

Isolation of an Immunodominant IgE Hapten from an Epitope Expression cDNA Library

DISSECTION OF THE ALLERGIC EFFECTOR REACTION*

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An epitope expression cDNA library was constructed from the randomly fragmented cDNA coding for *Phl p I*, the major grass pollen allergen. Using IgE from allergic patients, epitope clones were isolated and immunodominant fragments were selected. Among three epitope clones coding for a similar region of *Phl p I*, one clone expressed a 15-amino-acid epitope which was target for IgE antibodies from approximately 30% of grass pollen allergic patients. According to the prevalence of grass pollen allergy, 22% of all allergic patients are expected to display IgE reactivity with this epitope. Although the purified recombinant epitope specifically bound IgE, it did not release histamine from basophiles of most grass pollen allergic patients and thus represents an IgE hapten. Immunodominant IgE haptens may be useful as therapeutic agents to saturate mast cell-bound IgE prior to allergen exposure and may represent candidates for a safe immunotherapy of allergic diseases by reducing anaphylactic side effects.

Up to 20% of the population in industrialized countries suffer from type I allergic symptoms (rhinitis, conjunctivitis, and asthma bronchiale) (Myamoto, 1992). The cross-linking of IgE which is bound to mast cells and basophils via the high affinity receptor FcεRI is the key event leading to release of biological mediators such as histamine (Segal *et al.*, 1977). The cross-linking event by allergens represents, therefore, a potential target for the therapy of type I allergy. Such therapeutical approaches could either use portions of the IgE molecule or other ligands to interfere with the binding of IgE to the high affinity Fcε-receptor or reagents to block the subsequent signal transduction cascade, thus preventing the degranulation of mast cells and basophils (Dreskin and Metzger, 1988). An additional possibility for specific therapy would be to use haptens derived from complete allergens which by binding to IgE monovalently could block the cross-linking of IgE (Valenta *et al.*, 1993a). IgE haptens could also be used to modulate the immune response or to induce tolerance by immunotherapy with a minimum of anaphylactic side effects. Haptens can be obtained from complete allergens by proteolytic digestion. However, this often results in a mixture of fragments and enzymes that is

difficult to characterize. Synthesis of peptides based on the amino acid sequence of the allergens is an alternative approach. Recently, a number of cDNAs coding for important allergens (reviewed in Scheiner *et al.*, 1992) were isolated which can be used to determine IgE epitopes by molecular biological techniques.

Grass pollen allergy is spread world wide, and according to the prevalence of grass pollen allergy it can be expected that 75% of all allergic patients suffer from grass pollen allergy (Freidhoff *et al.*, 1986). Among grass pollen allergic patients more than 90% display IgE reactivity with group I allergens (Freidhoff *et al.*, 1986; Valenta *et al.*, 1992). For this reason the cDNA of the major timothy grass pollen allergen *Phl p I* (Valenta *et al.*, 1992; Laffer *et al.*, 1993) was selected to prepare an epitope expression cDNA library, starting from the randomly fragmented cDNA. IgE binding epitope clones were isolated by IgE immunoscreening, and using sera from different grass pollen allergic individuals immunodominant clones were selected. One of the epitope clones (c98) reacted with IgE from a high percentage (30%) of grass pollen allergic individuals tested. The cDNA of this clone encoded a hapten of 15 amino acids in length which did not induce histamine release in basophil degranulation assays. This hapten may represent a specific tool for safe immunotherapy without anaphylactic side effects.

EXPERIMENTAL PROCEDURES

Construction of an Epitope cDNA Library from the Randomly Fragmented *Phl p I* cDNA—The cDNA fragment coding for *Phl p I* (Valenta *et al.*, 1992; Laffer *et al.*, 1993) was excised from plasmid pUC 18 and purified by preparative agarose gel electrophoresis (Sambrook *et al.*, 1989; Ausubel, 1990). The cDNA was then randomly digested with *Nase I* (Sambrook *et al.*, 1989; Ausubel, 1990) and fragments shorter than 400 bp¹ were isolated by preparative agarose gel electrophoresis. The cDNA fragments were then end repaired with T4 polymerase (Boehringer, Mannheim, Germany), linked with 5'-phosphorylated 8-mer *EcoRI* linkers (Schmidheini, Windisch, Switzerland). After *EcoRI* digestion, linkers were removed using a nick column (Pharmacia, Uppsala, Sweden), and the inserts were ligated into dephosphorylated λgt11 arms (Pharmacia, Uppsala, Sweden). The phage DNA was then *in vitro* packaged using *in vitro* packaging extracts (Amersham, Buckinghamshire, United Kingdom).

