

A mimotope defined by phage display inhibits IgE binding to the plant panallergen profilin

Agnes Leitner¹, Monique Vogel², Christian Radauer¹, Heimo Breiteneder¹, Beda M. Stadler², Otto Scheiner¹, Dietrich Kraft¹ and Erika Jensen-Jarolim¹

¹ Department of General and Experimental Pathology, University of Vienna, Vienna, Austria

² Institute of Immunology and Allergology, University of Bern, Inselspital, Bern, Switzerland

Birch pollen and mugwort pollen allergies are often associated with hypersensitivity to plant foods. This clinical and serological cross-reactivity is mediated by IgE antibodies reacting with homologous proteins in pollen and food. Cross-reacting homologs of the important birch pollen allergen Bet v 2 (profilin) could be detected in other pollen, fruits, nuts, and vegetables, such as celery tuber. We purified IgG/IgE antibodies from the serum of an exclusively profilin-allergic patient using affinity columns either coupled with protein extracts from mugwort pollen, birch pollen, or celery tuber. Constrained and unconstrained random nonapeptide libraries were pooled and screened with the anti-profilin antibody preparations to define cross-reactive ligands. Specific ligands were enriched by successive panning rounds using the profilin-specific antibodies in series. After the last panning round enriched phage clones were screened with purified profilin-specific antibodies and IgE-binding clones were sequenced. Five out of eight positive clones (62.5 %) displayed the same circular peptide CAISGGYPVC. This peptide was synthesized and examined for its ability to inhibit IgE binding to blotted mugwort pollen, birch pollen, or celery tuber profilin. Inhibition studies showed reduction of IgE binding to profilins in all three protein extracts. As the sequence of the mimotope did not show any homology to the known birch profilin sequence this peptide is considered to mimic a common conformational IgE epitope for these examined profilins.

Received	22/5/98
Accepted	19/6/98

Key words: IgE cross-reactivity / Phage display / Profilin mimotope

1 Introduction

In the past few years plant profilins, which are actin-binding proteins with a molecular mass of approximately 14 kDa, were revealed as allergens in pollen, nuts, vegetables, and fruits [1–7]. About 20 % of pollen- and food-allergic patients display IgE binding to profilins [8]. As revealed by immunoblot techniques, IgE Ab are cross-reacting extensively with profilins from different plants [2–7]. Profilins are highly conserved proteins and sequence comparisons among several plant profilins showed an identity of at least 75 % which may explain broad IgE cross-reactivity [8–11].

Recently, IgE epitopes on birch profilin could be defined by screening of a random fragment expression cDNA library with patients' sera. These epitopes were mapped in two clusters at the N- and C-terminal helices of the allergen, of which the three-dimensional structure has

recently been determined [12]. Such continuous epitopes possess sufficient affinity to bind IgE Ab, but do not represent the relevant conformational epitopes present in the native profilin. During natural exposure Ab interact primarily with surface-exposed residues forming conformational epitopes which often are not in linear sequence on the antigen.

A new strategy to identify epitopes for monoclonal and polyclonal Ab was introduced by the use of random peptide libraries displayed on filamentous phages [13–18]. Recently two phage libraries containing either linear or circular random nonapeptides [13, 14] have been used to isolate ligands interacting with mAb recognizing conformational epitopes present on proteins such as *Bordetella pertussis* toxin [15] or human H ferritin [14]. These affinity-selected ligands were capable of mimicking the discontinuous epitopes and hence binding to the corresponding mAb. Moreover, it has been demonstrated that peptides isolated from the same libraries by screening of sera from hepatitis C virus-infected patients were able to induce an immune response in mice [16, 17]. Thus, these peptides, or so-called mimotopes, are not only able to

[1 18393]

mimic linear or conformational epitopes but they are additionally immunogenic and are therefore candidates for vaccine development.

We purified IgG/IgE Ab from the serum of an exclusively profilin-allergic patient using three affinity columns coupled with birch pollen, mugwort pollen, or celery tuber proteins. These affinity-purified Ab directed against different plant profilins were used for screening the two nonapeptide libraries [13, 14] to define mimics for cross-reactive IgE epitopes. Several panning rounds were performed using the affinity-purified Ab in successive order for isolating cross-reactive ligands from the peptide libraries. Isolated ligands were examined and the most frequently occurring was then used for IgE inhibition studies.

