Profilins Constitute a Novel Family of Functional Plant Pan-allergens

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Summary

Type I allergy is a major health problem in industrialized countries where up to 15% of the population suffer from allergic symptoms (rhinitis, conjunctivitis, and asthma). Previously, we identified a cDNA clone that encoded a birch pollen allergen as profilin. Profilins constitute a ubiquitous family of proteins that control actin polymerization in eukaryotic cells; in particular, profilin participates in the acrosomal reaction of animal sperm cells. Although profilins had been unknown in plants so far, our finding led to the assumption that profilins might have similar functions in pollens during plant fertilization and therefore represent allergenic components in almost all pollens. We show that profilins are prominent allergens that can be isolated from tree pollens (*Betula verrucosa*, birch), from pollens of grasses (*Phleum pratense*, timothy grass), and weeds (*Artemisia vulgaris*, mugwort). About 20% of all pollen allergic patients tested (n = 65) displayed immunoglobulin E (IgE) reactivity to recombinant birch profilin that was expressed in pKK223-3. An IgE inhibition experiment performed with recombinant birch profilin and purified natural profilins from timothy grass and mugwort indicates common IgE epitopes. Moreover, all pollen profilins purified from these far distantly related plant species, and likewise the purified recombinant birch profilin, are able to elicit dose-dependent histamine release via high affinity Fcε receptor of blood basophils from profilin allergic patients. The presence of profilin and possibly related proteins as crossreacting allergenic components in various plants therefore provides an explanation as to why certain allergic patients display type I allergic reactions with pollens and even food from distantly related plants. A functional pan-allergen, like profilin, available as purified recombinant protein, may be a useful diagnostic and probably therapeutic reagent.

Allergies of the immediate type are an important health problem in industrialized countries. One approach to get deeper insight into the pathogenesis of allergic diseases involves the characterization of allergens. The application of molecular biological techniques during the last few years has significantly improved our knowledge about the structure and biological function of atopic allergens (1). So far, the primary structure of allergens from the house dust mite (2), and pollens of trees (3), grasses (4), and weeds (5) have been determined by recombinant techniques. Computer-aided comparison of the deduced amino acid sequence revealed homology of the major birch pollen allergen *Bet v* I with a group of pathogenesis-related plant proteins (3). Yet all pollen allergens identified so far were expressed exclusively in pollens of one particular species and in those species that are botanically closely related (6). So far no ubiquitous allergen was identified that could provide an explanation for the clinical phenomenon that a high percentage of allergic patients are sensitized to a broad spectrum of pollens from distantly related plants.

Recently, we cloned and characterized a novel birch pollen allergen (*Bet v* II), which we could identify as profilin (7), by sequence homology and affinity to poly(L-proline)-Sepharose.

Profilins are ubiquitous components of the eukaryotic cytoskeleton. Proteins of this family function as actin-sequestering proteins (8) and were found to play an important role during the acrosomal reaction of echinoderm sperm (9). Furthermore, profilins participate in the phosphoinositide pathway, during signal transduction and reassembly of the cytoskeleton (10, 11).

So far no plant profilins have been described although the presence of a protein complexed to actin was reported (12). It was therefore tempting to speculate that profilins could

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constitute essential components of pollens and fulfill functions in plant fertilization that are similar to those already described for the acrosomal reaction of animal sperm (9).

The present study demonstrates that profilins are prominent allergens in pollens of trees, grasses, and weeds that are recognized by ~20% of all pollen-allergic patients. Thus, profilins are characterized as a novel type of pan-allergens sharing IgE epitopes. Profilin-allergic patients can be discriminated as a group of multivalent allergic patients suffering throughout the pollen season from allergic symptoms. Purified natural pollen profilins as well as recombinant birch profilin are able to induce histamine release from basophils of profilin-allergic patients but not from basophils of patients allergic to other allergens, in particular to recombinant and pollen-derived *Bet v* I (3), the major birch pollen allergen. In addition, a passive transfer experiment showed that pure chronic myeloic leukemia (CML)[1] basophils after short-term culture released significantly more histamine with recombinant birch profilin when they were preincubated with serum of an allergic patient containing profilin-specific IgE compared with a preincubation with serum of a nonallergic donor. These data identify the plant profilins as a group of pan-allergens that do not only bind to patients' IgE antibodies but are also functional in a cellular model of the allergic reaction.

Materials and Methods

Expression of the cDNA Encoding Birch Profilin and Bet v I. The cDNAs encoding birch profilin (7) and the major birch pollen allergen *Bet v* I (3) were subcloned into the EcoRI site of plasmid pKK223-3 (13). These constructs were transformed into *Escherichia coli* JM 105 (thi, str A, endA, sbcB15, hsdR4, lac-proAB, [F′, traD36, proAB, lacI^{q}ZM15]), and colonies producing the recombinant allergens were identified by IgE colony screening with serum IgE of profilin allergic patients as described (14). *E. coli* JM 105 containing the expression plasmid were grown at 37°C to an OD (600 nm) of 0.3, and then protein expression was induced with addition of 1 mM final concentration IPTG (isopropylthio-β-galactoside) for 3 h. *E. coli* cells were harvested and washed three times in 0.9% NaCl.