IgE Immunoscreening and Analysis of Epitope Clones—A 100,000 phage of the *Phl p I* epitope library were used to infect *Escherichia coli* Y1090 at a density of 5,000 phage/plate (140 mm diameter). The synthesis of recombinant proteins was induced by overlaying the plates with nitrocellulose filters (Schleicher & Schuell, Dassel, Germany) soaked in 10 mM isopropyl-1-thio-β-D-galactopyranoside. 114 *Phl p I* epitope clones were isolated using serum IgE from a *Phl p I* allergic patient and ¹²⁵I-labeled rabbit anti-human IgE (Pharmacia, Uppsala,

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¹ The abbreviations used are: bp, base pair(s); ELISA, enzyme-linked immunosorbent assay; aa, amino acid(s).

Sweden) as described (Breiteneder *et al.*, 1989; Valenta *et al.*, 1991; Vrtala *et al.*, 1993a). The epitope clones were then characterized by hybridization with three synthetic oligonucleotides spanning the *Phl p I* cDNA (oligo(A): 5'-GGG GGC TTG TCC ACA TCC TTG TAC CCG C-3' bp 191–218; oligo(B): 5'-GGA GAG GTC GAA GTG GTA GGG G-3' bp 372–393; oligo(C): 5'-CCG CCA CCA CGT CTC CGT CGC CG-3' bp 573–595). 19 clones that had hybridized with only one of the oligonucleotides were tested for IgE binding with sera from 12 *Phl p I* allergic patients. Clones 80, 97, and 98 bound IgE from most patients and were further tested for IgE reactivity with sera from 134 grass pollen allergic patients as described (Valenta *et al.*, 1992).

For DNA sequence analysis, phage DNA was prepared, the epitope encoding cDNAs were excised with *KpnI/SacI*, subcloned into plasmid pUC 18, and both DNA strands were sequenced using *λgt11* forward and reversed sequencing primers (Clontech, Palo Alto, CA) with [³⁵S]dCTP.

Expression and Purification of Recombinant *Phl p I* and Recombinant *Phl p I* Epitopes—Recombinant *Phl p I* was expressed in *E. coli* BL21 (DE3) (Novagen, Madison, WI) using plasmid pMW 175, a derivative of pMW172 (Studier *et al.*, 1990), and purified to more than 95% purity.² The epitopes encoded by clones 80, 97, and 98 were selected for purification. Recombinant phage were used to infect lysogenic *E. coli* Y1089 (Huynh *et al.*, 1985). The *Phl p I* epitopes were expressed as β -galactosidase fusion proteins in liquid culture (Huynh *et al.*, 1985) and were affinity purified using an anti- β -galactosidase affinity column (Promega) as described (Vrtala *et al.*, 1993a). β -Galactosidase was obtained upon infection of *E. coli* Y1089 with empty *λgt11* phage and purified in the same way.

IgE Binding of Natural Timothy Grass Pollen Allergens and Recombinant *Phl p I* Epitopes—Natural timothy grass pollen allergens were extracted from timothy grass pollen (Allergon, Valinge, Sweden) (Vrtala *et al.*, 1993b) separated by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970) and transferred to nitrocellulose (Towbin *et al.*, 1979). The recombinant *Phl p I* epitopes and β -galactosidase were purified and also nitrocellulose-blotted. IgE from grass pollen allergic patients was used to detect the nitrocellulose-blotted proteins as described (Jarolim *et al.*, 1989) whereas IgE binding to non-denatured recombinant epitopes was done using plaque lifts of phage clones as described elsewhere (Spitzauer *et al.*, 1993). An ELISA assay using recombinant *Phl p I* and the purified c98 fusion polypeptide, respectively, was used to estimate the proportion of *Phl p I*-specific IgE directed against the c98 epitope. In brief, 100 μ l of 1 μ g/ml antigen dissolved in phosphate-buffered saline 0.05%, Tween, 0.5% bovine serum albumin were coated per well at 4 $^{\circ}$ C overnight. Patients sera were diluted 1:10, and bound IgE was determined as described (Ferreira *et al.*, 1993). IgE binding to synthetic peptides which had been synthesized by Cambridge Research Biochemicals, U. K. was measured by dot-blot assays. 100 ng-2 μ g peptide/spot were dotted to nitrocellulose. Reactive peptides derived from other allergens and reactive sera were included as positive controls. Dot-blots were incubated with 1:10 diluted patient sera, and bound IgE was detected using ¹²⁵I-labeled anti-human IgE (Pharmacia, Uppsala, Sweden) as described (Jarolim *et al.*, 1989).