2 Results

2.1 Purification of human profilin-specific IgG/IgE Ab by affinity chromatography

We purified IgG/IgE Ab from a profilin-allergic patient's serum via affinity chromatography columns coupled with protein extracts of birch pollen, mugwort pollen, or celery tuber. The total yield was 8.2 µg Ab specific for birch pollen, 4.5 µg Ab specific for mugwort pollen, and 7.6 µg Ab specific for celery tuber profilin from 10 ml serum each. Comparing affinity-purified Ab with serial dilutions of monoclonal IgE and myeloma IgG Ab in immunodot assays indicated an IgG:IgE ratio of 10:1 among purified Ab (data not shown). Before using these Ab for the biopanning method, IgE immunoblots were performed to check the immunoreactivity of purified Ab. Immunoblot analysis revealed that the IgE binding capability of affinity-purified Ab to profilins of birch pollen, mugwort pollen, and celery tuber extracts remained unaffected by the purification procedure (Fig. 1, lanes C).

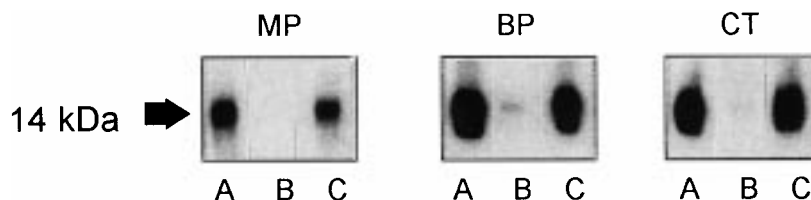


Figure 1. IgE immunoblots with serum from a patient with exclusive IgE reactivity to profilins and of Ab purified from the same serum by affinity columns coupled with mugwort pollen (MP), birch pollen (BP) or celery tuber (CT) extract. Diluted serum (A), effluents (B) and eluents (C) from affinity columns were tested for IgE binding to corresponding extracts. Bound IgE was detected by ^{125} I-labeled anti-human IgE Ab.

Table 1. Phage library screening^{a)}

Panning round	IgG/IgE Ab specific for	Eluted phages
1	Mugwort pollen profilin	10 ⁷
2	Birch pollen profilin	10 ⁵
3	Celery tuber profilin	10 ⁶
4	Mugwort pollen profilin	10 ⁷

a) Pooled constrained and unconstrained nonapeptide libraries were screened with affinity-purified IgG/IgE Ab specific for different profilins. The amounts of eluted phages during four subsequent rounds of biopanning were monitored.

2.2 Screening of a nonapeptide phage library pool with profilin-specific IgG/IgE Ab

We screened a phage library pool with a repertoire of 10⁸ different phage particles from the pVIII-9aa [13] and 10⁸ from the pVIII-9aa.Cys library [14] with the profilin-specific Ab. To select cross-reactive epitope mimics for IgE Ab directed against profilin from different sources, four panning rounds were performed using the following antibody preparations in series: (1) anti-mugwort pollen profilin Ab, (2) anti-birch pollen profilin Ab, (3) anti-celery tuber profilin Ab and (4) the anti-mugwort pollen profilin Ab again (Table 1).

After the first panning round 10⁷ phages were eluted. In the second panning round the number of eluted phages decreased to 10⁵ but increased in the third and fourth round to 10⁶ and 10⁷, respectively (Table 1).

Subsequently a dot assay was performed to test the specificity of eluted phages. Purified IgG/IgE Ab were

dotted on nitrocellulose and incubated with amplified phages eluted from each panning round. A peroxidase-labeled rabbit anti-phage Ab detected phages bound on profilin-specific Ab after the third and fourth panning rounds (Fig. 2). No signals were obtained by incubating dotted IgG/IgE Ab with phages eluted from the first and the second panning and in control experiments with the original library pool and the helper phage VCS M13. In addition, no reactions of the selected phages with monoclonal IgE and myeloma IgG Ab were observed (Fig. 2).