Purification of Natural Profilins from Pollen, and Recombinant Birch Profilin from E. coli. Pollen from birch (*Betula verrucosa*), timothy grass (*Phleum pratense*), and mugwort (*Artemisia vulgaris*) determined to be 95% pure by light and electron microscopy was obtained from Allergon AB (Engelholm, Sweden). For preparation of recombinant birch profilin, *E. coli* JM 105 transformed with pKK223-3 containing the cDNA encoding birch profilin was used. Biological material was lysed in PHEM-TX buffer (15) and the lysate was centrifuged for 1 h at 65,000 *g* at 4°C. The supernatants were incubated overnight at 4°C with poly(L-proline) coupled to BrCN-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) (16).

Then, the affinity matrix was washed three times with two volumes of TBS-ATP (20 mM Tris, 150 mM NaCl, 0.5 mM ATP, pH 7.6) each time and then eluted for 5 min at room temperature (RT) with 2 vol of elution buffer I (TBS-ATP with 2 M urea). This procedure was repeated twice with elution buffer II (TBS-ATP with 6 M urea). The supernatants were dialyzed against distilled water at 4°C and lyophilized. Purity and amount of the profilins were checked by polyacrylamide gel electrophoresis (17) and IgE immunoblots (Fig. 1) (18).

Purification of Natural and Recombinant Bet v I. Pollen-derived *Bet v* I was purified from birch pollen by affinity chromatography to anti-*Bet v* I mAb BIP 1, as described (19). Recombinant *Bet v* I was prepared from the *E. coli* pellet obtained from a 500-ml culture. The cells were broken in ~20 ml of 50 mM Tris-HCl, 220 mM NaCl, pH 7.5, by freezing twice in liquid nitrogen and thawing at 37°C. After centrifugation at 30,000 *g* for 25 min, the supernatant was dialyzed against 25 mM imidazole-HCl buffer, pH 7.4, and chromatofocussed in a PBE-94 exchanger column (Pharmacia) at room temperature. The column was washed with 25 mM imidazole-HCl buffer, pH 7.4, and the protein eluted with 12.5% (vol/vol) Polybuffer 74 (Pharmacia) that was titrated to pH 4.0 with HCl. The pooled fractions of recombinant *Bet v* I were subjected to HPLC using a linear gradient of 2-propanol created within 50 min at room temperature (C8, reversed-phase column; solvent A, 0.1% TFA in water; solvent B, 90% 2-propanol/0.1% TFA; gradient of 0–60% solvent B; flow rate 1.0 ml/min). Both proteins were checked on immunoblots for IgE binding property.

Characterization of Mugwort and Grass Pollen Allergic Patients. Grass pollen and mugwort pollen allergic patients were characterized by case history, skin prick tests, and RAST, as described earlier (18). Sera from all patients were tested for IgE reactivity with total pollen proteins from *P. pratense* and *A. vulgaris* on IgE immunoblots.

IgE Immunoblotting with Patients' Sera. 30 sera from grass pollen–allergic patients and 35 sera from mugwort pollen–allergic patients were tested for IgE binding to total pollen proteins from each species, for binding to natural profilins enriched from the respective species, and to purified recombinant birch profilin. Proteins had been separated by PAGE (17) and were blotted onto nitrocellulose (20). Bound serum IgE was detected as described (18).

IgE Immunoblot Inhibition. A serum pool of five patients with strong IgE reactivity to birch profilin was diluted 1:5 in buffer A (50 mM sodium phosphate, pH 7.5, 0.5% Tween 20, 0.5% BSA, 0.05% NaN_3) and preincubated either with purified recombinant birch profilin, profilin from timothy grass, control proteins from *E. coli* transformed with pKK223-3 without insert, or buffer A without addition of proteins, overnight at 4°C. The preincubated serum dilution was used for IgE binding to purified mugwort profilin that had been blotted onto nitrocellulose. Bound serum IgE was detected as described above.

Immunoblots with a Rabbit Anticelery Profilin Antibody. Strips of nitrocellulose containing purified profilins from pollens of birch (Fig. 4 *B*), timothy grass (*S*), and mugwort (*M*) were incubated with a 1:1,000 dilution of a rabbit anticelery profilin antibody (Vallier, P., Ch. Dechamp, R. Valenta, O. Vial, and P. Deviller, manuscript submitted for publication). Bound antibody was detected with a ^{125}I-labeled donkey anti-rabbit antibody (Amersham Corp., Amersham, UK) that was diluted at 1:1,000. For dilution of antibodies and incubation of blots, TBS-Tween (50 mM Tris-HCl, 150 mM NaCl, 0.5% Tween 20, pH 7.4) was used.

Histamine Release Experiments with Natural Pollen Profilins, Recombinant Birch Profilin (rBet v II), Pollen-derived Bet v I, and Recombinant Bet v I. Histamine release from blood basophils of profilin-allergic patients, patients that are allergic to the major birch pollen allergen *Bet v* I, and a group of nonallergic donors was done after informed consent was given. Allergic patients had been characterized as described previously (18) and, in addition, their sera were tested for IgE binding to recombinant birch profilin (r*Bet v* II) and recombinant *Bet v* I on immunoblots (14). Peripheral blood cells were

[1] *Abbreviation used in this paper:* CML, chronic myeloic leukemia.

