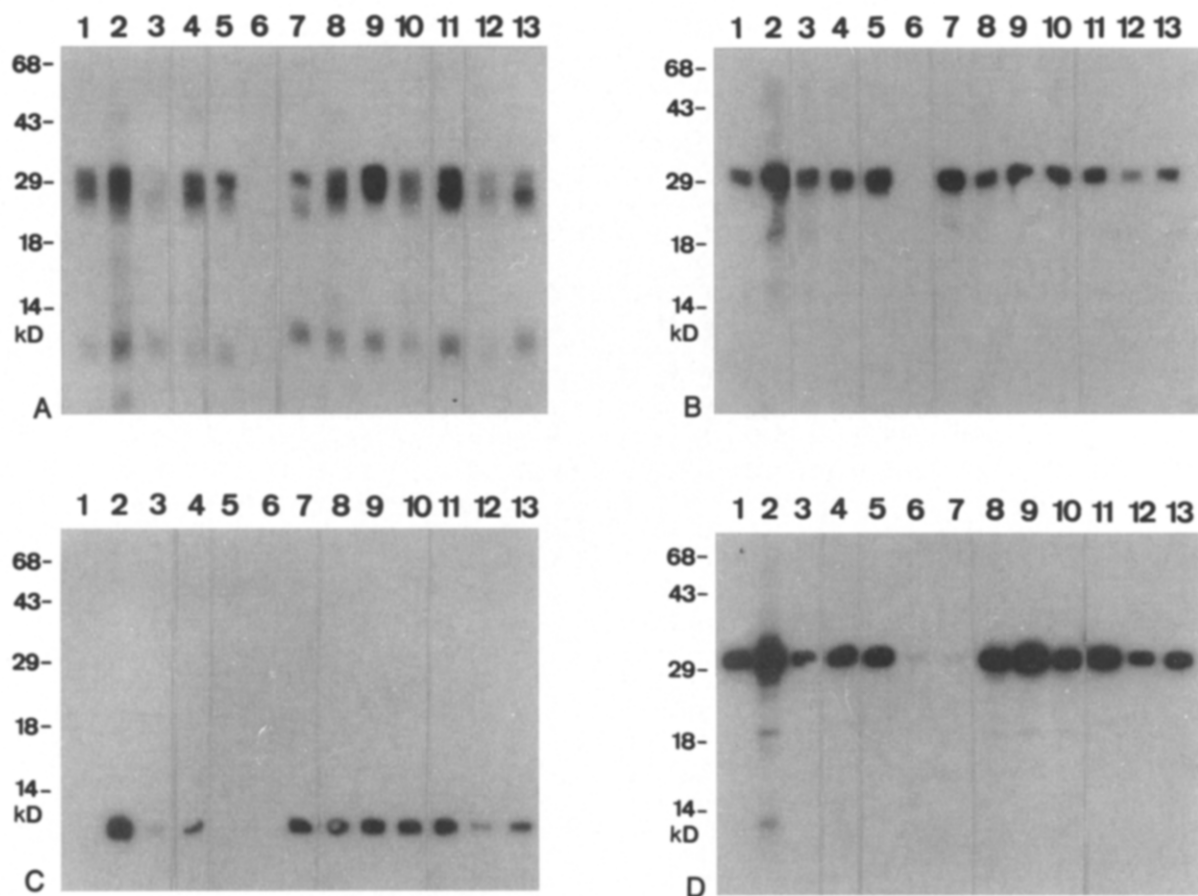


FIG. 2. IgE immunoblots with total timothy grass pollen proteins (A), purified rPhl p 1 (B), purified rPhl p 2 (C), and purified rPhl p 5 (D). Thirteen patients with timothy grass pollen allergy (lanes 1 to 13) were tested for IgE binding to nitrocellulose-blotted natural timothy grass pollen proteins (A) and purified recombinant timothy grass pollen allergens (B, C, and D).