To test immunoreactivity of individual affinity-selected phage clones, a colony screening with 50 clones from the fourth panning round with purified profilin-specific IgG/IgE Ab was performed. Eight clones which showed the most intense IgE binding of all three Ab preparations were sequenced. DNA sequencing revealed five clones containing an equivalent insert with the deduced amino acid sequence CAISGGYPVC, while one clone had the insert CSADASPDC, and two were “blind” clones.

2.3 Inhibition experiments

Using IgE immunoblot inhibition experiments, we investigated whether the mimotope CAISGGYPVC isolated by

affinity selection from the phage library pool was able to prevent IgE binding to different profilins. Inhibition studies were performed with affinity-purified profilin-specific IgG/IgE Ab and with profilin-allergic patients' sera. Diluted Ab preparations and sera were preincubated with equimolar concentrations of a randomly chosen unrelated peptide CNLWEVMHGTC, recombinant birch profilin (rBet v 2), or with the synthetic peptide CAISGGYPVC before incubation with blotted extracts of mugwort pollen, birch pollen, or celery tuber. Preincubation with the mimotope CAISGGYPVC and with rBet v 2 reduced substantially the IgE binding to profilins from mugwort pollen, birch pollen, and celery tuber profilin as compared to the control experiment with the unrelated peptide (Fig. 3). The intensity of IgE signals on autoradiographs was densitometrically evaluated showing a 50–70 % relative reduction of IgE binding with the mimotope CAISGGYPVC as compared to the control experiment.

To demonstrate the relevance of the observed IgE-peptide interactions in serum conditions, we tested the sera from three other profilin-allergic patients in the inhibition assay. Depending on the IgE repertoire of the patients, different values for inhibition of IgE binding to birch pollen, mugwort pollen and celery tuber profilin with the mimotope CAISGGYPVC were found which are summarized in Table 2.

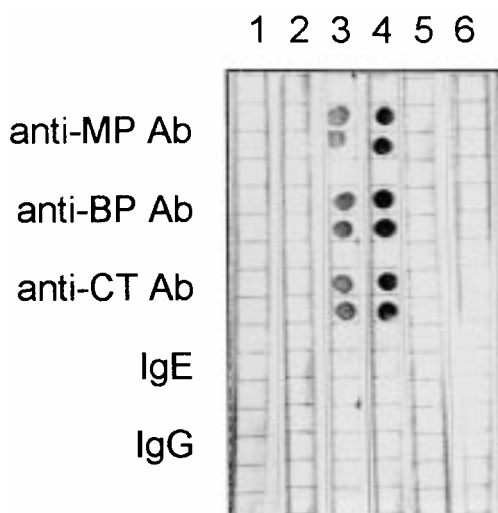


Figure 2. Specificity of affinity-selected phages shown by immunodot assay. Anti-mugwort pollen (MP), -birch pollen (BP) or -celery tuber (CT) IgG/IgE Ab, monoclonal myeloma IgE (IgE), and myeloma IgG (IgG) were dotted in duplicate and incubated with amplified phages after the first (lane 1), second (lane 2), third (lane 3), and fourth (lane 4) panning round, with helper phage (lane 5) or original library pool (lane 6). Bound phages were detected with a peroxidase-labeled rabbit anti-phage Ab.

2.4 Sequence alignment

Comparison of the amino acid sequence of birch profilin with the peptide CAISGGYPVC using the GCG package (Genetics Computer Groups, Inc., Madison, WI) did not

Table 2. Inhibition of IgE binding to allergens by the mimotope CAISGGYPVC^{a)}

Patient	MP	BP	CT
A	42 % ¹	58 %	57 %
B	71 %	none	82 %
C	none	none	56 %

a) Densitometrically evaluated reduction of IgE binding intensities in immunoblots using the profilin mimotope CAISGGYPVC for inhibition. Profilin-allergic patients' sera (A, B, C) were tested for IgE binding to blotted mugwort pollen (MP), birch pollen (BP) and celery tuber (CT) extracts after preincubation with the peptide CAISGGYPVC or an unrelated control peptide. The signal intensity with the control peptide was set equal 100 % and values obtained with the specific peptide compared hetero. The relative percentage of reduction of IgE binding to blotted MP, BP, and CT profilins is given.