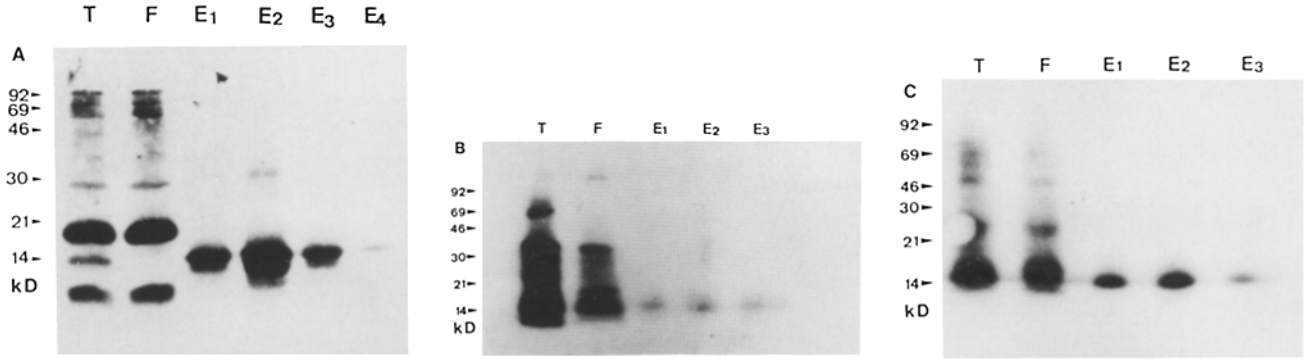

Figure 1. IgE Immunoblot of the purification of profilins from (*A*) birch pollen (*B. verrucosa*), (*B*) timothy grass pollen (*P. pratense*), and (*C*) mugwort pollen (*A. vulgaris*). Total IgE-binding pollen proteins from birch, timothy grass, and mugwort are shown in lane *T*, lane *F* is the flow through of the proteins not bound to poly(L-proline)-Sepharose, and subsequent elution steps of profilins are *E1–E3* (*E4* is birch) (*E1* elution with 2 M urea, *E2*, *3*, and *4* elutions steps with 6 M urea).

fractionated by incubation in 1.1% dextran 70 and 0.008 mM EDTA for 90 min at RT. Cells of the granulocyte-rich upper layer were then centrifuged (200 *g*, 4°C, 8 min) and washed twice in Pipes buffer (25 mM Pipes, 110 mM NaCl, 5 mM KCl, pH 7.35). Granulocytes were resuspended in Pipes buffer containing 2.0 mM $CaCl_2$. Cells were adjusted to a final concentration of 2.5 × 10^6/ml and incubated with various concentrations of agonists in multiwell plates. Basophils were incubated either with various concentrations (dose range, 10^{-4} to 1 μg/ml) of purified allergens or anti-IgE mAb E-124-2-8 as a positive control for 30 min at 37°C. Thereafter, cells were centrifuged (200 *g*, 4°C) and the cell-free supernatants recovered and analyzed for the presence of histamine by radioimmunoassay (Immunotech, Marseille, France), as described previously (22). Total histamine in cell suspensions was determined after cell lysis in distilled water. For passive sensitization experiments, >95% pure CML basophils (22) were used. Basophils were kept in culture for 4 d (RPMI plus 10% FCS [Gibco Laboratories, Grand Island, NY], 37°C, 5% CO_2) and were exposed to recombinant human (rh)IL-3 (Genetics Institute, Cambridge, MA) (100 U/ml) before being used in transfer experiments. Cells after this treatment lacked bound autologous IgE but were found to retain their ability to respond to release-inducing compounds. After culture the cells were exposed to 20% serum of a patient with specific IgE against profilin and a control serum of a nonallergic individual for 3 h. Thereafter, cells were washed three times in medium and incubated with various concentrations of recombinant birch profilin for 30 min. Histamine release into the supernatant was determined as above.

Results

Purification of Pollen Profilins and Recombinant Birch Profilin. Using the known affinity of profilins to poly(L-proline)-Sepharose (16), we could purify pollen profilins from birch (*B. verrucosa*), timothy grass (*P. pratense*), and mugwort (*A. vulgaris*), as well as recombinant birch profilin. To monitor the purification procedure of pollen profilins, blots that document the purification steps of profilins from pollens of birch (*B. verrucosa*) (Fig. 1 *A*), timothy grass (*P. pratense*) (Fig. 1 *B*), and mugwort (*A. vulgaris*) (Fig. 1 *C*) were incubated with serum IgE of a selected patient reacting in addition to profilin with most pollen allergens of the respective species. These blots show that profilins from tree, grass, and weed pollens can be purified to apparent homogeneity by affinity to poly(L-proline)-Sepharose.