three recombinant timothy grass pollen allergens bound a high percentage of grass pollen-specific IgE.⁹ Although β -galactosidase-fused timothy grass pollen allergens showed similar IgE-binding capacity compared with natural timothy grass pollen allergens, the expression of large amounts of rPhl p 1, rPhl p 2, and rPhl p 5 without leader peptides and fused protein was attempted. In this study the expression of functional rPhl p 1, rPhl p 2, and rPhl p 5 is reported. The recombinant allergens lack their hydrophobic leader peptides and other additionally fused polypeptides. In contrast to the natural allergens, an additional methionine is present at the N-terminus, and no carbohydrates or hydroxyprolines are present in the recombinant proteins because of the bacterial expression system used. As already demonstrated for the β -galactosidase-fused allergens, IgE binding to the native and denatured allergens was shown. The use of rPhl p 1, rPhl p 2, and rPhl p 5 allowed a precise determination of the

patients' sensitization patterns. A major advantage of recombinant allergens is the possibility to discriminate patients' IgE reactivity to allergens of a similar molecular weight, such as Phl p 1 or Phl p 5. Patient 6, who showed no detectable IgE reactivity to nitrocellulose-blotted natural grass pollen extract, was found to react with recombinant Phl p 1. Although considerable amounts of natural grass pollen extracts had been used to coat the ELISA plates, a combination of the three recombinant timothy grass pollen allergens yielded, for the most part, a higher extinction than the natural extract. Recombinant allergens therefore offer the advantage of measuring allergen-specific antibody levels, and it appears that carbohydrate moieties or amino acid modifications, such as hydroxyprolines, which frequently occur in natural grass pollen allergens, are not of relevance for patients' IgE binding.

In addition to the antibody-binding capacity, the biologic activity of rPhl p 1, rPhl p 2, and rPhl p 5

#	1	2	3	4	5	6	7	8	9	10	11	12	13	
E	0.16	2.26	0.18	0.40	0.44	0.05	0.36	0.34	0.88	0.22	0.78	0.16	0.24	IgE
I	0.10	0.52	0.11	0.10	0.29	0.05	0.29	0.13	0.47	0.13	0.16	0.09	0.10	
II	0.08	0.19	0.09	0.05	0.05	0.04	0.12	0.10	0.13	0.06	0.12	0.05	0.07	
V	0.17	1.54	0.14	0.43	0.34	0.05	0.06	0.32	1.33	0.18	0.99	0.16	0.24	
E	0.15	0.60	0.50	0.28	0.20	0.09	0.16	0.73	0.20	0.13	0.47	0.26	0.25	IgG1
I	0.08	0.28	0.27	0.11	0.10	0.10	0.12	0.58	0.18	0.11	0.10	0.16	0.20	
II	1.09	0.25	0.31	0.09	0.07	0.12	0.38	0.38	0.10	0.15	0.08	0.19	0.13	
V	0.10	0.45	0.48	0.30	0.09	0.10	0.10	0.81	0.65	0.16	0.14	0.25	0.19	
E	0.23	0.63	1.19	0.51	0.34	0.14	0.20	1.77	0.50	0.21	1.18	0.28	0.14	IgG2
I	0.23	0.50	0.64	1.13	0.61	0.94	0.64	0.55	1.04	0.24	1.32	1.16	0.36	
II	1.01	0.19	0.48	0.47	0.29	0.34	0.24	0.58	0.71	0.44	0.54	0.54	0.46	
V	0.14	0.20	0.66	0.25	0.21	0.12	0.14	1.25	0.83	0.18	0.37	0.47	0.13	
E	0.11	0.28	0.12	0.08	0.13	0.15	0.12	0.35	0.13	0.08	2.04	0.07	0.09	IgG3
I	0.06	0.05	0.05	0.05	0.06	0.13	0.09	0.08	0.09	0.05	0.08	0.05	0.07	
II	0.09	0.05	0.06	0.06	0.11	0.06	0.04	0.05	0.70	0.37	0.11	0.05	0.05	
V	0.26	0.07	0.05	0.05	0.06	0.11	0.05	0.09	0.08	0.28	0.07	0.58	0.05	
E	0.27	1.95	>2.5	2.15	0.22	0.06	0.28	>2.5	0.29	0.75	0.42	0.13	0.14	IgG4
I	0.08	0.40	2.31	0.36	0.21	0.06	0.18	2.05	0.16	0.35	0.27	0.08	0.10	
II	0.20	0.50	>2.5	0.19	0.07	0.07	0.12	>2.5	0.17	0.27	0.14	0.06	0.07	
V	0.14	0.58	>2.5	1.10	0.20	0.05	0.06	>2.5	0.33	0.54	0.48	0.10	0.15	

FIG. 3. IgE, IgG₁-IgG₄ reactivity to natural (E) and recombinant timothy grass pollen allergens (I, Phl p 1; II, Phl p 2; V, Phl p 5) estimated by ELISA in 13 patients with grass pollen allergy. Extinctions over baselines determined with a group of nonallergic individuals are displayed.