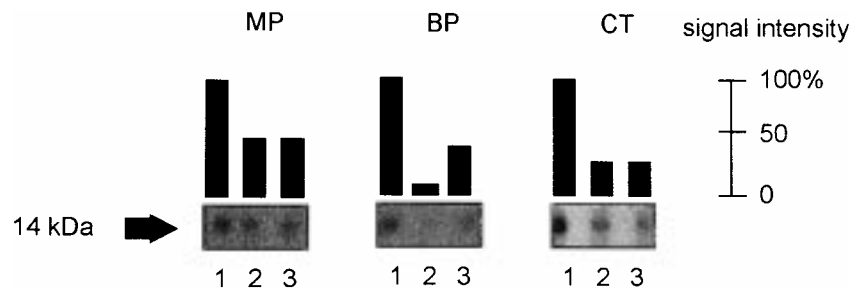


Figure 3. Autoradiographs and densitometrical analysis of an inhibition assay using equimolar amounts of peptides and recombinant birch pollen profilin (rBet v 2) for competition of IgE binding. Mugwort pollen (MP), birch pollen (BP) and celery tuber (CT) extracts were blotted and incubated with corresponding affinity-purified anti-MP, -BP or -CT profilin-specific IgG/IgE Ab, which were preincubated with a control peptide (lanes 1), with rBet v 2 (lanes 2), or with the peptide CAISGGYPVC (lanes 3). The signal intensity of IgE binding to the 14-kDa allergen of IgG/IgE Ab preincubated with the control peptide was set equal 100 % and inhibited signals compared hereto (black bars).

indicate any sequence homology. In addition, we were not able to define a distinct peptide corresponding area on the three-dimensional structure of birch profilin using the Swiss PdbViewer (Geneva Biomedical Research Institute, Glaxo Wellcome Research and Development S. A., Geneva, Switzerland) software program.

3 Discussion

On profilin, IgE-binding epitopes have been determined by screening of expression cDNA libraries with patients' sera [12]. With this approach it was possible to define sequential epitopes on profilin, but information about conformational epitopes is still not given although the three-dimensional structure of birch profilin is known.

The aim of our study was to define cross-reactive ligands for different profilin-specific IgE Ab by the use of random peptide phage libraries. We selected birch pollen, mugwort pollen and celery tuber protein extracts, as these are sources of allergens that are responsible for the celery-birch-mugwort-spice syndrome [19–21]. Profilin is known to cause immediate type reactions and has been described to be one of at least three cross-reactive structures inducing the celery-birch-mugwort-spice syndrome [21].

To eliminate unspecific binding of phage-displayed peptides with other serum Ab and to enrich profilin-specific IgE, Ab were affinity purified from a patient's serum. Mugwort pollen, birch pollen and celery tuber profilin-specific IgG/IgE Ab were panned in series with pooled constrained and unconstrained random nonapeptide libraries to define cross-reactive IgE epitope mimics [13, 14]. Although we used the libraries pooled, phage clones identified after the last panning round were derived

exclusively from the constrained library. An immunodot assay showed that isolated phages bound specifically to the variable domains of the purified Ab, as no binding occurred to myeloma IgE or IgG Ab. The biopanning with profilin-specific IgG/IgE Ab resulted in an affinity selection of the mimotope CAISGGYPVC which was present in five of eight IgE-binding clones (62.5 %). This peptide, also when isolated from a nonapeptide library, consists of eight amino acids flanked by two cysteines. Such variations may occur during construction of the library (F. Felici, personal communication).

Neither comparison of the mimotope with the birch profilin sequence [1] nor with known sequential epitopes for IgE mapped on the C and N termini of birch profilin showed any sequence homology. Therefore we speculate that the mimotope CAISGGYPVC represents a conformational IgE epitope on profilin, although we were not capable to define a corresponding area on the birch profilin of which the crystal structure has been published recently [12].