IgE Reactivity of Grass Pollen- and Mugwort Pollen–allergic Patients with Profilins. Sera of 30 grass pollen allergic patients were tested for IgE reactivity with crude pollen proteins extracted from timothy grass (*P. pratense*) (Fig. 2 *A*), profilins enriched from pollen of timothy grass (Fig. 2 *B*), and purified recombinant birch profilin (Fig. 2 *C*) on IgE immunoblots. All patients who bound with their IgE to the enriched grass profilin had comparable IgE reactivity to recombinant birch profilin, suggesting common IgE-binding epitopes. According to the percentage of patients with IgE reactivity (30%), profilin could be identified as important grass pollen allergen. On blots containing crude grass pollen extracts, profilin is difficult to identify because a series of other allergens (groups II and III) (23) ranges in a similar molecular mass. The reactivity of patients' IgE with the grass pollen profilin can therefore not be distinguished from the binding to group II and III allergens in crude pollen protein extracts (Fig. 2 *A*).

The same experiment was done with a group of 35 mugwort-allergic patients. Again the patients were tested for IgE reactivity with total pollen proteins from mugwort (Fig. 3 *A*), profilin enriched from mugwort pollen (Fig. 3 *B*), and recombinant birch profilin (Fig. 3 *C*). 33% of the patients were found to display IgE reactivity against profilin, which therefore is identified as an important allergen of mugwort pollen. Corresponding binding of patients' IgE to profilins suggests similar IgE-binding capacity of recombinant birch profilin and profilin from mugwort pollen. Within crude protein extracts of mugwort pollen, the profilin appears as distinct IgE binding band at ~14 kD.

Crossreactivity of a Rabbit Anticelery Profilin Antibody with Profilins from Different Plants. A rabbit anticelery profilin antibody that identifies profilins from celery and apples (Vallier et al., manuscript submitted for publication) as allergenic component in food crossreacted with pollen profilins purified from birch, grasses, and mugwort (Fig. 4).

Demonstration of Common IgE-binding Epitopes of Recombinant Birch Pollen Profilin and Pollen Profilins from Grasses and Mugwort. The presence of common IgE-binding epitopes between

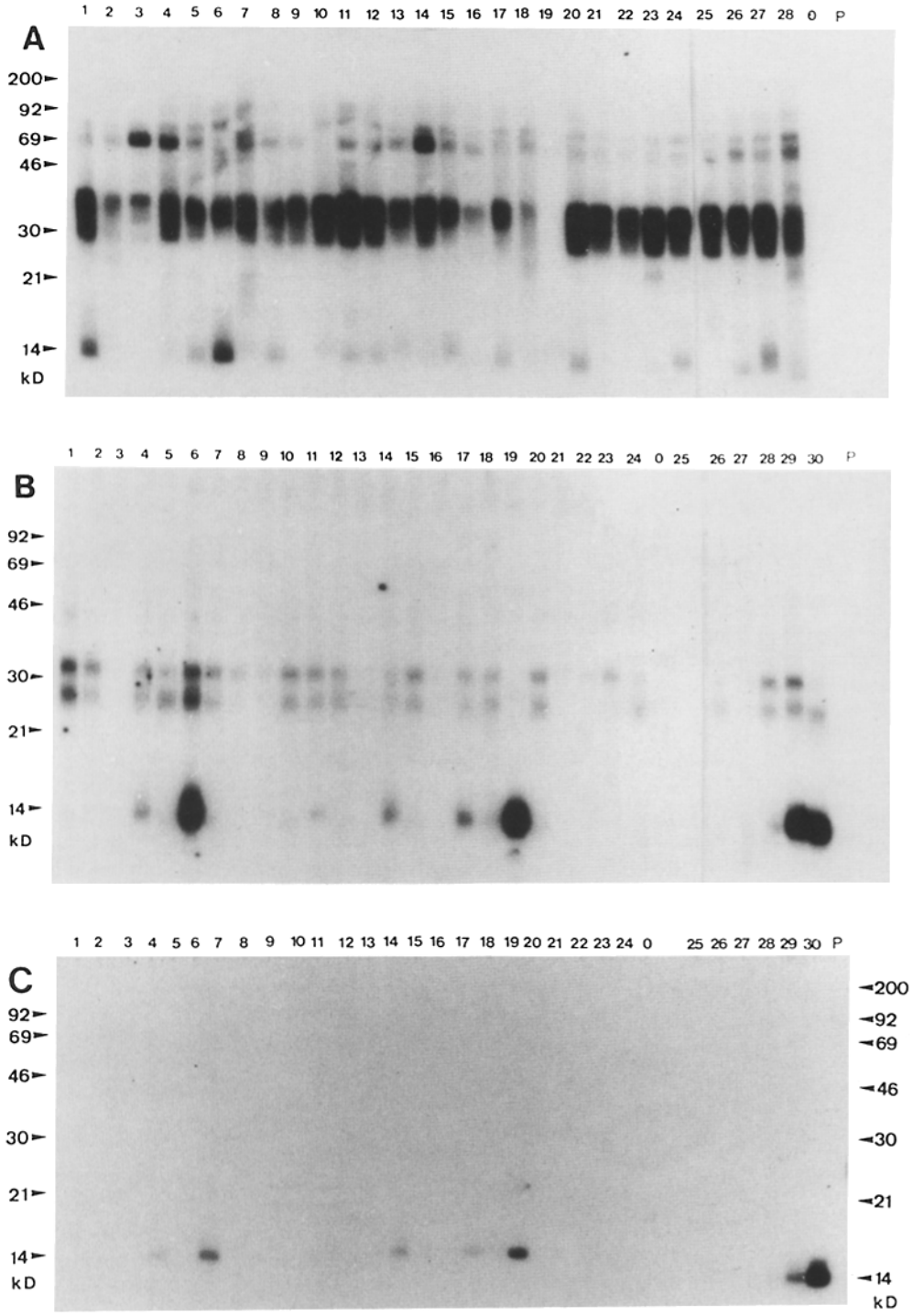

Figure 2. IgE immunoblot. Grass pollen–allergic patients were tested for IgE binding to total pollen proteins from *P. pratense* (*A*), to profilin enriched from pollen (*B*), and to purified recombinant birch profilin expressed in pKK223-3, *E. coli* JM105 (*C*). 0 is a serum pool from nonallergic individuals and *P* represents the buffer control without addition of serum.

