

Immunization with Purified Natural and Recombinant Allergens Induces Mouse IgG1 Antibodies That Recognize Similar Epitopes as Human IgE and Inhibit the Human IgE-Allergen Interaction and Allergen-Induced Basophil Degranulation¹

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Molecular characterization of allergens by recombinant DNA technology has made rapid progress in the recent few years. In the present study we immunized mice with aluminum hydroxide-adsorbed purified recombinant major timothy grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5), dog albumin, a major animal dander allergen, and proteins with low (β -lactoglobulin) or no (ribulose diphosphate carboxylase) allergenic potential in humans. Allergens that bind high levels of IgE in humans (Phl p 1, Phl p 5, dog albumin) induced high IgE and IgG1 levels in mice, whereas proteins with little or no allergenic activity in humans failed to induce significant IgE and IgG1 levels in mice. Continuous immunization for a period of 27 wk resulted in the production of mouse IgG1 Abs that recognized recombinant allergen fragments/epitopes defined by IgE Abs of allergic patients. As a consequence, allergen-specific mouse Abs strongly inhibited human IgE binding to the allergens and suppressed the allergen-induced histamine release from human basophils. In summary, our data indicate that 1) the allergenic potency of a protein may be related to its overall immunogenicity and 2) prolonged immunization with single purified recombinant allergens induces protective IgG Abs. The presented experimental *in vivo/in vitro* system allows the evaluation of Ag preparations (e.g., recombinant allergens) to be used for immunotherapy in humans. *The Journal of Immunology*, 1998, 160: 6137–6144.

Almost 20% of the population suffers from Type I allergy (1). The symptoms of Type I allergy (allergic rhinitis, conjunctivitis, asthma, and anaphylaxis) result from the cross-link of effector cell-bound IgE Abs by per se innocuous Ags (allergens). While the role of IgE Abs in the pathogenesis of Type I allergy is well established, much less is known concerning the effects of allergen-specific IgG Abs. In 1935, Cooke and colleagues had already described successful therapy of an allergic patient by transfer of blood from a hyposensitized patient (2), and it could be later shown that the transferable protective factor represented a thermostable “blocking Ab” that suppressed skin responses to injected allergens (3–5). In humans, blocking Abs were found to belong essentially to the IgG class (6–9), and it could be demonstrated that successful immunotherapy is paralleled by an increase of IgG, in particular IgG4 Abs (10). A later study monitoring pa-

tients who had undergone hyposensitization was, however, unable to correlate the clinical improvement with changes in the allergen-specific IgG and IgG-subclass levels (11). The concept that blocking Abs may have beneficial effects has recently gained back support by the description of a human mAb, BAB 1, established from a hyposensitized patient, which potently suppressed IgE binding to the major birch pollen allergen, Bet v 1, and Bet v 1-induced histamine release from the patient's basophils (12). Moreover, a series of mouse monoclonal anti-Bet v 1 Abs that blocked binding of human IgE to Bet v 1 were identified (13).

Here we revisit the concept of protective/blocking Abs using a mouse model established with defined purified natural and recombinant allergens. Murine IgE and IgG Ab responses, induced against aluminum hydroxide-adsorbed purified recombinant timothy grass pollen allergens, Phl p 1 (14), Phl p 2 (15), Phl p 5 (16), purified dog albumin (17, 18) and, for comparison, proteins with low or no allergenic activity in humans, were studied. The kinetics and levels of specific IgE and IgG1 Abs were investigated and the epitope recognition of human IgE Abs and mouse IgG1 Abs was compared using recombinant allergen fragments expressed in *Escherichia coli*. The capacity of mouse Igs to inhibit IgE binding to natural allergen extracts as well as to suppress the allergen-induced histamine release from patients' basophils was investigated. The results show that prolonged immunization with purified natural and recombinant allergens induced blocking mouse IgG1 Abs that bound to similar epitopes as human IgE, which inhibited the interaction of human IgE and allergens and blocked the allergen-induced histamine release from human basophils.

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Materials and Methods

Characterization of allergic patients and sera

Allergic patients were characterized by positive case history, skin prick testing, and radioallergosorbent test (RAST)³ analysis of the sera as described (19, 20). In addition, sera were tested for reactivity with natural allergen extracts by IgE immunoblotting and for IgE reactivity to purified recombinant timothy grass pollen allergens and dog albumin as described (20, 18).

Allergen extracts, purified recombinant timothy grass pollen allergens, dog albumin, and control proteins

Pollen from timothy grass (*Phleum pratense*), Kentucky bluegrass (*Poa pratense*), rye grass (*Lolium perenne*), and rye (*Secale cereale*) was purchased from Allergon AB (Välinge, Sweden). Aqueous protein extracts were prepared (21), checked by SDS-PAGE (22) and Coomassie Blue staining (23), and stored lyophilized at -20°C until use. Purified dog albumin, β -lactoglobulin, and ribulose diphosphate carboxylase were purchased from Sigma (St. Louis, MO). Recombinant timothy grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5) were expressed as nonfusion proteins in *E. coli* BL21 (DE3) and purified as described (24). Before immunization, recombinant allergens were tested for their capacity to bind human IgE and to induce specific histamine release from allergic patients' basophils.

Plasmids, phage, and *E. coli* strains

The cDNAs coding for the major timothy grass pollen allergens Phl p 1, Phl p 2, and Phl p 5 were inserted into plasmid pMW 172 (25), a derivative of pRK 172 (26). Plasmids were transformed into *E. coli* BL21 (DE3), derived from *E. coli* strain B (27) (Novagen, Madison, WI). *EcoRI*-cut, dephosphorylated λ gt11 DNA was purchased from Pharmacia (Uppsala, Sweden). *E. coli* strain Y1090 (*hsd* ($r_k^- m_k^-$) *lac* U169, *ProA*⁺, *Ion*⁻, *araD* 139, *StrA*, *Sup F* *trpC22:Tn10*(pMC9)) was obtained from Amersham (Amersham, Buckinghamshire, U.K.). *E. coli* XL-1 Blue *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* (F' *proAB lacIqZAM15 Tn10* (Tetr))^c was purchased from Stratagene (La Jolla, CA), and plasmid pUC18 from Boehringer (Mannheim, Germany).