Immunoblot inhibition studies with IgG/IgE Ab used for biopanning and the synthetic peptide CAISGGYPVC identified it as a mimotope shared by birch pollen, mugwort pollen, and celery tuber profilin. The magnitude of inhibitions was substantial with regards to the fact that the IgE response is polyclonal (Fig. 3). We speculate that the mimotope identified might represent a relatively important binding site for IgE on the profilin molecule because it also affected binding of IgE from three patients of our study to profilin. The limited amount of affinity-purified IgE Ab and the presence of other Ig isotypes in the preparations, however, did not allow to characterize binding affinities by measurement of dissociation constants.

We describe here the successful use of a constrained peptide library for isolating the mimotope CAISGGYPVC which represents a cross-reacting epitope mimic for IgE Ab directed against mugwort pollen, birch pollen and celery tuber profilin. Such peptides, defined by phage display techniques, may represent candidates for peptide therapies, as they might block the antigen binding sites of IgE without cross-linking and subsequent release of mediators that cause type I allergy symptoms.

4 Materials and methods

4.1 Characterization of patients and sera

Sera were collected from four exclusively profilin-allergic patients. The patients suffered from inhalant allergy during the flowering season of birch and mugwort and had experienced adverse reactions to foods, such as celery tuber. Skin prick tests with these allergen sources were positive and immunoblot analysis revealed an exclusive IgE binding to profilins of birch pollen, mugwort pollen and celery tuber.

4.2 Affinity purification of patients' IgG/IgE Ab

4.2.1 Preparation of protein extracts and immunosorbent

Birch pollen and mugwort pollen were obtained from Allergon AB (Engelholm, Sweden), celery tuber was purchased in a local retail shop. Protein extractions were performed as previously described [22] and protein concentrations were determined colorimetrically using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

Ten milligrams of birch pollen, mugwort pollen or celery tuber extract were dissolved in coupling buffer (0.2 M NaHCO₃, 0.5 M NaCl, pH 8.8) and mixed with 3 g CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). To block any remaining active groups the gels were incubated with 1 M ethanolamine (pH 9.0), subsequently extensively washed and packed into glass columns.

4.2.2 Affinity chromatography

Serum of one patient (30 ml) was diluted 1:2 in 0.9 % (w/v) NaCl and dialyzed against 0.9 % (w/v) NaCl prior to application to the affinity columns. For each separation run (two runs per type of affinity column were performed), 10 ml of diluted serum were loaded and recirculated overnight at 4 °C. After extensive washing with 0.9 % (w/v) NaCl, bound Ab were eluted with 3 M KSCN in 5 mM phosphate buffer, pH 6.5. Eluted fractions were immediately neutralized, dialyzed against 0.9 % (w/v) NaCl and lyophilized. Quality and specificity of eluents were checked on immunoblots (see below).

4.2.3 Dot assay with affinity-purified Ab

To determine the yield of affinity-purified human IgG/IgE Ab, a dot assay was performed. For quantification IgG/IgE Ab, serial dilutions of human monoclonal myeloma IgE (SU-11) [23] and human myeloma IgG Ab were dotted onto nitrocellulose. After blocking with 50 mM Tris, 150 mM NaCl, pH 7.4 (TBS) containing 0.15 % (w/v) casein (Fluka Chemie, Buchs, Switzerland) nitrocellulose strips were incubated with peroxidase-labeled polyclonal sheep anti-human IgE and polyclonal sheep anti-human IgG Fab Ab (The Binding Site, Birmingham, GB) for 1 h at room temperature. After washing the enzyme reaction was developed with 4-chloronaphthol substrate. Serial dilutions of the monoclonal myeloma IgE and myeloma IgG Ab were used to determine the concentration of affinity-purified Ab.

4.3 Biopanning: phage selection with profilin-specific IgG/IgE Ab

4.3.1 Panning procedure

Affinity-purified, profilin-specific IgG/IgE Ab were screened with a pool of two random nonapeptide phage libraries. One library, constructed by Felici et al. [13], consisted of nine random amino acids inserted in the major coat protein pVIII of the M13 bacteriophage. The other library was a similar nonamer library in which random nonapeptides are flanked by two cysteine residues and expressed in constrained form on the virus surface [14].