In Vitro Histamine Release from Patients' Basophils—Grass pollen allergic patients with IgE reactivity to group I grass pollen allergens were selected according to case history, serological testing, using RAST and immunoblotting with natural and recombinant grass pollen allergens, and skin prick tests as described (Valenta *et al.*, 1992). After informed consent was obtained, heparinized blood samples were taken and granulocytes were prepared by dextran sedimentation (Valent *et al.*, 1989). Granulocytes were then incubated with various doses of natural timothy grass pollen allergens, purified recombinant *Phl p I*, anti-IgE monoclonal antibody E-124-2-8 (positive control), the recombinant β -galactosidase-fused *Phl p I* epitopes, and β -galactosidase (negative control), respectively. Liberated histamine expressed as percentage of total histamine was measured in the cell-free supernatants by radioimmunoassay (Immunotech, Marseille, France) (Valent *et al.*, 1989). To ensure that IgE antibodies specific for *Phl p I* epitopes were present in the patients serum when the histamine release was performed, the supernatants obtained from the granulocyte preparation were probed in parallel with nitrocellulose-blotted timothy grass pollen allergens and the *Phl p I* epitopes as described (Valenta *et al.*, 1993b).

RESULTS

Isolation and Characterization of Immunodominant IgE Epitope Clones from *Phl p I*—100,000 phage from the *Phl p I*

epitope library were screened using serum IgE from a grass pollen allergic individual with IgE reactivity to group I grass pollen allergens. 114 IgE-binding phage clones were obtained and subsequently tested for hybridization with three synthetic oligonucleotides spanning the *Phl p I* cDNA. 19 clones which hybridized with only one oligonucleotide were further tested with serum IgE from 12 different *Phl p I* allergic patients. All tested patients displayed IgE reactivity with clone 98 which therefore contained an immunodominant IgE epitope. Clone 80 reacted with 10 out of 12 *Phl p I* reactive patients and clone 97 with 11 out of 12 patients. The cDNAs of the three immunodominant epitope clones were sequenced and found to code for almost the same portion of the *Phl p I* molecule. Fig. 2 shows the alignment of the deduced amino acid sequences of the epitope clones with the amino acid sequences of the major grass pollen allergens from rye grass (*Lolium perenne*), *Lol p I* (Perez *et al.*, 1990; Griffith *et al.*, 1991), from timothy grass (*Phleum pratense*), *Phl p I* (Laffer *et al.*, 1993), and from rye (*Secale cereale*), *Sec c I*.³ Clone 98 which had reacted with IgE from all 12 patients (Fig. 1) expressed a 15-amino-acid immunodominant IgE epitope of *Phl p I* which is highly conserved among the other group I allergens and covers amino acid 101–115 of the mature *Phl p I* protein. The COOH-terminal portion of the peptide could be critical for IgE binding in some patients because clone 80 and 97 were less frequently recognized (Fig. 1). Peptides of 12 amino acids length were synthesized according to the deduced amino acid sequence of *Phl p I* with 3 amino acids overlaps, spanning the complete allergen. No reactivity of the patients' IgE with these peptides could be detected indicating that the critical length for IgE binding of the clone 98 epitope might be in the range between 13 and 15 amino acids (data not shown) or that synthetic peptides cannot form a conformation suitable for IgE binding. Two synthetic peptides (D: aa 76–87, E: aa 117–129) which flanked the clone 98 IgE epitope also did not bind IgE (data not shown).

Frequency of IgE Binding to Recombinant *Phl p I* Epitope Clones—To estimate the percentage of grass pollen allergic patients with IgE reactivity to the epitope clones, 134 grass pollen allergic patients were tested in parallel for IgE binding to nitrocellulose-blotted timothy grass pollen extract, recombinant *Phl p I*, and epitope clones 80, 97, and 98, respectively. 94% of grass pollen allergic patients showed IgE reactivity with recombinant *Phl p I* and 30% bound to clone 98 (data not shown).

Comparison of Different Methods for the Determination of B-cell Epitopes—Different methods were used to determine IgE epitopes of *Phl p I*. Overlapping peptides (Geysen *et al.*, 1987) of 12 amino acids length which were synthesized according to the deduced amino acid sequence of *Phl p I* were tested by dot-blotting for reactivity with serum IgE from grass pollen allergic patients. In addition, 10 peptides covering regions of a predicted high antigenicity (Jameson and Wolf, 1988) of the mature *Phl p I* allergen (peptide A, IPKVPPGPNTATYGDK aa 1–17; peptide B, DAKSTWYGKPTGAGPKDNGG aa 20–39; peptide C, KDVDKPPFSGMTGCGNTPIFKSGRGC aa 44–69; peptide D, KCTKPEACSGEP aa 76–87; peptide E, KKGDE-QKLRSAGE aa 117–129; peptide F, RVCKYKEGTVK aa 136–147; peptide G, VEKGSNPY aa 151–159; peptide H, DIK-EKGKDKWIELKESW aa 177–193; peptide I, IDTPDKLTGPF aa 199–209; peptide J, TTEGGTKTEAEDVIPEGWKADTSY aa 214–237) were also tested for IgE reactivity with 35 sera from grass pollen allergic patients by dot-blotting. None of the peptides tested reacted with IgE from more than 5% of the grass pollen allergic patients (data not shown).