the various plant profilins was further tested in an IgE immunoblot inhibition experiment (Fig. 5). Purified mugwort profilin was subjected to electrophoresis and blotted to nitrocellulose. A serum pool of five patients with IgE reactivity against birch profilin was diluted as described in Materials and Methods, preincubated either with recombinant birch profilin (Fig. 5, lane *2*), profilin from *P. pratense* (Fig. 5, lane *3*), proteins from *E. coli* (Fig. 5, lane *1*), or with serum dilution buffer only (Fig. 5, lane *4*), and then used for IgE binding. Addition of nondenatured profilin from *P. pratense* or recombinant birch profilin in the fluid phase inhibits binding of patients' IgE to blotted mugwort profilin. These data provide strong evidence for the presence of common IgE-binding epitopes on the pollen profilins tested.

Histamine Release from Blood Basophils of Profilin Allergic Patients. To assess whether the similar capacity of the different

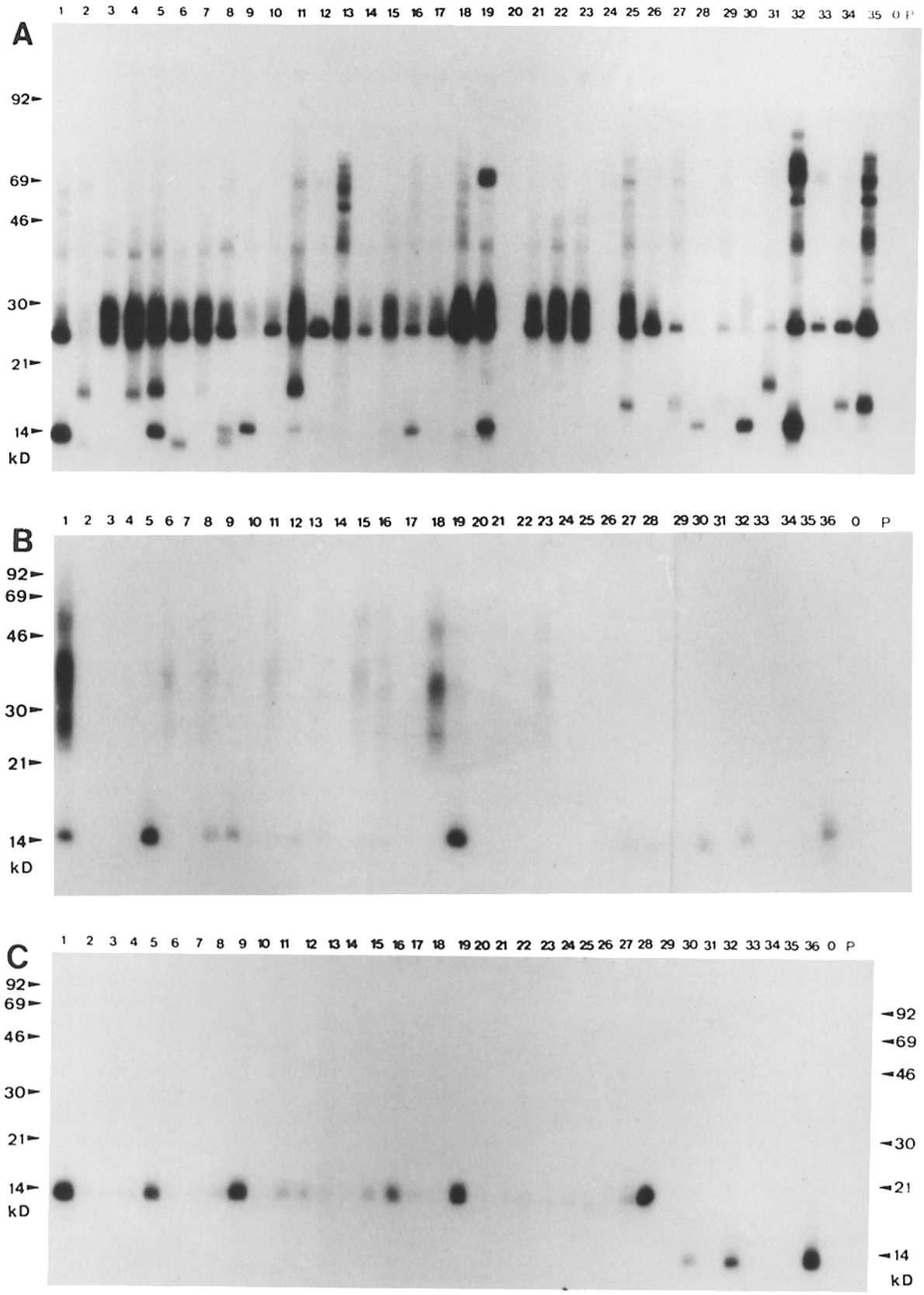

Figure 3. IgE immunoblot. Mugwort allergic patients were tested for IgE reactivity with total pollen proteins from *A. vulgaris* (*A*), with profilin enriched from pollen (*B*), and with recombinant birch profilin as above (*C*). Lanes *O* and *P* are as in Fig. 2.