Immunization of mice and measurement of specific IgE and IgG Ab levels

Eight-week-old BALB/c mice were obtained from Charles River (Kislegg, Germany). Animals were maintained in the animal care unit of the Institute of General and Experimental Pathology of the University of Vienna according to the local guidelines for animal care. Groups of five mice each were immunized for 27 wk with 5 μg of each purified protein (recombinant timothy grass pollen allergens Phl p 1, Phl p 2, and Phl p 5; dog albumin, ribulose-diphosphate carboxylase; and β -lactoglobulin) adsorbed to 200 μl of AluGel-S (Serva, Heidelberg, Germany) s.c. in the neck as described (28, 29). Mice were immunized and bled at approximately 3-wk intervals. Sera were stored at -20°C until analysis. IgE and IgG1 responses were measured by ELISA as described (28, 29).

SDS-PAGE and immunoblotting

IgE and IgG1 detection of nitrocellulose-blotted allergens and allergen extracts was performed by immunoblotting. Proteins were separated by SDS-PAGE and transferred to nitrocellulose (Schleicher & Schuell, Dassel, Germany) by electroblotting (30). Nitrocelluloses were blocked in buffer A (50 mM sodium phosphate, pH 7.5, 0.5% w/v BSA, 0.5% v/v Tween-20, 0.05% NaN_3) two times for 5 min and once for 30 min and incubated with 1:20 (IgE) or 1:1000 (IgG1) in buffer A-diluted mouse sera at 4°C overnight. Nitrocelluloses were then washed three times in buffer A and exposed to 1:1000 in buffer A-diluted monoclonal rat anti-mouse IgE or anti-IgG1 Abs (PharMingen, San Diego, CA) at 4°C overnight. After washing as above, bound secondary Abs were detected with an ^{125}I -labeled sheep anti-rat Ig antiserum (Amersham) and visualized by autoradiography.

IgE epitope mapping of recombinant Phl p 1, Lol p 5, and dog albumin by gene fragmentation

Recombinant IgE epitopes of Phl p 1. A random fragment expression cDNA library was constructed using the cDNA coding for the major timothy grass pollen allergen, Phl p 1, as a source (31). Phage clones coding

for IgE epitopes were isolated by immunoscreening with serum IgE from grass pollen-allergic individuals. IgE-binding phage clones were characterized by sequence analysis of the inserted cDNA fragments (Ref. 31; and T. Ball and R. Valenta, unpublished observations).

Recombinant IgE epitopes of Lol p 5. Phage clones containing IgE binding portions of the major rye grass pollen isoallergens, Lol p 5a and Lol p 5b, which share IgE epitopes and sequence homology with the major timothy grass pollen allergen, Phl p 5, were isolated by IgE immunoscreening of a random fragment expression cDNA library prepared from the Lol p 5a and 5b cDNAs as described (32). The IgE-binding phage clones were purified to homogeneity by rescreening with serum IgE from a grass pollen-allergic individual. cDNAs coding for Lol p 5 IgE epitopes were amplified by PCR using λ gt11 forward (5'-CGG GAT CCC GGT TTC CAT ATG GGG ATT GGT GGC 3') and reversed (5' CGC GGA TCC CGT TGA CAC CAG ACC AAC TGG TAA TG-3') primers and phage DNA as a template. Both primers contained *Bam*HI restriction sites (underlined) that allowed subcloning of the PCR products into plasmid pUC18 (33). Plasmids were transformed into *E. coli* XL-1 Blue using the calcium chloride method, and plasmid DNA was isolated using Qiagen tips (Qiagen, Hilden, Germany). The sequence of the subcloned fragments was determined by DNA sequence analysis according to the method of Sanger et al. (34) using the λ gt11 primers described above, [^{35}S]dCTP, and a T7 polymerase sequencing kit (Pharmacia).

Recombinant IgE epitopes of dog albumin. IgE binding fragments comprising different regions of dog albumin were obtained by IgE immunoscreening of a dog liver expression cDNA library constructed in phage λ gt11 (Ref. 17; and B. Pandjaitan and R. Valenta, unpublished observation). Representative epitope clones from the N terminus, C terminus, and a middle region of dog albumin as well as a clone expressing complete dog albumin were used in this study.

Comparison of human IgE and mouse IgG1 binding to recombinant allergen fragments

E. coli Y1090 were grown overnight in LB medium containing 0.4% w/v maltose and 50 $\mu\text{g}/\text{ml}$ ampicillin, harvested by centrifugation (3000 rpm, 10 min, 4°C), and resuspended in 1/10 vol of 10 mM MgSO_4 . One hundred microliters of *E. coli* were plated with 4 ml of 0.6% Top agarose onto LB plates containing 50 mg/l ampicillin. Aliquots of phage lysates (1 μl ; $>10^5$ plaque-forming units), expressing β -galactosidase (β -gal)-fused IgE epitopes, β -gal-fused control proteins, or β -gal alone were dotted onto the plates. Plates were incubated at 43°C until lysis of *E. coli* was visible. The synthesis of recombinant β -gal-fused allergen fragments was induced by overlay with nitrocellulose filters soaked in 10 mM isopropyl- β -thiogalactoside and further incubation of the plates at 37°C for 4 h. Nitrocellulose filters containing the recombinant allergen fragments and control proteins were then probed with 1:10 diluted sera from allergic patients or 1:1000 diluted mouse sera. Bound human IgE Abs were detected with ^{125}I -labeled anti-human IgE Abs (RAST; Pharmacia). Bound mouse IgG1 Abs were stained with an alkaline phosphatase-conjugated monoclonal rat anti-mouse IgG1 Ab (PharMingen).