Since the epitope mapping based on the use of synthetic peptides was unsuccessful, a recombinant epitope mapping

² M. Susani, unpublished data.

³ S. Laffer, unpublished data.

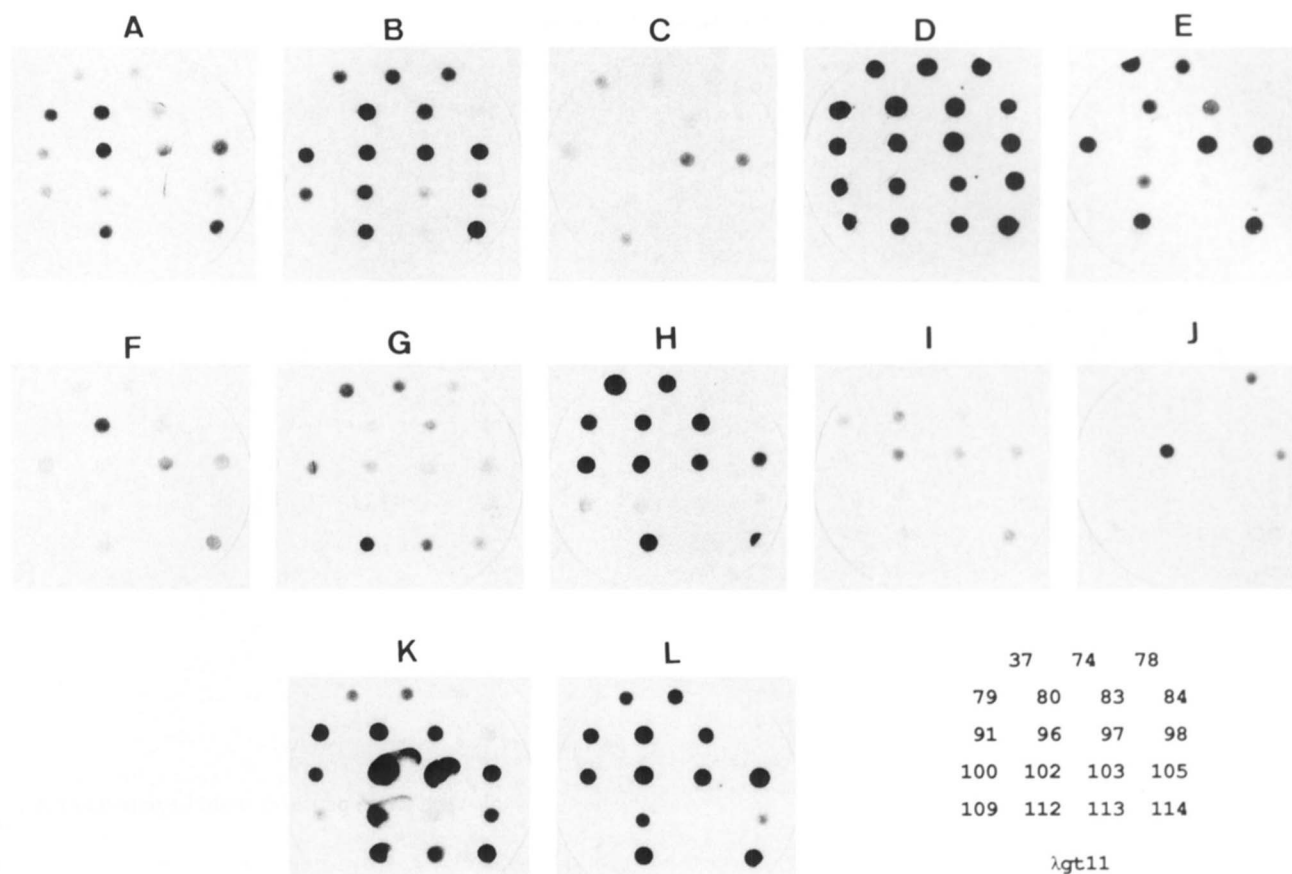


FIG. 1. **IgE binding capacity of recombinant *Phl p I* epitope clones.** 19 phage clones expressing selected *Phl p I* epitopes and λ gt11 phage (negative control) without insert were induced to synthesize recombinant proteins which were then transferred to nitrocellulose filters. Filter circles containing the recombinant proteins (the position of the phage clones on each filter is shown in the right lower corner) were then probed with serum IgE from 12 different grass pollen allergic patients (A-L).