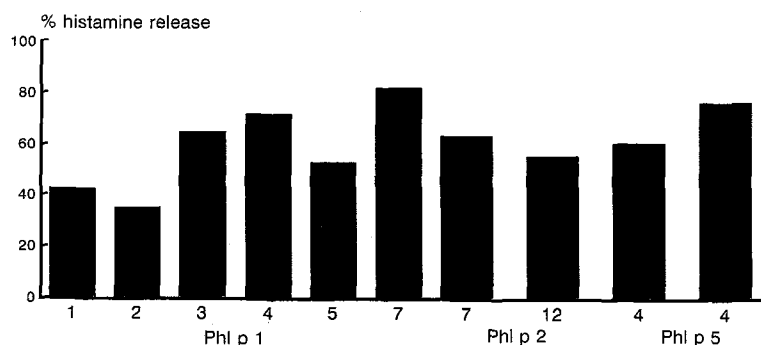


FIG. 4. Induction of histamine release from basophils of patients with grass pollen allergy with purified recombinant Phl p 1, Phl p 2, and Phl p 5. Basophils from six patients allergic to Phl p 1 were incubated with recombinant Phl p 1; basophils from two donors were incubated with recombinant Phl p 2 and basophils from one patient allergic to Phl p 5 (two experiments with the same patient). The percentage of histamine release at the optimal allergen concentration (1 µg/ml) is displayed. Patient numbers are the same as in Fig. 2 to allow a comparison of histamine-releasing capacity and IgE-binding capacity of recombinant timothy grass pollen allergens.

was demonstrated in basophil degranulation assays. The basophil degranulation assay¹⁹ has been shown to closely reflect the cascade of the allergic effector reaction, and in the case of recombinant birch pollen allergens, has already served as a valuable tool for testing recombinant allergens in a

close to in vivo system.^{15, 19} In this study purified recombinant timothy grass pollen allergens induced dose-dependent and specific histamine release from basophils of sensitized patients. Even at concentrations up to 10 µg/ml, no cell toxic effects were observed.

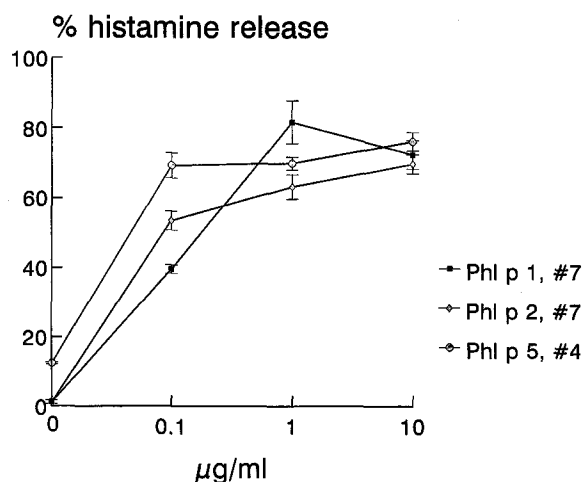


FIG. 5. Dose dependency of histamine release induced with basophils from representative patients allergic to grass pollen by use of recombinant Phl p 1, Phl p 2, and Phl p 5. Basophils from three representative patients with grass pollen allergy were incubated with different concentrations of recombinant timothy grass pollen allergens, and histamine release was determined in the cell-free supernatants by radioimmunoassay. Histamine release induced from basophils of patient 7 with rPhl p 1 and rPhl p 2 and the dose dependency of a histamine release obtained with recombinant Phl p 5 in patient 4 are shown. Patient numbers are the same as in Fig. 2.

In conclusion we believe that the expression of large amounts of recombinant timothy grass pollen allergens will substantially add to current forms of allergy diagnosis by allowing determination of the patients' sensitization patterns (allergogram). The recombinant nonfusion allergens can be produced in gram amounts with consistent batch-to-batch quality and can be used for current in vitro allergy tests, as well as for skin prick testing. On the basis of the determination of the patients' individual sensitization patterns and specific antibody levels, immunotherapy with selected recombinant allergens is a realistic prospect for the near future.²⁰

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