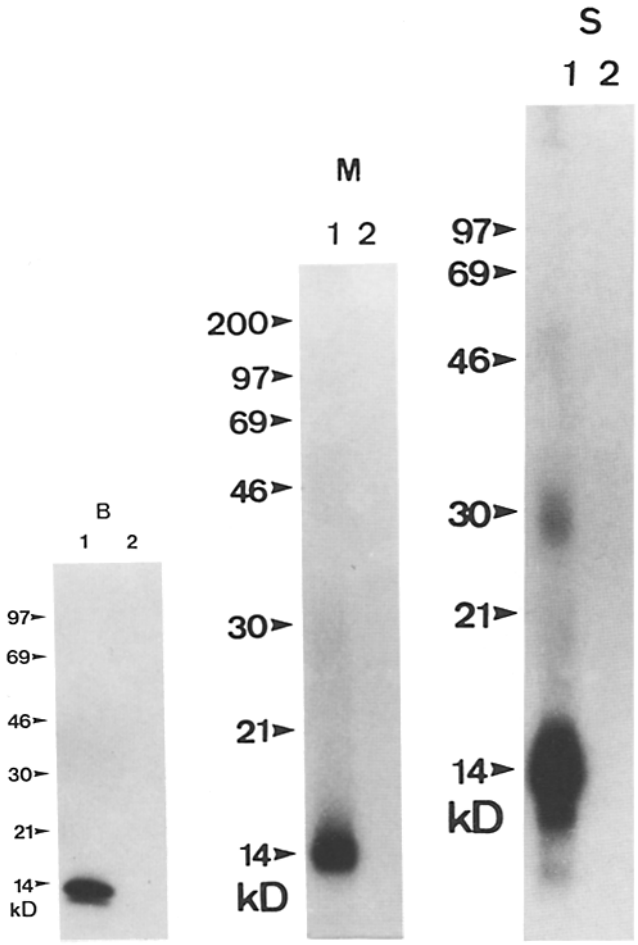

Figure 4. IgG immunoblot. Purified profilins from pollens of birch (*B*) (*B. verrucosa*), timothy grass (*S*) (*P. pratense*), and mugwort (*M*) (*A. vulgaris*) were tested with a rabbit anticelery profilin antibody (lanes *1*). Lanes *2* shows the buffer control without the addition of antibody.

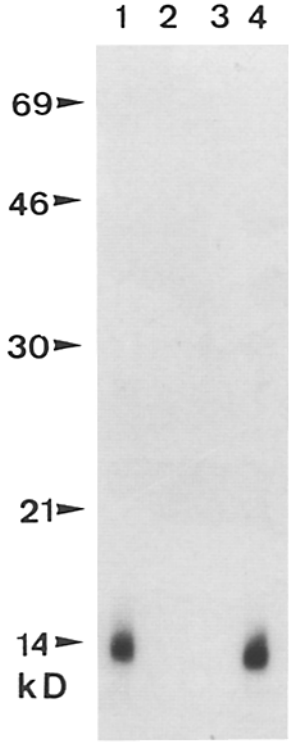

Figure 5. IgE-immunoblot inhibition. Purified profilin from mugwort *A. vulgaris* had been blotted to nitrocellulose. A serum pool from five patients with IgE reactivity to recombinant birch profilin was used for IgE binding to profilin. After dilution (1:5 in buffer A), the serum pool was preincubated either with 10 μg of recombinant birch profilin (lane *2*), with profilin purified from *P. pratense* (lane *3*), or with control proteins from *E. coli* JM 105 transformed with pKK223-3 containing no inserted cDNA (lane *1*). In lane *4*, no protein was added (buffer control).

plant profilins to bind patients' IgE can also lead to histamine release, basophils of patients who are allergic against *Bet v* I and profilin (Fig. 6 *A*), and patients allergic to *Bet v* I only (Fig. 6 *B*), were incubated with the different plant profilins and, to ensure specificity of the in vitro system, with purified birch pollen–derived *Bet v* I and purified recombinant *Bet v* I. All purified pollen-derived profilins as well as the recombinant birch profilin induced significant histamine release in patients allergic to profilin and *Bet v* I, whereas no release was seen with basophils from patients allergic to *Bet v* I only and a healthy control group. The results of these experiments are summed up in Table 1. In all cases tested the natural and recombinant allergens released histamine in the same way, and perfect correlation was found between IgE reactivity of the patient's sera on immunoblots and capacity of their basophils to release histamine towards the purified natural as well as recombinant allergens. Furthermore, these data show that the structural similarity of all plant profilins derived from different species leads to common IgE-binding capacity and hence to comparable functional effects on the cellular level. Passive transfer experiments (Fig. 6 *c*) furthermore indicate that it is possible to reconstitute in vitro an allergic effector mechanism leading to histamine release using purified CML basophils, specific serum IgE from a profilin allergic patient, and recombinant birch profilin. Although the release obtained in the passive transfer was less pronounced than in the direct assay with basophils from allergic donors, it was significant when compared with the control performed with serum from a nonallergic donor.