strategy was used (Mehra *et al.*, 1986). An epitope expression cDNA library was constructed using the randomly fragmented *Phl p I* cDNA. 114 IgE epitope clones could be isolated out of 100,000 phage with patients serum IgE. 19 epitope clones containing cDNAs that hybridized only with one oligonucleotide and thus representing small *Phl p I* fragments were selected. These clones were further tested with sera from 12 different grass pollen allergic patients to determine immunodominant IgE epitopes. One prominent IgE epitope encoded by clones 80, 97, and 98 was isolated (Fig. 2). This sequence was not predicted to be antigenic by computer analysis (Jameson *et al.*, 1988) nor was it identified by testing overlapping synthetic peptides spanning the complete *Phl p I* molecule for IgE binding (Geysen *et al.*, 1987).

IgE Binding Capacity of Recombinant *Phl p I* Epitopes—Recombinant *Phl p I* epitopes were expressed as β -galactosidase fusion proteins and tested for IgE binding in different assays as native and denatured proteins. Plaque lifts containing native recombinant IgE epitope clones were tested for IgE binding as described (Valenta *et al.*, 1992; Spitzauer *et al.*, 1994). Fig. 1 shows the IgE reactivity of 12 grass pollen allergic individuals to *Phl p I* epitope clones. Although clone 98 represented the immunodominant epitope other epitope clones seemed to possess a higher IgE binding capacity (Fig. 1, patients A, F, H, I, and J). The clone 98 β -galactosidase fusion protein was purified by affinity to anti- β -galactosidase antibodies to be tested by ELISA and Western blot. Comparing the IgE binding to recombinant *Phl p I* and the clone 98 epitope in ELISA assays, 5–10% of IgE directed against recombinant *Phl p I* bound to the clone 98 epitope as determined in 19 patients (data not shown).

Fig. 3A shows that the denatured immunoblotted (Laemmli, 1970; Towbin *et al.*, 1979) clone 98 β -galactosidase fusion epitope bound IgE from group I allergic patients whereas no IgE reactivity with β -galactosidase (negative control) was observed (Fig. 3C).

Identification of the Immunodominant Recombinant *Phl p I* IgE Epitope (Clone 98) as an IgE Hapten by in Vitro Histamine Release Assays—The recombinant *Phl p I* IgE epitope clones were tested for their capacity to release histamine from basophils of grass pollen allergic patients. To ensure that specific IgE was present in the sera of patients when the histamine release experiments were done, the supernatants obtained during the preparation of granulocytes were tested for IgE reactivity with the nitrocellulose blotted clone 98 β -galactosidase fusion protein (Fig. 3A), with natural timothy grass pollen extract (Fig. 3B) and with β -galactosidase (Fig. 3C), respectively. Three representative patients who displayed distinct IgE reactivity to the nitrocellulose blotted clone 98 epitope (Fig. 3, lanes B–D) showed no histamine release when their granulocytes were incubated with the purified clone 98 epitope (Fig. 4, B–D). Dose-dependent and specific histamine release was measured when granulocytes from these patients were incubated with natural grass pollen allergens and anti-IgE monoclonal antibodies (positive control). No release was obtained upon incubation with β -galactosidase (negative control). As a negative control, a grass pollen allergic patient without IgE reactivity to *Phl p I* and the clone 98 epitope but with high levels of IgE against group V allergens was included in the histamine release assays (Fig. 4, patient G). Again no histamine release with the clone 98 epitope was observed whereas dose-dependent histamine re-

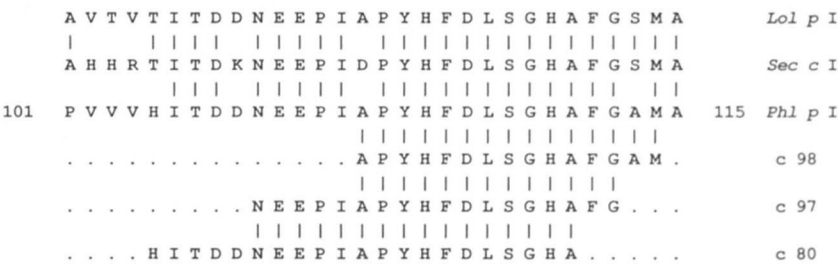


FIG. 2. Alignment of the deduced amino acid sequences of three immunodominant *Phl p I* IgE epitope clones with the amino acid sequences of group I grass pollen allergens from different grass species. The deduced amino acid sequence of epitope clones 80, 97, and 98 was aligned with the deduced amino acid sequences from the major rye grass (*L. perenne*) allergen, *Lol p I*, with the major timothy grass (*P. pratense*) allergen, *Phl p I*, and the major allergen from rye (*S. cereale*), *Sec c I*. Vertical bars indicate identical amino acids.