Four successive panning rounds with affinity-purified human IgG/IgE Ab directed against (1) mugwort pollen, (2) birch pollen, (3) celery tuber, and (4) mugwort pollen profilin were performed according to the method of Parmley and Smith [24]. For each round, 1 µg/0.1 µg IgG/IgE Ab in 0.1 M NaHCO₃, pH 8.5 were coated on ELISA plates (Nunc, Roskilde, Denmark). After blocking with PBS/3 % (w/v) BSA at 37 ° for 1 h, approximately 10⁸ PFU of freshly prepared phages from each library were pooled and incubated with coated Ab. After extensive washing, bound phages were eluted and amplified by infecting freshly grown *E. coli* XL-1 blue and superinfection with helper phage VCS M13 (10¹² PFU/ml). Amplified phage particles were precipitated with PEG, resuspended in TBS/0.15 % (w/v) casein and used for the following panning round.

4.3.2 Dot assay with selected phages

Affinity-purified human IgG/IgE antibodies (5 ng and 10 ng, respectively), 10 ng of monoclonal myeloma IgE (SU-11) and IgG Ab were dotted onto nitrocellulose. After blocking with TBS/0.15 % (w/v) casein, nitrocellulose strips were incubated with 10⁷ PFU of amplified phage after each panning round, with 2 × 10⁸ PFU from the library pool and with 10¹² PFU of the helper phage VCS M13. Bound phages were detected with a 1:1000 diluted peroxidase-labeled rabbit anti-phage Ab [25].

4.3.3 Colony screening of selected phages

Isopropyl, β -D-thiogalactopyranoside (IPTG) (5 mM)-treated nitrocellulose filters were applied on LB/carbenicillin plates with 50 phage clones from the fourth panning round and transferred to new LB/carbenicillin plates for 6 h at 37 °C. Nitrocellulose filters were removed, blocked with TBS/0.15 % (w/v) casein and incubated overnight at 4 °C with affinity-purified IgG/IgE Ab directed against birch pollen-, mugwort pollen- and celery tuber profilin. Bound IgE Ab were detected with 100 000 cpm of ^{125} I-labeled anti-human IgE Ab (Pharmacia, Uppsala, Sweden) per ml blocking buffer.

4.3.4 DNA sequencing

Phagemid DNA was prepared from overnight cultures of single phage clones selected from colony screening and sequenced by the Sanger dideoxy method using a Thermo Sequenase cycle sequencing kit (Amersham, Little Chalfort, GB) with fluorescent-labeled primers, and analyzed by a LI-COR DNA Sequencer 4000L (LI-COR Inc., Lincoln Nebraska).

4.3.5 Synthetic peptides

Peptides were synthesized and purified by HPLC by piChem (Graz, Austria).

4.4 SDS-PAGE, IgE immunoblot and inhibition experiments

SDS-PAGE was performed according to the method of Laemmli [26] in a vertical slab gel apparatus (Biometra, Göttingen, Germany) with modifications as described previously [22]. Protein extracts of birch pollen, mugwort pollen and celery tuber were separated by denaturing SDS-PAGE and blotted onto nitrocellulose according to the method of Towbin et al. [27]. After blocking with 50 mM sodium phosphate buffer, pH 7.5, 0.5 % Tween-20, 0.5 % BSA, nitrocellulose strips were incubated with patient's serum (1:10), or with effluent and eluent of affinity chromatography (1 ml each). For inhibition experiments, diluted sera (1:100) from three profilin-allergic patients or affinity-purified Ab (100 ng) were preincubated with either 2.5 μ g (0.175 nmol) of rBet v 2 [1] or with synthetic peptides (0.175 nmol). Incubations were carried out under continuous shaking over night at 4 °C. Bound IgE Ab were detected with 100 000 cpm of ^{125} I-labeled anti-human IgE Ab (Pharmacia, Uppsala, Sweden) per ml blocking buffer.