Inhibition of human IgE binding to allergens with mouse immune Ig

Approximately 10 ng/cm of purified rPhl p 1, rPhl p 5, and dog albumin as well as 1 $\mu\text{g}/\text{cm}$ natural grass pollen extracts were separated by preparative 12.5% SDS-PAGE and blotted onto nitrocellulose. Nitrocellulose strips of exactly 0.5-cm width were cut from the same preparative sheet and blocked with buffer A as described for immunoblotting. Strips were then preincubated with 1:10 diluted mouse immune sera or, for control purposes, with 1:10 diluted preimmune sera. After washing, strips were incubated with 1:5 diluted sera from allergic patients at 4°C overnight, washed, and bound IgE Abs were detected with ^{125}I -labeled anti-human IgE Abs (RAST; Pharmacia) and visualized by autoradiography. The percentage reduction of IgE binding after preincubation with mouse immune sera vs preimmune sera was determined by gamma counting of the strips in a gamma counter (Wallac, LKB, Turku, Finland) as follows: % inhibition of IgE binding = $100 - (100 \times \text{cpmI})/\text{cpmP}$. CpmI and cpmP represent cpm after preincubation of strips with the mouse immune serum and preimmune serum, respectively.

Inhibition of allergen-induced histamine release from human basophils after preincubation of allergens with mouse sera

Heparinized blood samples from an allergic patient were obtained by venipuncture and granulocytes were isolated by dextran sedimentation

³ Abbreviations used in this paper: RAST, radioallergosorbent test; β -gal, β -galactosidase.

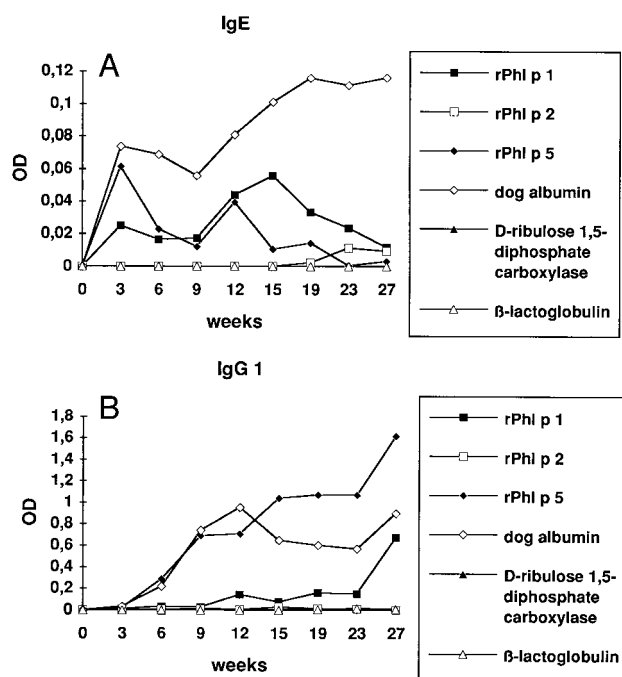


FIGURE 1. Time course and intensity of mouse IgE and IgG1 responses. Groups of five mice each were immunized with rPhl p 1, rPhl p 2, rPhl p 5, dog albumin, D-ribulose 1, 5-diphosphate carboxylase, and β -lactoglobulin, respectively. IgE (A) and IgG1 (B) levels of sera collected at different times (x-axis) were determined by ELISA. The mean levels of the allergen-specific IgE and IgG1 levels in each group are displayed on the y-axis as ODs.

(35). Granulocytes were then incubated with recombinant allergens dissolved in histamine release buffer (20 mM PIPES, pH 7.4, 110 mM NaCl, 5 mM KCl, 1 mM CaCl_2 , 1 g/L glucose, 0.3 mg/ml human serum albumin) at different concentrations (0.1, 0.01, and 0.001 $\mu\text{g/ml}$) that had been preincubated with an equal volume of mouse immune serum, or, for control purposes, with mouse preimmune serum or buffer alone, for 1 h at room temperature. Histamine released into the cell-free supernatant was determined by radioimmunoassay (Immunotech, Marseille, France) and is expressed as percentage of total histamine release

determined after cell lysis. All measurements represent means of triplicate determinations.