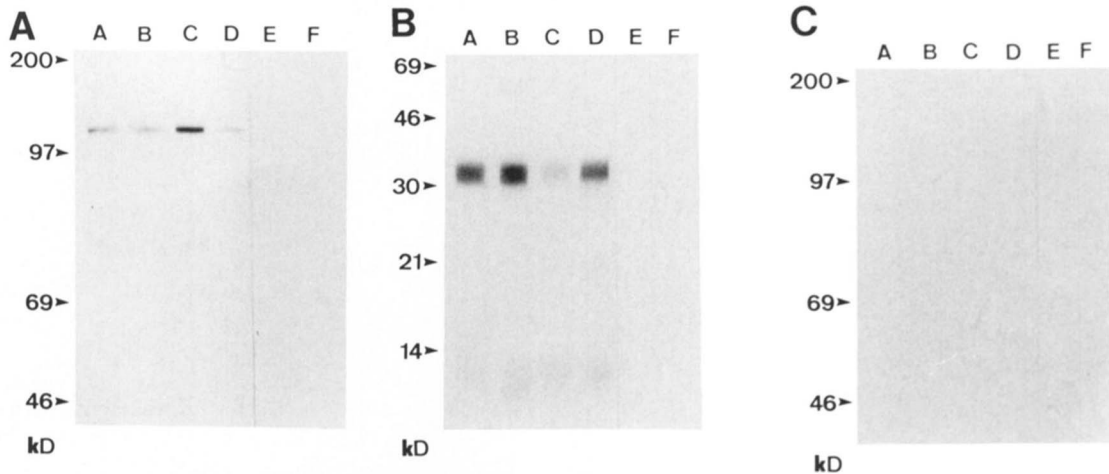


FIG. 3. Presence of specific IgE antibodies in sera from grass pollen allergic individuals which were tested in histamine release experiments. Supernatants obtained from four grass pollen allergic individuals (lanes A–D) and from a non-allergic control individual (lane E) during the preparation of granulocytes for histamine release were probed for IgE reactivity with the nitrocellulose blotted clone 98 fusion protein (panel A), with nitrocellulose-blotted natural timothy grass pollen extract (panel B), and with β -galactosidase (panel C), respectively. Lane F in all three figures represents the buffer control without addition of a supernatant.