Acknowledgments: We acknowledge IRBM (Istituto di Ricerche di Biologia Molecolare P. Angeletti SPA; Rome, Italy) for kindly providing the pVIII 9aa and the pVIII 9aa.Cys phage libraries. We are also grateful to Dr. Franco Felici for inspiring discussions. Furthermore, we thank Dr. Christof

Ebner from the Allergy Clinic Reumannplatz in Vienna, Austria for providing the patients' sera.

This work was supported by S06074-Med from the Fonds zur Förderung Wissenschaftlicher Forschung (FWF), Vienna, Austria.

5 References

- 1 Valenta, R., Duchêne, M., Pettenburger, K., Sillaber, C., Valent, P., Bettelheim, P., Breitenbach, M., Rumpold, H., Kraft, D. and Scheiner, O., Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991. **253**: 557–560.
- 2 Valenta, R., Duchêne, M., Ebner, C., Valent, P., Sillaber, C., Deviller, P., Ferreira, F., Tejkl, M., Edelmann, H., Kraft, D. and Scheiner, O., Profilins constitute a novel family of functional plant pan-allergens. *J. Exp. Med.* 1992. **175**: 377–385.
- 3 Hirschwehr, R., Valenta, R., Ebner, C., Ferreira, F., Sperr, W. R., Valent, P., Rohac, M., Rumpold, H., Scheiner, O. and Kraft, D., Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J. Allergy Clin. Immunol.* 1992. **90**: 927–936.
- 4 Van Ree, R., Vointenko, V., Van Leeuwen, W. A. and Aalberse, R. C., Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int. Arch. Allergy Immunol.* 1992. **98**: 97–104.
- 5 Valenta, R., Ferreira, F., Grote, M., Swoboda, I., Vrtala, S., Duchêne, M., Deviller, P., Meagher, R. B., McKinnley, E., Heberle-Bors, E., Kraft, D. and Scheiner, O., Identification of profilin as an actin-binding protein in higher plants. *J. Biol. Chem.* 1993. **268**: 22777–22781.
- 6 Ebner, C., Hirschwehr, R., Bauer, L., Breiteneder, H., Valenta, R., Ebner, H., Kraft, D. and Scheiner, O., Identification of allergens in fruits and vegetables: IgE-crossreactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J. Allergy Clin. Immunol.* 1995. **95**: 962–969.
- 7 Calkhoven, P. G., Aalbers, M., Koshte, V. L., Pos, O., Oei, H. D. and Aalberse, R. C., Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 1987. **42**: 382–390.
- 8 Valenta, R., Ball, T., Vrtala, S., Duchêne, M., Kraft, D. and Scheiner, O., cDNA cloning and expression of timothy grass (*Phleum pratense*) pollen profilin in *Escherichia coli*: comparison with birch pollen profilin. *Biochem. Biophys. Commun.* 1994. **199**: 106–118.