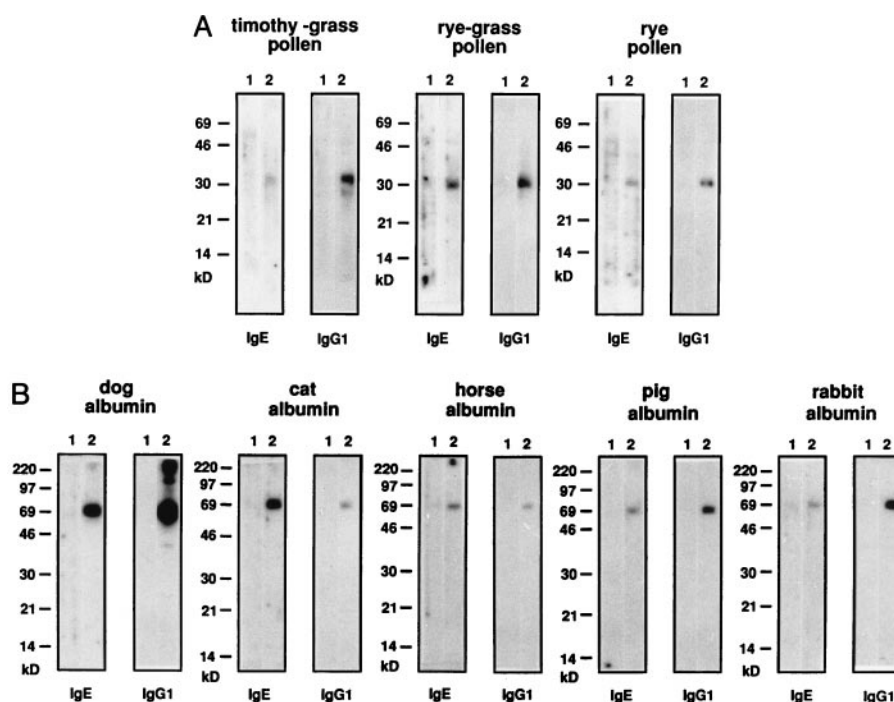
Results

Kinetics, levels, and cross-reactivity of mouse IgE and IgG1

Allergen-specific IgE and IgG1 responses were studied after immunization of mice with purified allergens (recombinant timothy grass pollen allergens rPhl p 1, rPhl p 2, rPhl p 5 and dog albumin) and with proteins that are rare (β -lactoglobulin) or no (D-ribulose 1, 5-diphosphate carboxylase) targets for human IgE Abs. The analysis of IgE and IgG1 reactivities (Fig. 1, A and B) determined in the mouse sera revealed that 1) at the given serum dilutions, allergen-specific IgE reactivity could be detected earlier after immunization (after 3 wk) than IgG1 responses (after 6 wk); 2) in comparison to allergens that bind lower levels of IgE in humans (Phl p 2 (15, 24)), there was a trend supporting the contention that allergens that cause greater IgE responses in humans (Phl p 1 (14, 24), Phl p 5 (16, 24), dog albumin (17, 18)) induce greater IgE and IgG1 responses in mice. Proteins that are rare (β -lactoglobulin) or no (D-ribulose 1, 5-diphosphate carboxylase) targets for human IgE Abs did not elicit detectable IgE or IgG1 responses in mice; and 3) it appeared that during prolonged immunization, allergen-specific IgE levels decreased (e.g., Phl p 5), whereas allergen-specific IgG1 levels showed a tendency to rise.

As exemplified for a Phl p 5- and dog albumin-immunized mouse (Fig. 2, A and B), IgE and IgG1 Abs cross-reacted with homologous allergens from different sources. The deduced amino acid sequences of group 5 allergens from various grass species share an average sequence identity of 75% (16). Accordingly, rPhl p 5-induced mouse IgE and IgG1 Abs cross-reacted with natural group 5 allergens present in timothy grass, rye grass, and rye pollen (Fig. 2A). An average sequence identity of 80% exists among albumins from various animals (17). As a consequence, dog albumin-induced IgE and IgG1 cross-reacted with cat, horse, pig, and rabbit albumin (Fig. 2B).

FIGURE 2. Cross-reactivity of mouse IgE and IgG1 Abs with homologous allergens. The preimmune sera (lane 1) and immune sera (lane 2) of a representative rPhl p 5 and dog albumin-immunized mouse were tested for IgE and IgG1 reactivity with blotted natural allergens by immunoblotting. Serum from a rPhl p 5-immunized mouse showed IgE and IgG1 reactivity to natural group 5 allergens at approximately 30 kDa in timothy grass, rye grass, and rye pollen extracts (A). The dog albumin-immunized mouse serum displays IgE and IgG1 cross-reactivity with cat, horse, pig, and rabbit albumin at approximately 69 kDa (B).