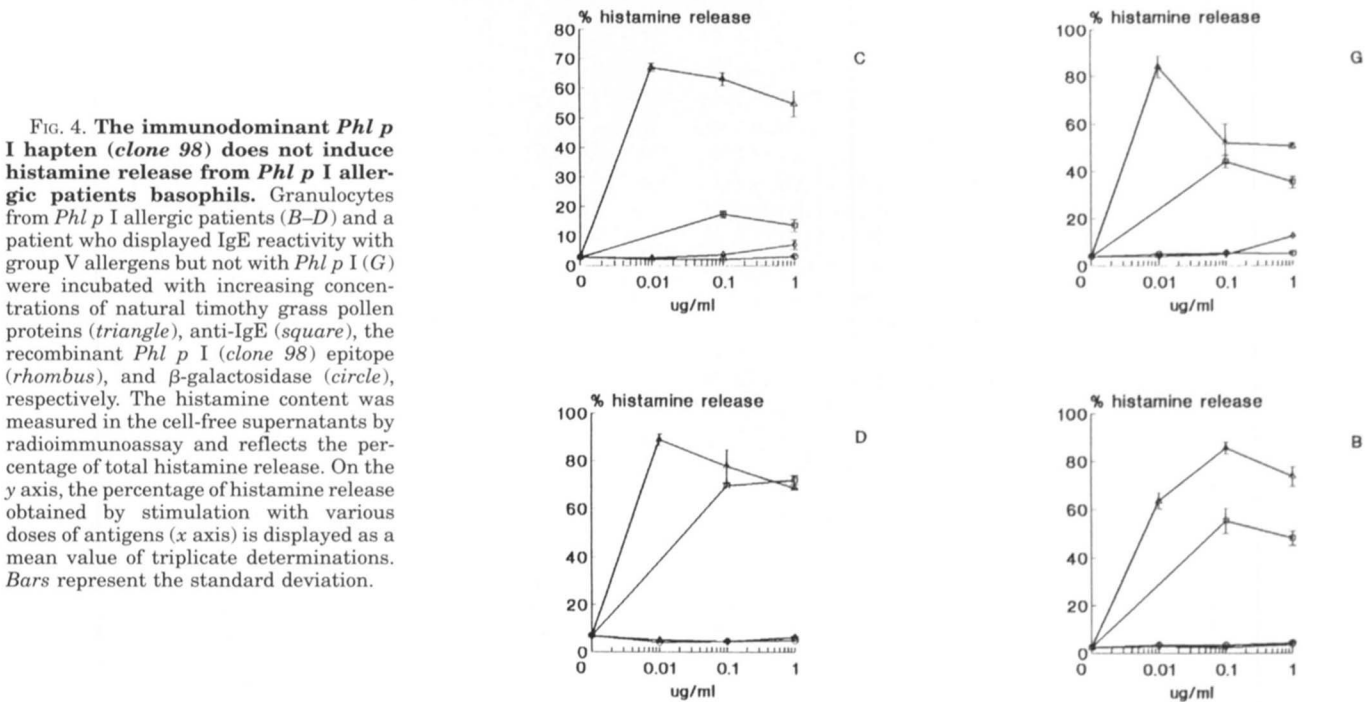


FIG. 4. The immunodominant *Phl p I* hapten (clone 98) does not induce histamine release from *Phl p I* allergic patients basophils. Granulocytes from *Phl p I* allergic patients (B–D) and a patient who displayed IgE reactivity with group V allergens but not with *Phl p I* (G) were incubated with increasing concentrations of natural timothy grass pollen proteins (triangle), anti-IgE (square), the recombinant *Phl p I* (clone 98) epitope (rhombus), and β -galactosidase (circle), respectively. The histamine content was measured in the cell-free supernatants by radioimmunoassay and reflects the percentage of total histamine release. On the y axis, the percentage of histamine release obtained by stimulation with various doses of antigens (x axis) is displayed as a mean value of triplicate determinations. Bars represent the standard deviation.

lease could be obtained with total grass pollen extracts containing group V allergens. Fig. 5 shows a comparison of the histamine release induced in two patients with specific IgE antibodies against *Phl p I* and three epitope clones (clone 80, 97, and 98). In both patients dose-dependent histamine release could be induced with

recombinant *Phl p I* whereas all three epitope clones did not lead to release of histamine up to a concentration of 1 μ g/ml indicating that clone 80 and clone 97 epitopes behave as haptens. In total the clone 98 epitope was tested in nine patients for its capacity to induce histamine, the clone 80 epitope was tested in eight patients and the clone 97 epitope was investi-

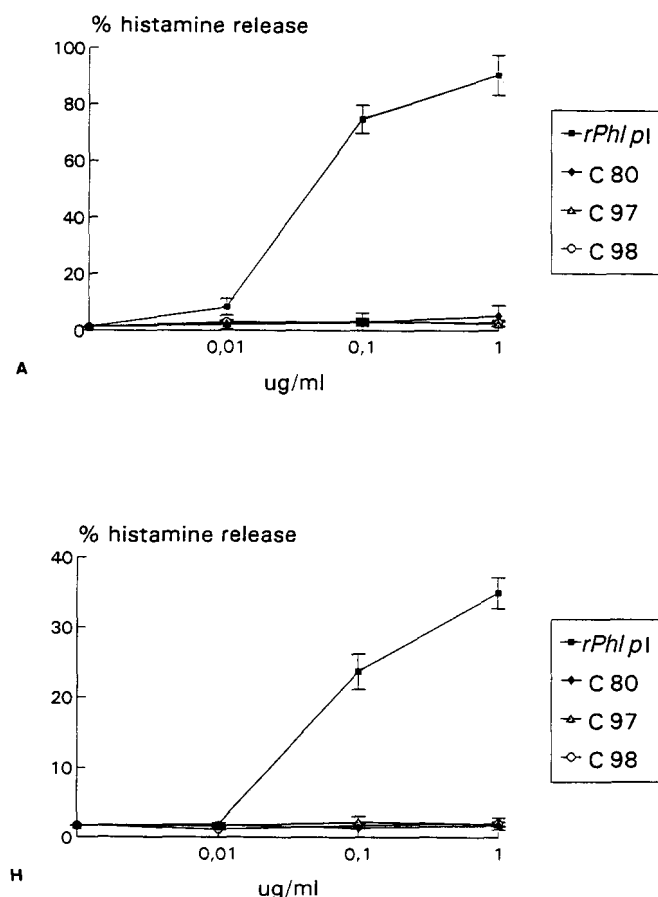


FIG. 5. Comparison of recombinant *Phl p I* and epitope clones (c80, c97, and c98) in histamine releases. As in Fig. 4, granulocytes from two grass pollen allergic patients (A and H) were incubated with various doses of recombinant *Phl p I* and epitope clones (c80, c97, and c98) respectively. The percentage of histamine release is displayed on the y axis. All measurements reflect means of triplicate determinations.

gated in two patients. Only in one patient did significant histamine release become induced with the clone 98 epitope whereas in none of the other experiments could histamine release be induced with any of the three epitope clones.