- 9 Mittermann, I., Swoboda, I., Pierson, E., Eller, N., Kraft, D., Valenta, R. and Heberle-Bors, E., Molecular cloning and characterization of profilin from tobacco (*Nicotiana tabacum*): increased profilin expression during pollen maturation. *Plant. Mol. Biol.* 1995. **27**: 137–146.
- 10 Vidali, L., Perez, H. E., Valdes-Lopes, V., Noguez, R., Zamudio, F. and Sanches, F., Purification, characterization, and cDNA cloning of profilin from *Phaseolus vulgaris*. *Plant. Physiol.* 1995. **108**: 115–123.
- 11 Staiger, C. I., Goodbody, K. C., Hussey, P. J., Valenta, R., Drobak, B. K. and Lloyd, C. W., The profilin multi-gene family of maize: differential expression of three isoforms. *Plant J.* 1993. **4**: 631–641.
- 12 Fedorov, A. A., Ball, T., Mahoney, N. M., Valenta, R. and Almo, S. C., The molecular basis for allergen cross-reactivity: crystal structure and IgE-epitope mapping of birch pollen profilin. *Structure* 1997. **5**: 33–45.
- 13 Felici, F., Castagnoli, L., Musacchio, A., Jappelli, R. and Cesareni, G., Selection of antibody ligands from a large library of oligopeptides expressed on a multivalent exposition vector. *J. Mol. Biol.* 1991. **222**: 301–310.
- 14 Luzzago, A., Felici, F., Tramontano, A., Pessi, A. and Cortese, R., Mimicking of discontinuous epitopes by phage-displayed peptides. I. Epitope mapping of human H ferritin using a phage library of constrained peptides. *Gene* 1993. **128**: 51–57.
- 15 Felici, F., Luzzago, A., Folgori, A. and Cortese, R., Mimicking of discontinuous epitopes by phage-displayed peptides, II. Selection of clones recognized by a protective monoclonal antibody against the *Bordetella pertussis* toxin from phage peptide libraries. *Gene* 1993. **128**: 21–27.
- 16 Prezzi, C., Nuzzo, M., Meola, A., Delmastro, P., Galfrè, G., Cortese, R., Nicosia, A. and Monaci, P., Selection of antigenic and immunogenic mimics of hepatitis C virus using sera from patients. *J. Immunol.* 1996. **156**: 4504–4513.
- 17 Folgori, A., Tafi, R., Meola, A., Felici, F., Galfrè, G., Cortese, R., Monaci, P. and Nicosia, A., A general strategy to identify mimotopes of pathological antigens using only random peptide libraries and human sera. *EMBO J.* 1994. **13**: 2236–2243.
- 18 Motti, C., Nuzzo, M., Meola, A., Galfrè, G., Felici, F., Cortese, R., Nicosia, A. and Monaci, P., Recognition by human sera and immunogenicity of HBsAg mimotopes selected from an M13 phage display library. *Gene* 1994. **146**: 191–198.
- 19 Wüthrich, B. and Hofer, T., Nahrungsmittelallergie: das "Sellerie-Beifuß-Gewürz-Syndrom". *Dtsch. Med. Wochenschr.* 1984. **109**: 981–986.
- 20 Wüthrich, B., Stöger, P. and Johannson, S. G. O., RAST-spezifische IgE auf Gewürze bei Sensibilisierung gegen Birke-, Beifußpollen und Sellerie. *Allergologie* 1992. **11**: 380–383.
- 21 Bauer, L., Hirschwehr, R., Wüthrich, B., Pichler, C., Fritsch, R., Scheiner, O., Ebner, C. and Kraft, D., IgE-cross-reactivity between birch pollen, mugwort pollen and celery is due to at least three distinct cross-reacting allergens. Immunoblot investigations of the birch-mugwort-celery syndrome. *Clin. Exp. Allergy* 1996. **26**: 1161–1170.
- 22 Jarolim, E., Rumpold, H., Endler, A. T., Ebner, H., Breitenbach, M., Scheiner, O. and Kraft, D., IgE and IgG antibodies of patient with allergy to birch pollen as tools to define the allergen profil of *Betula verrucosa*. *Allergy* 1989. **44**: 385–395.
- 23 Zürcher, A. W., Lang, A. B., Aebischer, I., Miescher, S. and Stadler, B. M., IgE-producing hybridomas established after B-cell culture in the CD40 system. *Immunol. Lett.* 1995. **46**: 49–57.
- 24 Parmley, S. F. and Smith, G. P., Antibody-selectable filamentous fd phage vectors: affinity purification of target genes. *Gene* 1988. **73**: 305–318.
- 25 Vogel, M., Miescher, S., Biaggi, C. and Stadler, B. M., Human anti-IgE antibodies by repertoire cloning. *Eur. J. Immunol.* 1994. **24**: 1200–1207.
- 26 Laemmli, U. K., Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970. **227**: 680–685.
- 27 Towbin, H., Staehelin, T. and Gordon, J., Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* 1979. **76**: 4350–4354.

Correspondence: Erika Jensen-Jarolim, Dept. of General and Experimental Pathology, AKH, EBO 3Q, Währinger Gürtel 18–20, A-1090 Vienna, Austria
Fax: +43-40400-5130
e-mail: erika.jensen-jarolim@akh-wien.ac.at