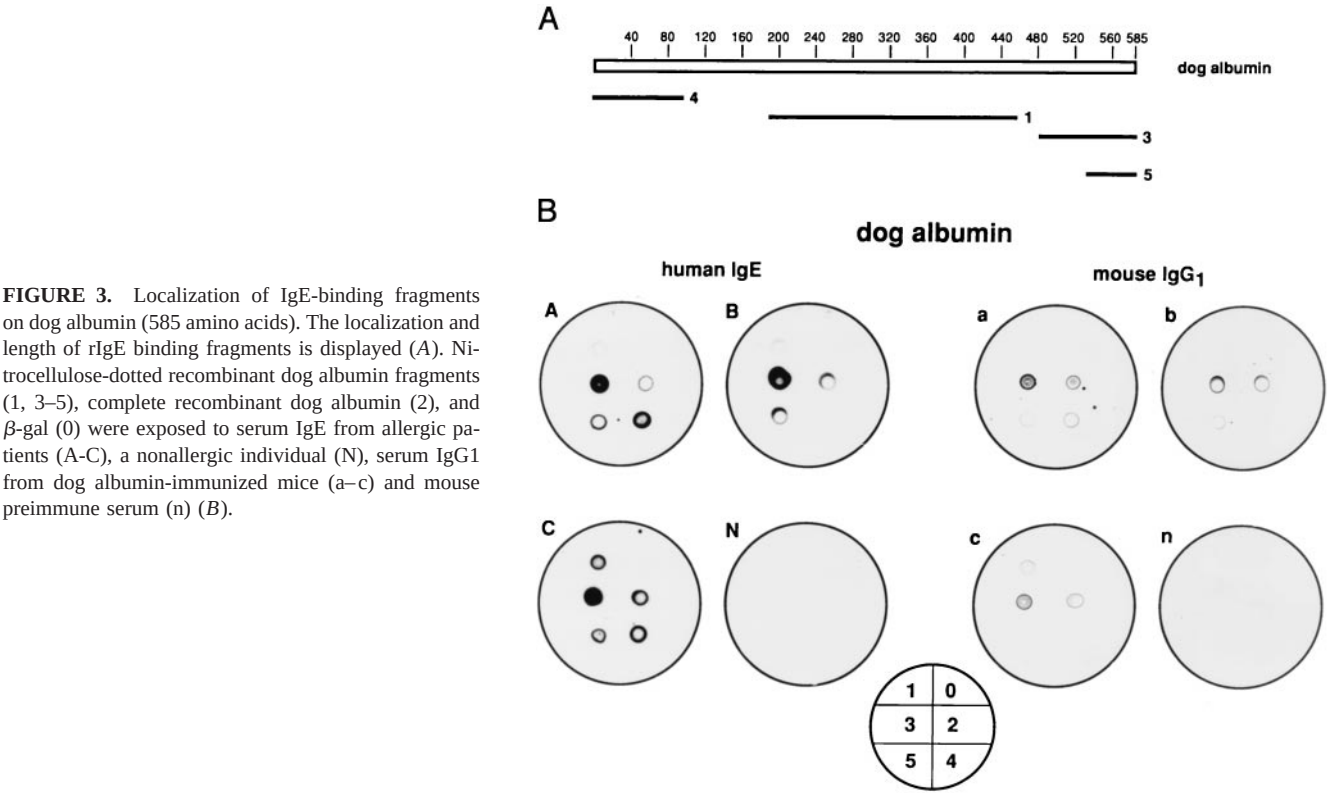


FIGURE 3. Localization of IgE-binding fragments on dog albumin (585 amino acids). The localization and length of rIgE binding fragments is displayed (A). Nitrocellulose-dotted recombinant dog albumin fragments (1, 3–5), complete recombinant dog albumin (2), and β -gal (0) were exposed to serum IgE from allergic patients (A–C), a nonallergic individual (N), serum IgG1 from dog albumin-immunized mice (a–c) and mouse preimmune serum (n) (B).

Mapping of mouse IgG1 epitopes using recombinant allergen fragments: mouse IgG1 Abs recognize similar epitopes as human IgE Abs

We have expressed IgE binding fragments of dog albumin, and the major grass pollen allergens, Phl p 1 and Lol p 5, as β -gal fusion proteins using phage λ gt11 and *E. coli* Y1090. Fragments of the allergen-encoding cDNAs were inserted into phage λ gt11 and clones that bound IgE Abs from allergic patients were selected. IgE-binding regions in dog albumin were found at the N terminus, C terminus, and in the middle of the protein (Fig. 3A). IgE epitopes of Phl p 1 were found at the N and C termini as well as in the center

of the protein (Fig. 4A). Lol p 5a contains a major IgE binding site in the center, close to the C terminus (Fig. 5A, upper part). Major IgE binding areas of Lol p 5b were found in the center of the molecule, one represented by clones 123 and 81 at the more N-terminal part and one represented by clone 21 at the C-terminal portion (Fig. 5A, lower part). The testing of the recombinant allergen fragments with serum IgE from allergic patients showed that certain immunodominant portions (e.g., the C-terminus of dog albumin, the central portion of Phl p 1, and the two central portions of Lol p 5 represented by clones 81 and 117) were recognized by all sera. While no significant IgG1, IgG2, and IgG3 reactivity to

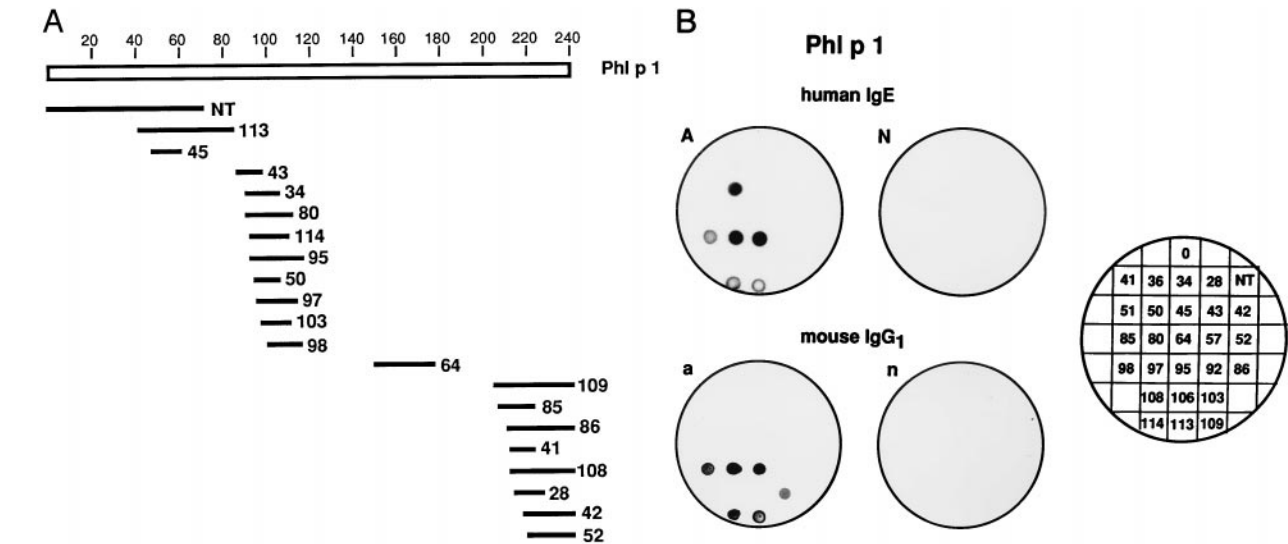
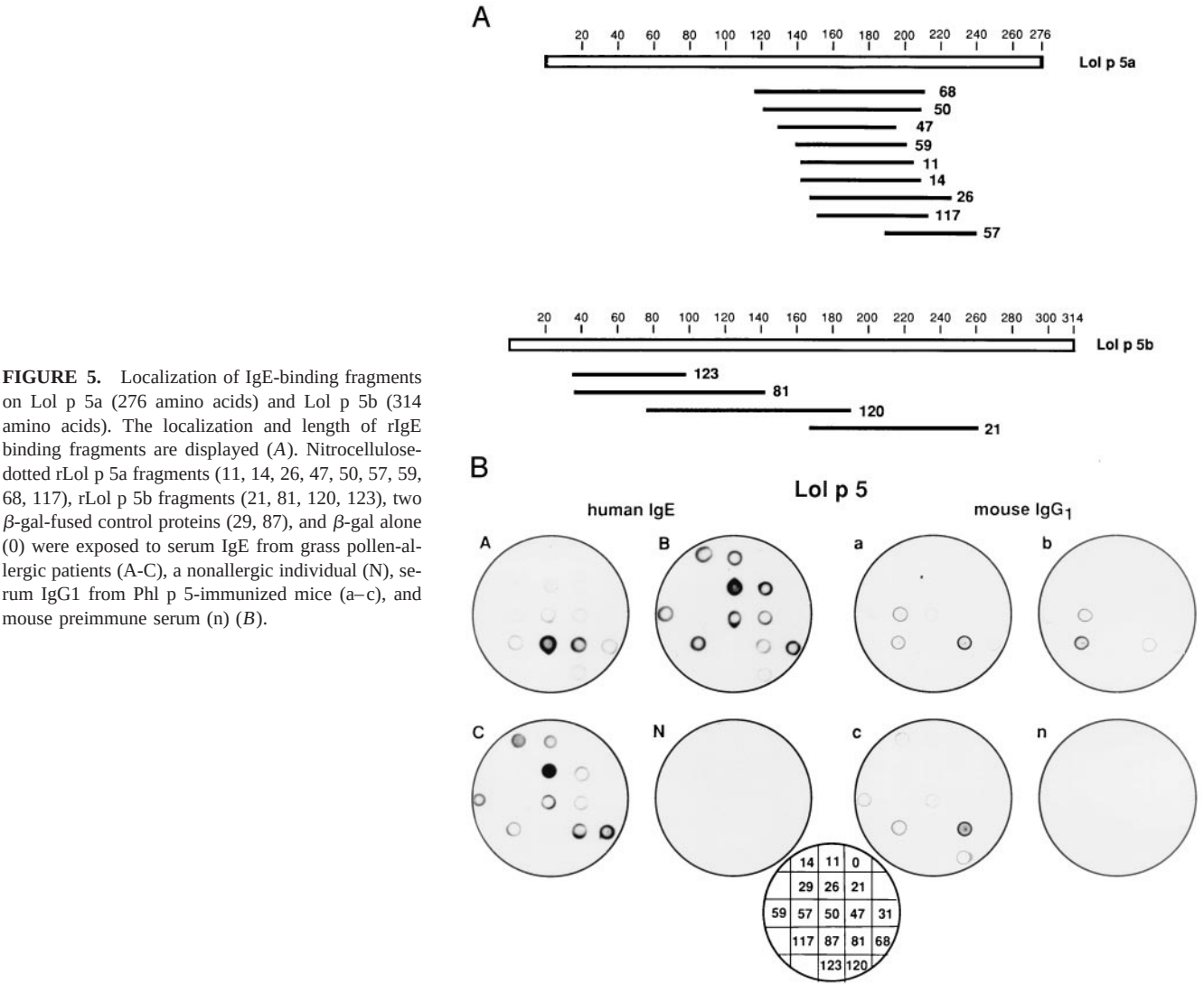