Discussion

In a previous paper we described profilin as novel birch pollen allergen sharing IgE epitopes even with human profilin (7). This particular birch pollen allergen, designated *Bet v* II, was isolated from a cDNA library constructed from birch pollen mRNA. Sequence homology with profilins from yeast (24), slime mold (25), *Ancanthamoeba* (26), mouse (27), calf (28), and human (29) was found by computer-aided comparison of the deduced amino acid sequence of the birch profilin with known proteins. The natural as well as the recombinant birch profilin bound to poly(L-proline)-Sepharose (16) and IgE autoreactivity was found to the human counterpart.

So far nothing was known about a function or even presence of profilins in plants. The important role of profilins in animal fertilization made it tempting to speculate about a similar role in plants. We show that profilins can be isolated from pollens of far distantly related plant species (trees, grasses, and weeds) by affinity to poly(L-proline)-Sepharose. All pollen profilins bind to a rabbit anticelery profilin antibody (Vallier et al., manuscript submitted for publication),

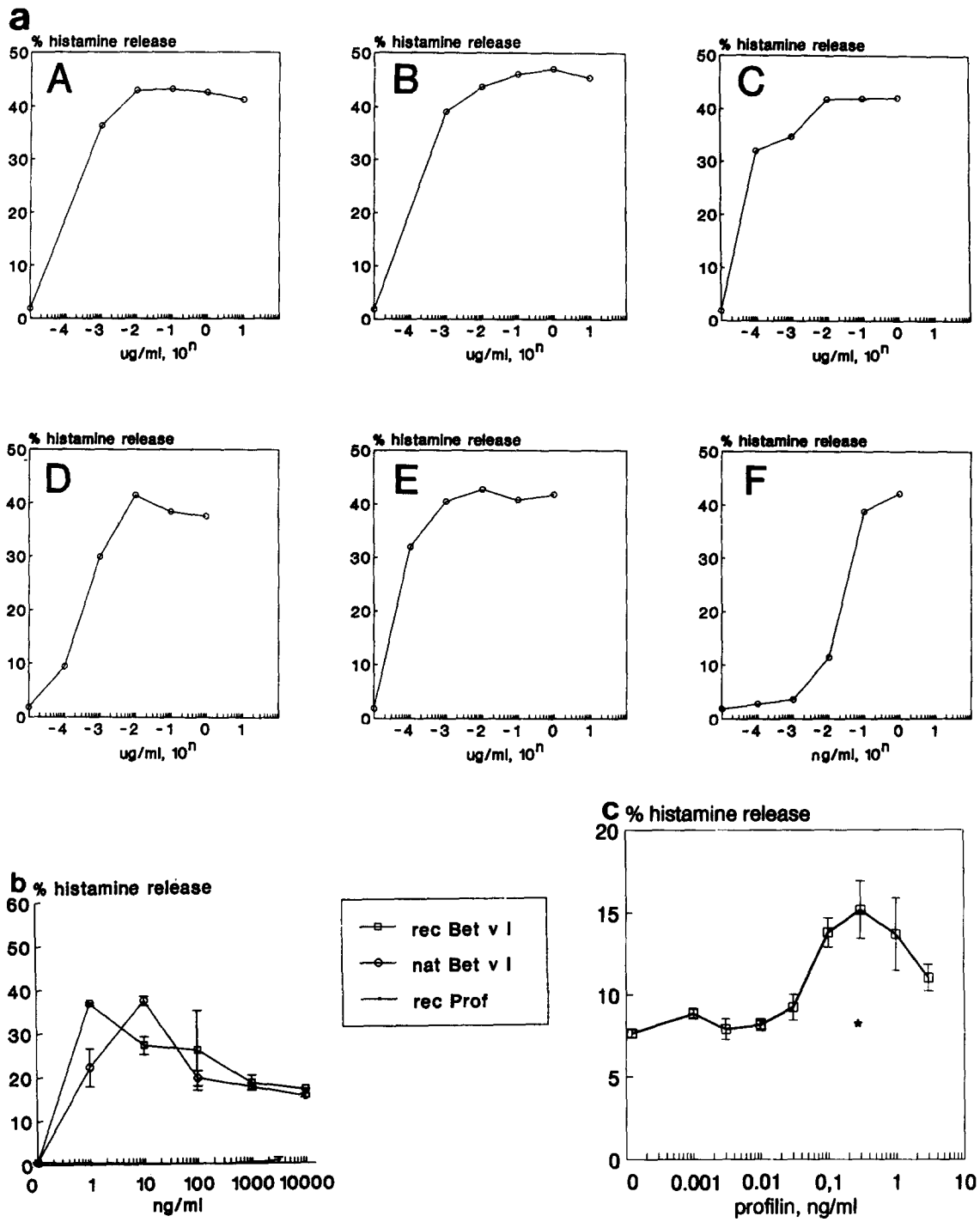

Figure 6. (*a*) Histamine release with purified *Bet v* I and profilins. Blood granulocytes from a patient (Table 1, no. 4) allergic to the major birch pollen allergen, *Bet v* I, and to profilin were incubated with various concentrations of purified pollen-derived *Bet v* I (*A*), recombinant *Bet v* I (*B*), and purified profilins (recombinant birch profilin [*C*], profilin from pollens of timothy grass [*P. pratense*] [*D*], birch [*B. verrucosa*] [*E*], and mugwort [*A. vulgaris*] [*F*]). Histamine release is given as percentage of total histamine and results represent the mean of duplicate determinations. The release obtained with optimal concentrations of anti-IgE mAb E-124-28 was 47.2% of total histamine. (*b*) Histamine release with basophils from a patient allergic to *Bet v* I but not to profilin (Table 1, no. 5). (*c*) Passive sensitization of human basophils with an allergic patient's serum containing profilin-specific IgE. Pure basophils treated as described in Materials and Methods were sensitized either with profilin-specific serum IgE (□) and serum from a nonallergic donor (maximal histamine release is indicated with an asterisk). Histamine release after addition of various concentrations of recombinant profilin is expressed as percentage of total histamine. Results represent the means of triplicate determinations.

Table 1. *Comparison of Histamine Release and IgE Reactivity of Different Allergic Patients to Pollen-derived and Recombinant Allergens*

Patient no.	Allergen	Maximum release	IgE reactivity
1	nprof	nt	–
	rprof	3.4	–
	r*Bet v* I	38.8	+
	n*Bet v* I	38.7	+
2	nprof	nt	–
	rprof	4.5	–
	r*Bet v* I	29.1	+
	n*Bet v* I	31.4	+
3	nprof	nt	+
	rprof	55.3	+
	r*Bet v* I	nt	–
	n*Bet v* I	nt	–
4	nprof	nt	+
	rprof	42.1	+
	r*Bet v* I	47	+
	n*Bet v* I	43.2	+
5	nprof	nt	–
	rprof	0.7	–
	r*Bet v* I	36.8	+
	n*Bet v* I	37.3	+
6	nprof	nt	–
	rprof	2.4	–
	r*Bet v* I	4.1	–
	n*Bet v* I	6.4	–

nprof, pollen-derived birch profilin; rprof, recombinant birch profilin; n*Bet v* I, pollen-derived *Bet v* I; r*Bet v* I, recombinant *Bet v* I; nt, not tested.