DISCUSSION

The present study demonstrates the efficient determination of IgE epitopes using recombinant DNA techniques. The cDNA coding for the major grass pollen allergen, *Phl p I* (Valenta *et al.*, 1992; Laffer *et al.*, 1993), which is a target for IgE antibodies of 90% of the grass pollen allergic patients, and thus is a target for IgE antibodies of up to 75% of all allergic patients (Freidhoff *et al.*, 1986; Valenta *et al.*, 1992), was selected as the starting material to construct an epitope expression cDNA library. Using grass pollen allergic patients IgE immunodominant IgE epitope clones were isolated among which clone 98 expressed a *Phl p I* peptide of 15 amino acids fused to β -galactosidase.

The described 15 amino acid epitope was not predicted by a computer algorithm (Jameson *et al.*, 1988) nor was it detected by overlapping peptide synthesis technology (Geyssen *et al.*, 1987).

Knowledge of IgE epitopes is of particular importance because the release of biological mediators such as histamine during the allergic effector reaction requires a divalent cross-link of mast cell and basophil-bound IgE by allergens (Segal *et al.*, 1977). IgE haptens derived from allergens contain only one IgE epitope and thus cannot trigger allergic effector mechanisms unless they are polymerized.

The immunodominant IgE epitope which we derived from the major grass pollen allergen *Phl p I* was therefore purified and tested for its capacity to induce histamine release from grass pollen allergic patients basophils. Although in all experiments histamine release could be induced with natural timothy grass pollen allergens or recombinant *Phl p I* in a dose-dependent way, no histamine release could be elicited with the recombinant *Phl p I* epitope encoded by clone 98 in eight out of nine patients suggesting that the clone 98 epitope represents an immunodominant IgE hapten.

IgE haptens may be useful for two therapeutical approaches of allergic diseases. Haptens could be used to block mast cell- and basophil-bound IgE thereby directly inhibiting mediator release. Synthetic epitopes could be synthesized in large amounts and used directly in the effector organs (lung, nasal mucosa, and conjunctiva). Such an approach would, however, require the characterization of many different haptens according to the patients' IgE-binding pattern. Using recombinant DNA techniques for the characterization of allergens and the determination of IgE epitopes, such an approach might be feasible. In addition, it is expected that due to extensive immunological cross-reactivities among most allergens it might be possible to define a limited number of IgE epitopes (Valenta *et al.*, 1993a). As was demonstrated for *Phl p I*, IgE epitopes can be determined by immunoscreening of expression libraries derived from the allergens cDNAs. A representative number of patients can then be tested for IgE reactivity with recombinant epitope clones to obtain immunodominant structures. In a second step most of the additional relevant IgE epitopes of each allergen have to be characterized. This may be possible because B-cell epitopes, unlike T-cell epitopes, assemble a larger conformation that also has to be available on the surface of the allergen (Berzofsky, 1985; Chothia, 1991; Laver *et al.*, 1990). Thus it can be assumed that the diversity of B-cell epitopes may be much more restricted than that of T-cell epitopes.

In addition to the blocking of the allergic effector reaction, IgE haptens could be used to modulate IgE responses by applying vaccination strategies which aim at the induction of immunological tolerance. Hyposensitization treatment of Type I allergies which is established world-wide requires the increasing application of allergens by injection or oral administration. Although hyposensitization has been used successfully since 1911 (Noon, 1911), many patients undergoing this therapy suffer from severe side effects such as anaphylactic shock. The use of IgE haptens derived from the allergens could contribute considerably to the improvement of this therapy by reduction of such side effects. Various other methods to modulate the IgE response in allergic patients which are currently developed could also take advantage of the use of IgE haptens.

In conclusion our study demonstrates that by use of recombinant techniques an immunodominant IgE hapten from the major timothy grass pollen allergen *Phl p I* could be obtained. This allergen was selected as a model allergen because a high proportion (>90%) of all grass pollen allergic patients, and thus almost 75% of all allergic patients show IgE reactivity with this molecule. Although the obtained 15-amino-acid epitope bound IgE from approximately 30% of grass pollen allergic patients, it did not release histamine from most patients' basophils and may therefore be considered to be a hapten. We are aware that in addition to the described *Phl p I* epitope certainly more epitopes from the same molecule and other allergens must be defined to block histamine release by saturating mast cell- and basophil-bound IgE. However, the results encourage continuation with the characterization of IgE haptens for their future use in a specific therapy of allergic diseases.

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