FIGURE 4. Localization of IgE-binding fragments on Phl p 1 (240 amino acids). The localization and length of recombinant IgE-binding fragments are displayed (A). Nitrocellulose-dotted rPhl p 1 fragments (NT, 28–114), and β -gal (0) were exposed to serum IgE from a grass pollen-allergic patient (A), a nonallergic individual (N), serum IgG1 from a Phl p 1-immunized mouse (a), and mouse preimmune serum (n) (B).



nitrocellulose-immobilized recombinant allergen fragments could be detected in sera from nonatopic and allergic individuals, sera from allergic patients who had received immunotherapy contained IgG4 Abs that strongly reacted with IgE epitopes (Ref. 36, and R. Valenta and T. Ball, unpublished observations). The immunodominant IgE epitopes were also detected by IgG1 Abs of all mouse sera. Serum IgE from nonallergic individuals and mouse preimmune sera failed to react with the epitope clones. Phage clones with control inserts or without inserts were not bound by any serum tested.

Allergen-specific mouse Abs block binding of human IgE to purified allergens and allergen extracts

To investigate whether mouse IgG1 Abs are able to inhibit the binding of human IgE Abs to allergens, competition experiments were performed. In the first set of experiments, nitrocellulose-blotted purified allergens were preincubated with sera from immunized mice, and for control purposes, with preimmune sera. As exemplified in Figure 6, IgE binding of six allergic patients (panels A–F) to purified allergens (panels: rPhl p 1, rPhl p 5, dog albumin) was strongly inhibited by preincubation of the respective allergen with the mouse immune sera (lane 2) but not after preincubation with the preimmune sera (lane 1). Table I displays the percentage of inhibition of IgE binding to recombinant Phl p 1 and

Phl p 5 determined for sera from several grass pollen-allergic patients by gamma counting. Phl p 5-specific mouse sera inhibited human IgE binding to recombinant Phl p 5 between 45 and 89%

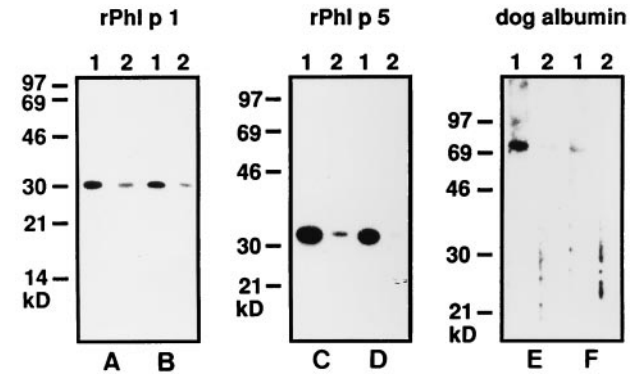


FIGURE 6. Mouse Ig inhibits human IgE binding to purified allergens. Nitrocellulose-blotted rPhl p 1, rPhl p 5, and dog albumin were preincubated with preimmune sera (lane 1) and sera from immunized mice (lane 2). Strips were then exposed to sera from six allergic patients (panels A–F) and bound IgE was detected with ¹²⁵I-labeled anti-human IgE Abs and visualized by autoradiography.