indicating common antigenicity of all these proteins. Moreover, purified profilins from timothy grass (*P. pratense*) and from mugwort (*A. vulgaris*) share IgE-binding epitopes with the recombinant birch profilin, as was demonstrated by testing these proteins on immunoblots with serum IgE from 30 grass pollen allergic patients and with serum IgE from 35 mugwort allergic patients. All patients who displayed IgE reactivity to the enriched profilin from grass and mugwort pollen bound also to purified recombinant birch profilin.

Since antigen-antibody interactions can be modified when proteins are blotted to nitrocellulose (30), competitive inhibition studies were performed. IgE antibodies to mugwort profilin that had been blotted to nitrocellulose can be blocked by preincubation of patient's IgE with nondenatured recombinant birch profilin or grass pollen profilin (*P. pratense*) in the fluid phase. To further test the functional properties of these plant profilins in an in vitro cellular system, histamine release tests with blood basophils from allergic patients were carried out. As a result, we show that in addition to common IgE-binding capacity, all these purified profilins are able to induce significant histamine release in profilin-allergic patients and characterize these proteins as functional pan-allergens. A series of control experiments performed with an allergen different from profilin, natural and recombinant *Bet v* I, and patients with different patterns of IgE reactivity towards these allergens, ensure specificity of the bioassays. Less pronounced, a passive transfer experiment showed that it was possible to reconstitute in vitro the cascade leading to an allergic response by using a recombinant allergen (recombinant birch profilin), profilin specific serum IgE, and highly purified CML basophils.

Our data demonstrate that profilin-allergic patients constitute ~20% of all pollen-allergic individuals. All these patients display similar IgE reactivity to pollen profilins from different species, as well as to profilins in plant food (21) and various fruits (31, Hirschwehr, R., R. Valenta, C. Ebner, F. Ferreira, O. Scheiner, and D. Kraft, manuscript submitted for publication). This may be of importance for diagnosis and possibly therapy of Type I allergy because it modifies the current view that patients are allergic towards a certain species to a view in which a certain allergenic protein is the cross-sensitizing agent. The identification of such pan-allergens that despite of their different origin are likely to cross-sensitize patients may help to see the puzzling variety of allergens more clearly and can explain the clinical observation that some patients suffer from allergic symptoms (rhinitis, conjunctivitis, and asthma) throughout the pollen season and even show intolerance towards food of plant origin. The availability of such a purified pan-allergen as a recombinant protein for diagnostic purposes (14) therefore allows discrimination of a novel group of multivalent allergic patients and probably improves our knowledge of mechanisms leading to type I allergy. In addition, the purified recombinant birch profilin might be a useful tool for hyposensitization treatment of allergic patients.

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