Table I. Quantification of the inhibition of human IgE binding to purified recombinant timothy grass pollen allergens, rPhl p 1 and rPhl p 5, after preincubation with mouse immune sera^a

		% Inhibition of IgE Binding				
		A	B	G	H	I
rPh1 p 1		15	47	59	77	43
		C	D	J	K	L
rPh1 p 5		84	89	46	45	65
		M				

^a The percentage inhibition of IgE binding to the allergens after preincubation with mouse sera is shown. Capital letters indicate the patients' sera and correspond to Figures 6 and 7.

and Phl p 1-specific mouse sera reduced human IgE binding to recombinant Phl p 1 between 15 and 77%. Serum Ig from rPhl p 5-immunized mice neither cross-reacted with rPhl p 1 nor inhibited human IgE binding to Phl p 1. Likewise, rPhl p 1-specific mouse Abs failed to react with Phl p 5 and to inhibit human IgE binding to Phl p 5 (data not shown).

Sera from mice that were immunized with single purified recombinant grass pollen allergens also significantly blocked IgE binding to natural grass pollen extracts. Serum Ig from a Phl p 5-immunized mouse but not the preimmune Ig blocked IgE binding of two sera from grass pollen-allergic patients (C and D) to natural pollen extracts from Kentucky blue grass, rye grass, and rye (Fig. 7). The quantification of the inhibition of IgE binding of an additional 13 sera from grass pollen-allergic patients to natural grass pollen extracts is summarized in Table II. Sera from rPhl p 5-immunized mice inhibited IgE binding to natural grass pollen extracts up to 66% and sera from rPhl p 1-immunized mice suppressed IgE binding up to 76%. Although IgE binding of several sera to certain extracts was not inhibited (Table II, dashes) a significant inhibition of IgE binding was observed for most of the sera tested.

Preincubation of rPhl p 5 with mouse immune sera inhibits the allergen-induced histamine release from human basophils

To evaluate whether blocking mouse Igs are also able to inhibit allergen-induced effector reactions, histamine release experiments were performed with basophils from a grass pollen-allergic individual. As exemplified in Figure 8, preincubation of the major timothy grass pollen allergen Phl p 5 with serum from a Phl p 5-immunized mouse, but not with buffer alone or preimmune serum, strongly suppressed the allergen-induced histamine release in a dose-dependent manner.

Discussion

In the present study, we investigated whether it is possible to use single purified natural or recombinant allergens to induce blocking mouse Abs that interfere with the interaction of allergic patients' IgE and allergens. Mice were immunized with aluminum hydroxide-adsorbed purified recombinant major timothy grass pollen allergens, rPhl p 1 (14), rPhl p 2 (15), rPhl p 5 (16), dog albumin (a relevant pet allergen (17, 18)), and two proteins with low (β -lactoglobulin) or no (α -ribulose-1,5-diphosphate carboxylase) allergenic activity in humans. Results obtained indicated that there is a trend supporting the contention that allergens that cause greater IgE responses in humans (Phl p 1, Phl p 5, dog albumin) (14, 16–18, 24) induce greater IgE and IgG₁ responses in mice. Ags that bind lower levels of IgE (Phl p 2) (24) and represent rare (β -lactoglobulin) or no (α -ribulose 1, 5-diphosphate carboxylase) targets for human IgE Abs induced low or no detectable Ab responses in mice. In a previous study performed with the recombinant birch pollen allergens rBet v 1 and rBet v 2 in mice (29) and rhesus monkeys (37), comparable results were obtained for the two birch pollen allergens. Bet v 1 (38), the major birch pollen allergen, binds high levels of IgE in humans (39, 40) and induced higher IgE levels both in mice and rhesus monkeys, compared with Bet v 2 (41), a less frequently detected allergen (40). Taken together, these data and the present results suggest immunogenicity as a general feature of a prominent allergen.

In this study, we noted pronounced cross-reactivity of timothy grass pollen specific- and dog albumin-specific IgE and IgG₁ Abs with homologous allergens from other sources. Also, this finding was not limited to the allergen panel investigated in this study as we had previously noted that IgE and IgG Abs induced in mice and rhesus monkeys with recombinant birch pollen allergens, Bet v 1 and Bet v 2, cross-reacted with immunologically related allergens present in various pollens and plant-derived food (29, 37). On the basis of the presence of cross-reactive IgE Abs, sensitized rhesus monkeys displayed skin reactivity with a series of allergen sources containing Bet v 1 and Bet v 2-related proteins (37). The present and previously obtained data would indicate that many of the B cell epitopes present on structurally related tree pollen, grass pollen, plant food, and animal hair/dander allergens are similar. The concept that relevant allergens bear a limited number of prominent B cell epitopes gained further support by our demonstration that mouse IgG₁ Abs recognized recombinant allergen fragments/epitopes defined by IgE Abs of allergic patients. These data strongly suggest that major allergens contain a few prominent B cell epitopes that may represent conserved immunodominant structures. In fact, the cDNA and deduced amino acid sequences of

FIGURE 7. Mouse Ig from a Phl p 5-immunized mouse inhibits human IgE binding to natural grass pollen extracts. Nitrocellulose-blotted timothy grass, Kentucky blue grass, rye grass, and rye pollen extracts were preincubated with mouse preimmune serum (lane 1) and serum from a rPhl p 5-immunized mouse (lane 2). Strips were then exposed to sera from two allergic patients (panels C and D), and bound IgE was detected with ¹²⁵I-labeled anti-human IgE Abs and visualized by autoradiography.

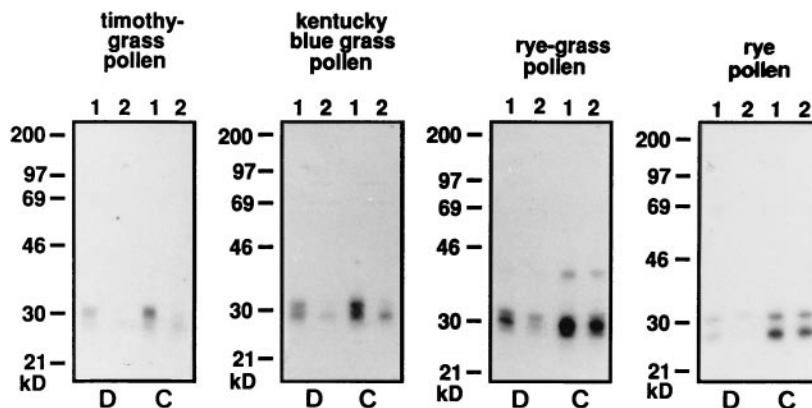


Table II. Quantification of the inhibition of human IgE binding to natural grass pollen extracts (Kentucky blue grass: *P. pratensis*; rye grass: *L. perenne*; rye: *S. cereale*) after preincubation with mouse immune sera^a

% Inhibition of IgE binding							
Preincubation with Phl p 1-Specific Mouse Ig				Preincubation with Phl p 5-Specific Mouse Ig			
Patients' serum	Kentucky blue grass	Rye grass	Rye	Patients' serum	Kentucky blue grass	Rye grass	Rye
B	16	—	23	J	66	35	—
G	11	—	—	K	42	39	15
H	11	36	—	L	31	13	—
I	51	39	12	M	47	—	35
Q	—	—	6	N	57	15	—
R	20	76	11	O	7	—	15
				P	44	39	53

^a The percentage inhibition of human IgE binding to the allergens after preincubation with mouse sera is shown. Dashes indicate no inhibition. Capital letters indicate the patients' sera and correspond to Figures 6 and 7.

the relevant IgE epitopes of the major timothy grass pollen allergen, Phl p 1 and dog albumin, are highly homologous to those found in related allergens (31, 17). More formal proof for the assumption that B cell epitopes of major allergens are conserved among their homologues will certainly come from the combined structural (x-ray, nuclear magnetic resonance) and epitope analysis of relevant allergens, as has been recently reported for the major birch pollen allergen Bet v 1 (42) and birch profilin (43). The analysis of the three-dimensional structures of both allergens indicated that the relevant B cell epitopes mapped to surface-exposed areas that are highly conserved across species and overlap with the binding sites of natural ligands.

The fact that IgG₁ Abs of immunized mice strongly recognized epitopes defined by human IgE Abs that are present on homologous allergens of various origin (e.g., group 1 or 5 allergens of different grass species) explains why preincubation of purified allergens as well as of natural allergen extracts with mouse immune sera lead to a strong reduction of human IgE binding. Support for the biologic relevance of the *in vitro* IgE competition comes from our finding that blocking mouse Abs inhibited the allergen-induced histamine release from basophils of an allergic patient. The presented mouse model hence not only allowed us to test whether blocking Abs can inhibit the

human IgE allergen interaction, but also to study their effects on allergen-induced effector mechanisms (basophil histamine release). It may therefore serve as a valuable *in vivo/in vitro* test system for the evaluation of recombinant Ag preparations or modified allergens sought for immunotherapy in humans.

Our finding that immunization with single purified recombinant allergens induced blocking Abs that protected against the original immunogen as well as against homologous allergens present in natural allergen extracts may be of great relevance for allergen-specific immunotherapy. Evidence for the hypothesis that blocking IgG Abs are elicited by immunotherapy and may account for the clinical efficacy of this treatment has been provided by several studies that measured increases of the levels of allergen-specific IgG Abs throughout the course of therapy (44, 45). However, due to the lack of defined allergens/allergen epitopes for immunotherapy as well as for serologic and *in vivo* (skin test diagnosis) assessment, it has been impossible to definitively answer whether the induction of blocking Abs, or perhaps a modulation of effector cell and/or T cell activity, is responsible for the clinical outcome (46, 47). Studies performed with recombinant allergens and allergen epitopes have shown that allergic as well as nonallergic individuals mount rather low levels of allergen-specific IgG Abs (39) and most failed to display IgG reactivity against IgE epitopes (36). However, high levels of IgG₄ Abs against IgE epitopes were detected in sera from patients who had received grass pollen-specific immunotherapy (36).

One immediate advantage of using recombinant allergens (48) or defined Ag preparations for patient-tailored immunotherapy would be that therapy-induced sensitization against unwanted components might be avoided (49). Ultimately, it may be expected that rDNA technology will provide us with recombinant hypoallergenic allergen fragments (31, 50, 51) or genetically engineered allergen derivatives (52–54) for specific immunotherapy that can be injected at much higher doses and thus will yield higher titers of blocking Abs.

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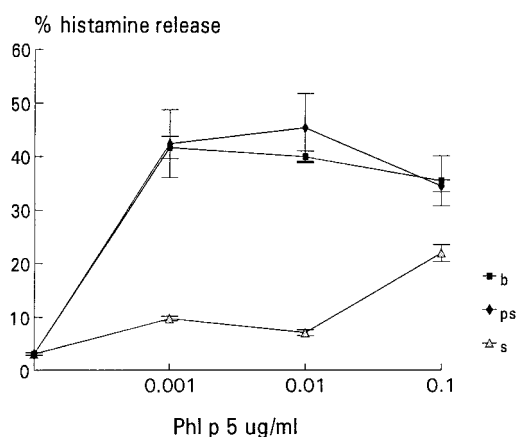


FIGURE 8. Preincubation of rPhl p 5 with serum from a Phl p 5-immunized mouse inhibits the allergen-induced histamine release from basophils of a grass pollen-allergic patient. Different concentrations of rPhl p 5 (0.001 µg/ml, 0.01 µg/ml, 0.1 µg/ml; x-axis) were preincubated with buffer alone (b), mouse preimmune serum (ps), or serum from the Phl p 5-immunized mouse (s). Histamine release is displayed on the y-axis as a percentage of the total histamine release measured after cell lysis.

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